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EVALUATING THE EFFECTS OF NEONICOTINOID SEED TREATMENTS ON AGRICULTURAL MICROBIOMES

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	ANalysis Of VAriance
ASV	Amplicon Sequence Variant
cp	c -colonizers, p -persisters
DESeq2	Differential Expression Analysis of Sequence data
FDR	False-Discovery Rate
NINJA	Nematode INdicator Joint Analysis
NMDS	Non-metric MultiDimensional Scaling
NST	Neonicotinoid Seed Treatment
PCoA	Principal Coordinate Analysis
PERMANOVA	PERmutational Multivariate ANalysis Of VAriance
RefSeq	Reference Sequence
RIN	RNA Integrity Number
SAMSA	Simple Analysis of Metatranscriptomes through Sequence Annotation
SE	Standard Error
SD	Standard Deviation

RÉSUMÉ

Les néonicotinoïdes, une classe d'insecticides systémiques, sont largement appliqués pour contrôler les ravageurs des cultures agricoles. Des études antérieures indiquent que l'application généralisée des néonicotinoïdes a des effets négatifs sur les macro- et micro-organismes bénéfiques, notamment les pollinisateurs. Les microbiotes de la phyllosphère et du sol jouent un rôle clé dans la régulation de l'écosystème, incluant les bactéries et les nématodes libres qui contribuent à la performance des cultures et sont essentiels pour la croissance et la santé des plantes et la fertilité du sol. Les nématodes sont également des bioindicateurs de la qualité du sol et, en tant qu'invertébrés, ils sont des cibles potentielles des néonicotinoïdes. Il est donc essentiel de comprendre la variation de la composition et des fonctions de ces communautés microbiennes en réponse aux perturbations environnementales, notamment dans le contexte de l'application de néonicotinoïdes. Peu d'études ont porté sur les impacts non ciblés des néonicotinoïdes sur les communautés microbiennes. De plus, la plupart de ces études ont été réalisées en laboratoire et se limitent à une seule espèce. Il y a donc encore un manque de connaissances sur ces impacts dans les agroécosystèmes.

Dans ma thèse, j'ai étudié les effets non ciblés du traitement de semences aux néonicotinoïdes sur les communautés bactériennes de la phyllosphère et du sol, les communautés de nématodes et l'expression génique microbienne du sol dans un agroécosystème soya-maïs. Pour améliorer notre compréhension des impacts des néonicotinoïdes sur les microbiomes agricoles, un projet de recherche structuré visant à étudier la variation des taxons et de l'expression des gènes en réponse à l'application de néonicotinoïdes était nécessaire. En plus, la détermination des facteurs de variation et des modèles de cooccurrence et d'interactions de ces communautés microbiennes pourrait nous permettre de mieux comprendre la dynamique des communautés microbiennes et leurs réponses aux néonicotinoïdes en tant que stress environnemental.

Ma thèse aborde trois objectifs principaux : (1) quantifier la composition taxonomique bactérienne de la phyllosphère et du sol, la composition taxonomique des nématodes, et l'expression génique chez les bactéries et les eucaryotes du sol, (2) déterminer les moteurs de variation de la composition des communautés microbiennes et de l'expression génique, et (3) détecter les changements dans la structure et la diversité des communautés microbiennes, la composition taxonomique, l'expression génique et les modèles de cooccurrence nématode-bactérie en réponse aux néonicotinoïdes dans un agroécosystème soya-maïs. À cette fin, nous avons utilisé le séquençage d'amplicons (gènes 16S rRNA bactérien et 18S rRNA des nématodes) et la métatranscriptomique.

Nos résultats révèlent des impacts des néonicotinoïdes sur la communauté bactérienne de la phyllosphère et du sol et la composition taxonomique des nématodes. Les néonicotinoïdes ont modifié l'abondance relative de certaines bactéries et nématodes, y compris une augmentation des genres bactériens liés à la biodégradation des néonicotinoïdes, une diminution de plusieurs genres de bactéries potentiellement bénéfiques pour la croissance des plantes et la fixation de l'azote, et de la famille des nématodes omnivores, Dorylaimidae, qui est un indicateur de stress environnemental. Cependant, les espèces hôtes et le temps ont également été des facteurs importants de variation des communautés microbiennes qui expliquent souvent les changements dans la composition de ces communautés, mieux que les néonicotinoïdes. Les néonicotinoïdes ont également affecté de manière significative la structure et la composition taxonomique des réseaux de cooccurrence nématodes-bactéries. De plus, les effets des néonicotinoïdes sur l'expression des gènes microbiens varient dans le temps. Certains gènes associés aux protéines de choc thermique, aux fonctions de régulation et aux processus métaboliques étaient sous-exprimés, tandis que d'autres gènes associés à la photosynthèse et à la réparation de l'ADN étaient surexprimés à différents moments en réponse aux néonicotinoïdes. À notre connaissance, cette étude est le premier exemple d'un plan expérimental représentant les conditions réelles d'exploitation agricole pour évaluer les impacts non ciblés du traitement de semences aux néonicotinoïdes sur la composition taxonomique microbienne et l'expression génique. Elle améliore notre compréhension des effets des néonicotinoïdes sur la variation des communautés microbiennes dans une rotation de cultures de soya-maïs au Québec, Canada. Elle met également en évidence le rôle des espèces hôtes et du temps comme facteurs importants de la variation des communautés microbiennes dans les agroécosystèmes.

Mots clés: néonicotinoïdes, traitement de semences, composition de la communauté microbienne, réseaux de cooccurrence microbienne, expression génique microbienne, facteurs de variation de la communauté, métatranscriptomique

ABSTRACT

Neonicotinoids, a class of systemic insecticides, are widely applied to control early-season and foliar-feeding pests. Previous studies indicated that the widespread application of neonicotinoids had adversely affected beneficial macro- and micro-organisms, especially insect pollinators. In agroecosystems, the phyllosphere and soil microbiota play a key role in regulating the ecosystem, including bacteria and free-living nematodes that contribute to crop performance and are vital for plant growth and health and soil fertility. Nematodes are also bioindicators of soil quality and ecological functions and, as one of the most abundant soil invertebrates, are potential targets of neonicotinoids. Thus, it is critical to understand variation in the composition and functions of these microbial communities in response to environmental disturbances, such as neonicotinoid application. Few studies have focused on the non-target impacts of neonicotinoids on microbial communities. However, most of these studies are lab-based and limited to a single species and there is still a lack of knowledge on these impacts in agroecosystems.

In my thesis, I have investigated the non-target effects of neonicotinoid seed treatment on phyllosphere and soil bacterial communities, soil free-living nematode communities and soil microbial gene expression in a soybean-corn agroecosystem. To improve our understanding of the impacts of neonicotinoids on agricultural microbiomes, a structured research project to investigate the taxa and gene community-wide variation in response to neonicotinoid application was required. In addition, determining the drivers of variation and patterns of co-occurrence and interactions of these microbial communities may allow us to better understand the dynamics of microbial communities and how they respond to neonicotinoid application as an environmental stress.

Therefore, in my thesis, I address three main objectives: (1) quantifying phyllosphere and soil bacterial taxonomic composition, soil nematode taxonomic composition, and soil bacterial and eukaryotic gene expression, (2) determining the drivers of microbial community composition and gene expression variation, and (3) detecting changes in microbial community structure and diversity, taxonomic composition, gene expression and nematode-bacteria co-occurrence patterns in response to neonicotinoid seed treatment. We used amplicon sequencing (bacterial 16S and nematode 18S rRNA genes) and metatranscriptomics to characterize the diversity, taxonomic composition and gene expression of phyllosphere and soil microbial communities and quantify their variation in response to neonicotinoid seed treatment in a soybean-corn agroecosystem. We also investigated soil nematode-bacteria co-occurrence networks and their changes in response to neonicotinoid application.

Our findings indicated impacts of neonicotinoid seed treatment on phyllosphere and soil bacterial community and soil nematode taxonomic composition. Neonicotinoid seed treatment altered the relative abundance of some bacteria and nematodes, including an increase in the relative abundance of bacterial genera related to neonicotinoid biodegradation, a decrease in the relative abundance of several potentially beneficial soil bacteria genera, such as those involved in plant growth and nitrogen fixation, as well as the relative abundance of the omnivorous nematode family, Dorylaimidae, which is an indicator of environmental stress. However, host species and time were also strong drivers of microbial community variation and often explained the changes in microbial community composition better than neonicotinoid seed treatment. Neonicotinoids also significantly affected the structure and taxonomic composition of soil nematode-bacteria co-occurrence networks. Moreover, the effects of neonicotinoid seed treatment on soil microbial gene expression varied with time. Some genes associated with heat shock protein, regulatory functions and metabolic processes were underexpressed, while some other genes associated with photosynthesis and DNA repair were overexpressed at different time points in response to neonicotinoid seed treatment. To our knowledge, this study is the first example of an experimental design representing real farming conditions to evaluate the nontarget impacts of neonicotinoid seed treatment on microbial taxonomic composition and gene expression. It improves our understanding of the effects of neonicotinoids on community-wide variation in the phyllosphere and soil microbiome in a soybean/corn crop rotation in Quebec, Canada. It also highlights the role of host species and time as strong drivers of microbial community variation in agroecosystems.

Keywords: neonicotinoids, seed treatment, microbial community composition, microbial co-occurrence networks, microbial gene expression, drivers of community, metatranscriptomics

INTRODUCTION

In this thesis, I present three chapters studying the non-target effects of neonicotinoids, a class of widely used insecticide pesticides, on the microbial communities of the phyllosphere (the aerial surfaces of plants, especially leaves) and soil in a soybean-corn agroecosystem. This project aims to characterize the diversity, taxonomic composition and gene expression in these microbial communities and determine the changes in microbial community diversity, composition and gene expression in response to neonicotinoid seed treatment.

In modern agriculture, pesticides have widely contributed to crop yield and food production by preventing crop losses caused by insect pests, diseases and weeds. However, the excessive use of chemical pesticides during the past decades, along with their non-target impacts on agriculturally beneficial macro- and micro-organisms have led to environmental stress and disturbances, which may negatively affect plant growth and development, and soil quality and fertility (Handa et al., 1999; Fox et al., 2007; Hussain et al., 2009; Lo, 2010; Fishel, 2011; Sofo et al., 2012). Among the frequently used pesticides, neonicotinoids are a family of systemic, neuro-active insecticides discovered in the late 1980s (Kagabu, 1996; Tomizawa & Casida, 2005). Neonicotinoids are chemically similar to nicotine and act on nicotinic acetylcholine receptors (nAChRs). They disrupt the central nervous system's neurotransmission by binding to nAChRs, particularly in invertebrates (e.g., arthropods, annelids, nematodes, etc.). Invertebrates are selectively more sensitive to neonicotinoids due to the structural differences between their nAChRs and those of vertebrates (Tomizawa et al., 1999; Tomizawa & Casida, 2005).

Since 2000, due to the serious economic damage to soybeans in North America caused by soybean aphids, producers have started using foliar or seed neonicotinoid treatments for a wide variety of crop plants (Koch et al., 2010; Simon-Delso et al., 2015). Among different ways of applying this pesticide, neonicotinoid seed treatment is the most popular approach to control various foliar and soil early-season pests, such as aphids (*Aphis glycines* Matsumura, Homoptera: Aphididae), chinch bugs (*Blissus leucopterus* Say, Hemiptera: Lygaeidae), flea beetles (*Epitrix cucumeris* Harris, Coleoptera: Chrysomelidae), seedcorn maggots (*Delia platura* Meigen, Diptera: Anthomyiidae), southern corn leaf beetles (*Myochrous denticollis* Say, Coleoptera: Chrysomelidae), thrips (*Thrips* sp., Thysanoptera: Thripidae), whiteflies (*Bemisia tabaci*, Homoptera: Aleyrodidae), white grubs (*Phyllophaga* sp, Coleoptera: Scarabaeidae) and wireworms (*Melanotus cribulosus*, Coleoptera: Elateridae) (Wilde et al., 2004; Elbert et al., 2008; Labrie et al., 2020). Many questions are being raised about the widespread and prophylactic use of seed treatments with insecticides of the neonicotinoid family without any verification of the presence of the targeted pests. In Canada, since 2008, almost 100% of Ontario and Quebec's corn seeds and about 60% of soybean seeds have been coated with neonicotinoids (Grant, 2014; Giroux, 2019). In the US, these amounts were 94% for corn seeds and one-third for soybeans in 2010 (America, 2012). Studies have shown that most of the time, neonicotinoids have no significant effects on crop yield (Cox & Cherney, 2011; Reisig et al., 2012; Penn & Dale, 2017; Alford & Krupke, 2018), especially when the pest pressure is low (Labrie et al., 2020). In addition, many pests are reported to have developed some level of neonicotinoid resistance after decades of worldwide application of these pesticides (Cahill et al., 1996; Elbert & Nauen, 2000; Zhao et al., 2000; Wang et al., 2002; Gorman et al., 2007; Karunker et al., 2008; Karatolos et al., 2010; Puinean et al., 2010; Bass et al., 2011; Tiwari et al., 2011; Kshirsagar et al., 2012; Huseth et al., 2018).

Neonicotinoid compounds are small molecules that are highly soluble in water. They are very persistent and may remain from 20 to 200 days in herbaceous plants, one year in woody plants and up to three years in soil (Goulson, 2013; Myers & Hill, 2014; Bonmatin et al., 2015; Zhang et al., 2016; Alford & Krupke, 2017). Their systemic nature lets them spread from the seed covering to all parts of the plant and so contaminate the pollinators through flowers, guttation, nectar and pollen (Sur & Stork, 2003; Bonmatin et al., 2005; Girolami et al., 2009). The pollinators may also be affected by neonicotinoids during sowing because of the high concentration of this pesticide in the generated dust (Krupke et al., 2012; Bonmatin et al., 2015; Chagnon et al., 2015; Simon-Delso et al., 2015). Hence, neonicotinoid molecules can easily be found in different environments including air, water, soil and plants and contaminate non-target species. The non-target effects of neonicotinoids on insect pollinators, particularly honeybees, beneficial soil invertebrates, such as earthworms, and other agriculturally beneficial organisms, have drawn a lot of attention during the last decades (Iwasa et al., 2004; Samson-Robert et al., 2014, 2017; Sanchez-Bayo & Goka, 2014; Bonmatin et al., 2015; Pisa et al., 2015). For example, studies have shown that exposure to neonicotinoids can interfere with honeybee reproduction and ability to navigate and fly (Hopwood et al., 2012; Whitehorn et al., 2012; Vanbergen & the Insect Pollinators Initiative, 2013), which can also affect birds, as they lose a portion of their food supply (Hopwood et al., 2013). Other studies have reported negative effects of neonicotinoids on the natural control of pest populations by affecting their predators or natural enemies (Seagraves & Lundgren, 2012; Frewin et al., 2014; Douglas & Tooker, 2016).

Following the concerns about the negative effects of neonicotinoids on pollinators and other beneficial insects, in 2018, the European Union banned the outdoor uses of three types of neonicotinoids (imidacloprid, clothianidin, and thiamethoxam) (EFSA, 2018). In 2020, the United States Environmental Protection Agency banned the spray application of neonicotinoids and proposed restrictions on the amount and time of their application, especially on blooming crops (EPA & OCSPP, 2020). Beginning in 2018, farmers in Quebec (Canada) need a prescription from agronomists before applying neonicotinoids on crops (MELCC, 2020). In 2021, Health Canada decided not to completely ban neonicotinoid application but did apply additional restrictions, including buffer zones around affected sectors and limiting neonicotinoid seed treatment rate for some crops, including soybean and corn (Health Canada, 2021).

The proposed restrictions on neonicotinoid application are mostly based on studies on the effects of the pesticides of this family on beneficial invertebrates. However, the impacts of neonicotinoids on microbial communities have not yet been thoroughly explored. There are few studies that indicate neonicotinoids have non-target impacts on the phyllosphere and soil beneficial microbial community composition and functions, including bacteria, fungi and free-living nematodes (Singh & Singh, 2006; Cycoń et al., 2013; Cycoń & Piotrowska-Seget, 2015; Filimon et al., 2015; Hopewell et al., 2017; Li et al., 2018; Bradford et al., 2020; Parizadeh et al., 2021). Most of these studies are lab-based and limited to a single species. In this thesis, I focus on the non-target impacts of neonicotinoid seed treatment on the community composition and diversity of the phyllosphere and soil bacteria and soil free-living nematodes, as well as community-wide soil microbial gene expression in real farming conditions.

Microbial communities (microbiota) colonize different ecosystems, including the phyllosphere and soil and provide several essential functions to regulate their host ecosystem. The phyllosphere microbiota includes prokaryotes (bacteria and archaea) and eukaryotes (such as fungi) (Lindow & Brandl, 2003). These microbial communities are abundant and diverse, help protect the host plant against infection and diseases, and have positive impacts on plant fitness, growth, mortality, and function (Innerebner et al., 2011; Vorholt, 2012; Maignien et al., 2014; Ritpitakphong et al., 2016). The global population of phyllosphere bacterial communities, as the most abundant colonies on leaves, is estimated to be 10^{26} cells (Morris & Kinkel, 2002), while bacterial cell densities in the phyllosphere are about 10^6 to 10^7 cells/cm2 of leaf (about 10^8 cells/gram) (Lindow & Brandl, 2003). Soil microbial communities include prokaryotic (bacteria and archaea) and eukaryotic (fungi, yeasts, protozoa, algae, and nematodes) organisms (Shannon et al., 2006; Ferris et al., 2012). Bacteria, fungi and many free-living nematodes, especially bacterial- and fungal-feeding nematodes, play a key role in soil fertility, organic matter production and nutrient mineralization (nitrogen fixation, carbon digestion for respiration and assimilation, and phosphorus, sulfur, and potassium digestion for assimilation) (Doran & Zeiss, 2000; Garbeva et al., 2004; Wang et al., 2004; Holtkamp et al., 2011; Ferris et al., 2012). They can improve water management in soil and reduce the need for fertilizers by increasing available nutrients. Previous studies have estimated that there are about 10^7 microbial species/gram and 10^{10} bacterial cells/gram of soil (Torsvik et al., 1990; Gans et al., 2005).

Studying the characteristics, drivers of variation and patterns of co-occurrence and interactions of microbial communities can lead us to an understanding of the system responses to environmental stress and perturbations, such as pesticide application. This will help us to predict the system dynamics and the shifts in microbial communities due to the environmental changes or disturbances through time (succession), and the mechanisms that cause this variation (Ulanowicz, 2004; Bascompte, 2009; Konopka, 2009; Dini-Andreote et al., 2015). The community composition of microbial taxa changes after perturbation and during the succession process (Schimel et al., 2007; Itoh et al., 2014). Some microbial communities become resistant to perturbations due to an increased abundance of resistant or resilient taxa or by altering their habitat (Schimel et al., 2007; Fierer et al., 2010). In contrast, low abundance taxa may have less chance of dispersal during succession (Sogin et al., 2006; Fierer et al., 2010). Furthermore, the microbial communities that coexist and inhabit the same ecosystem may be in relationships and interactions with each other and with the environment (Little et al., 2008). The factors that influence the diversity, composition and the patterns of succession and co-occurrence of the phyllosphere and soil microbial communities are still largely unknown. However, some previous studies show that they are governed by a variety of environmental factors, especially host plant species identity, time and site (Knief et al., 2010; Kembel et al., 2014; Sugiyama et al., 2014a; Laforest-Lapointe et al., 2016b; Hannula et al., 2019). Past studies also declare that there is a relationship between microbial community composition and diversity and microbial community functions (Bell et al., 2005; Fierer et al., 2007; Strickland et al., 2009). In addition, it is possible that some individual microbes change, but the functional capability of the community remains stable (Beisner et al., 2003; Walker et al., 2004). Previous studies show a high percentage of functional similarity among taxonomically diverse communities, indicating more consistency of functional traits than the taxonomic composition of microbial communities in different ecosystems (Burke et al., 2011; Steffen et al., 2012; Staley et al., 2014). However, current knowledge on microbial biodiversity and ecological functions is still in its very early stages in general, and for agroecosystems in particular, there is a lack of data on how microbial biodiversity varies as a function of pesticide application such as neonicotinoid treatment, space, host species and time. To address these issues, we first need to characterize microbial communities that inhabit the phyllosphere and soil.

Most microorganisms of the phyllosphere and soil cannot be grown or reproduced in the lab, which made their study complicated until the recent development of culture-independent molecular methods based on nucleic acid sequences (Müller & Ruppel, 2014). There are different technologies developed to study microbial communities in recent years, including next-generation technologies, such as amplicon sequencing and meta'omics (metagenomics, metatranscriptomics, metaproteomics and metabolomics). There is very little information documenting the impact of neonicotinoid seed treatment and their interactions with other potential drivers of microbial community variation, such as host species and time, on the phyllosphere and soil microbial community diversity, composition and their patterns of co-occurrence and interactions while the consequences of these effects could be significant in an agroecosystem. To address these questions, in the present study, we used amplicon sequencing and metatranscriptomics to quantify microbial diversity, composition and gene expression. We used amplicon sequencing, also referred to as metabarcoding sequencing, which is widely being used to determine the taxonomic composition of microbial communities by sequencing the variable regions of highly conserved genes (Neefs et al., 1993), as well as metatranscriptomics, also referred to as RNA-seq, to study the expressed genes (Bailly et al., 2007; Carvalhais et al., 2012). Using these approaches, we characterized the microbial communities of the phyllosphere and soil of two important agricultural crops, soybean and corn, which are usually being cultured in rotation and are among the crops whose seeds are covered by neonicotinoids. We then determined how microbial community taxonomic composition and gene expression and the patterns of microbial co-occurrence and succession were influenced in response to neonicotinoids.

0.1 Thesis structure and objectives

The main goal of this Ph.D. project was to assess the effects of neonicotinoid seed treatments on the bacterial community composition and diversity of the phyllosphere and soil, the free-living nematode community composition and diversity of soil, the co-occurrence patterns of soil bacterial and nematode communities, and the soil bacterial and eukaryotic gene expression in real farming conditions in a soybean/corn crop rotation in an experimental farm in Quebec, Canada. This study is structured in three chapters focusing on (i) characterizing the phyllosphere and soil bacterial taxonomic composition, soil nematode taxonomic composition, and soil bacterial and eukaryotic gene expression, (ii) determining the drivers of microbial community composition and gene expression variation, and (iii) identifying shifts in microbial community structure and diversity, taxonomic composition, gene expression and co-occurrence patterns in response to neonicotinoid seed treatment.

To address these goals, we performed bacterial 16S rRNA gene amplicon sequencing in soybean and corn phyllosphere and soil samples, as well as nematode 18S rRNA gene amplicon sequencing in soybean and corn soil samples collected over three years in l'Acadie, Quebec, Canada. We also applied a metatranscriptomic approach to evaluate the microbial gene expression of the soil samples retrieved from the first two years of the same soybean/corn crop rotation project. Here, I briefly describe the objectives and hypotheses for each of the following chapters:

0.1.1 Chapter 1: Neonicotinoid Seed Treatments Have Significant Non-target Effects on Phyllosphere and Soil Bacterial Communities

In the first chapter, we studied the changes in the phyllosphere and soil bacterial community composition and diversity in response to neonicotinoid seed treatment in a three-year soybean/corn crop rotation. In an agroecosystem, the phyllosphere and soil bacterial communities play a crucial role in plant growth and health and soil quality and fertility. Some previous studies showed that a variety of environmental factors, for example, host plant species and time, contribute greatly to the bacterial community variation in different ecosystems (Kembel et al., 2014; Laforest-Lapointe et al., 2016b; Hannula et al., 2019). However, the main drivers of the phyllosphere and soil bacterial community composition and diversity in an agroecosystem and the impacts of pesticide application, such as neonicotinoid seed treatment, on these communities are not thoroughly explored. To address these issues, we first characterized the phyllosphere and soil bacterial community composition in a soybean-corn agroecosystem using bacterial 16S rRNA gene amplicon sequencing. We then evaluated the proportion of bacterial community variation explained by habitat (phyllosphere and soil), host species (soybean and corn) and time (years and months) as potential drivers of bacterial community variation. We finally determined the effects of neonicotinoid seed treatment on the bacterial community composition and diversity and the bacterial temporal variation, as well as the changes in bacterial taxonomic composition in response to neonicotinoid application in both phyllosphere and soil. This chapter was published in 2021 in *Frontiers in Microbiology, Terrestrial Microbiology* (Parizadeh et al., 2021).

Objectives

1.1 Characterize the drivers of bacterial community variation in the soybean and corn phyllosphere and soil using amplicon sequencing;

1.2 Determine the effects of neonicotinoid seed treatment on the soybean and corn phyllosphere and soil bacterial community composition variation and diversity.

Hypotheses

H1.1 Habitat, host species and time will all contribute to variation in bacterial community composition and diversity;

H1.2 Neonicotinoid seed treatment will cause a shift in the bacterial community composition and a decrease in bacterial diversity of both phyllosphere and soil.

0.1.2 Chapter 2: Neonicotinoid Seed Treatments Influence Soil Nematode Taxonomic Composition and the Soil Microbial Co-occurrence Networks

In the second chapter, we explored the impacts of neonicotinoid seed treatment on the soil nematode community composition and diversity in a three-year soybean/corn crop rotation. Nematodes are important bioindicators to evaluate soil quality and ecosystem functions (Kennedy & Stubbs, 2006; Sharma et al., 2010) and play a key role in shaping soil microbial communities and influencing soil functions (Kennedy & Stubbs, 2006; Ferris, 2010a; Sharma et al., 2010). They are one of the most abundant soil invertebrates and, thus, one of the potential targets of neonicotinoid pesticides that are selectively more toxic to invertebrates. However, there are only a few lab-based studies on single nematode species indicating the negative neurological effects of neonicotinoids on nematodes (Hopewell et al., 2017; Bradford et al., 2020). We hypothesized that the non-target impacts of neonicotinoid seed treatment on soil bacterial communities could be related to the top-down regulation of bacteria by affecting nematode communities, as higher trophic levels that feed on them. We first used nematode 18S rRNA gene amplicon sequencing to identify the soil free-living nematode taxonomic composition in a soybean-corn agroecosystem and then classified nematode families to their trophic functional groups, also referred to as feeding guilds, according to references (Yeates et al., 1993). We also determined the proportion of nematode community variation explained by neonicotinoid seed treatment, as well as host species (soybean and corn) and time (years and months), as potential drivers of community variation. Finally, we assessed the impacts of neonicotinoid application on the nematode community structure and taxonomic composition, trophic groups, soil food web and the co-occurrence patterns of nematode and bacterial families. An article based on this chapter is in preparation.

Objectives

2.1 Characterize the soil free-living nematode communities using amplicon sequencing;

2.2 Determine the nematode community composition and trophic functional variation in response to neonicotinoid seed treatment;

2.3 Qualify the impacts of neonicotinoid seed treatment on the nematode and bacterial families co-occurrence patterns.

Hypotheses

Neonicotinoid seed treatment:

H2.1 affects the community composition of soil free-living nematodes;

H2.2 decreases soil nematode diversity;

H2.3 modulates soil nematode taxonomic composition and trophic functions;

H2.4 influences the co-occurrence patterns of bacteria and the free-living nematodes that feed on them.

0.1.3 Chapter 3: Effects of Neonicotinoid Seed Treatments on Soil Microbial Gene Expression Vary with Time in an Agricultural Ecosystem

In the third chapter, we aimed to study the effects of neonicotinoid seed treatment on the soil microbial gene expression in a two-year soybean/corn crop rotation. Given the vital role that microbial communities play in maintaining soil fertility and quality, to assess how these communities respond to neonicotinoid seed treatment, in addition to characterizing the present microbial communities in soil and their taxonomic composition variation, it is crucial to identify the active microbial communities and the variability in microbial gene expression. Few previous amplicon and metagenomic sequencing-based and biochemical studies have shown the effects of neonicotinoids on soil microbial functional activities, such as a decrease in soil respiration, nitrification and enzymatic activity, or an increase in the neonicotinoid-degrading bacterial functions (Singh & Singh, 2005; Filimon et al., 2015). However, none of these studies have evaluated the non-target impacts of neonicotinoid seed treatment on the community-wide variation of soil microbial gene expression. We addressed this objective by applying a metatranscriptomic approach to determine the soil microbial gene expression variation in response to neonicotinoid seed treatment. Therefore, we first characterized and profiled the soil microbial expressed genes in a soybean-corn agroecosystem based on the following databases: the SEED Subsystems hierarchical database (with four hierarchy levels) and the NCBI's RefSeq bacterial genomes and eukaryotic genomes. Then, we evaluated the proportion of the variability in soil microbial expressed genes explained by neonicotinoid seed treatment, as well as time (years and months) as a potential driver of community variation. Finally, we determined the changes in the composition and diversity of soil microbial expressed genes in response to time and neonicotinoid treatment. An article based on this chapter is in preparation.

Objectives

3.1 Characterize soil microbial gene expression, including bacterial and eukaryotic expressed genes, in a two-year soybean/corn crop rotation using metatranscriptomic sequencing;

3.2 Assess the effects of neonicotinoid seed treatment on soil microbial gene expression in this agroecosystem.

Hypotheses

H3.1 Neonicotinoid seed treatment and time affect soil microbial gene expression;

H3.2 The expression of pesticide degradation-related genes increases, while the expression of nitrification-related genes decreases in response to neonicotinoid seed treatment.

CHAPTER I

NEONICOTINOID SEED TREATMENT HAVE SIGNIFICANT NON-TARGET EFFECTS ON PHYLLOSPHERE AND SOIL BACTERIAL COMMUNITIES

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1.1 Abstract

The phyllosphere and soil are dynamic habitats for microbial communities. Non-pathogenic microbiota, including leaf and soil beneficial bacteria, plays a crucial role in plant growth and health, as well as in soil fertility and organic matter production. In sustainable agriculture, it is important to understand the composition of these bacterial communities, their changes in response to disturbances, and their resilience to agricultural practices. Widespread pesticide application may have had non-target impacts on these beneficial microorganisms. Neonicotinoids are a family of systemic insecticides being vastly used to control soil and foliar pests in recent decades. A few studies have demonstrated the long-term and non-target effects of neonicotinoids on agroecosystem microbiota, but the generality of these findings remains unclear. In this study, we used 16S rRNA gene amplicon sequencing to characterize the effects of neonicotinoid seed treatment on soil and phyllosphere bacterial community diversity, composition and temporal dynamics in a three-year soybean/corn rotation in Quebec, Canada. We found that habitat, host species and time are stronger drivers of variation in bacterial composition than neonicotinoid application. They respectively explained 37.3%, 3.2% and 2.9% of the community variation. However, neonicotinoids did have an impact on bacterial community structure, especially on the taxonomic composition of soil communities (2.6%) and over time (2.4%). They also caused a decrease in soil alpha diversity in the middle of the growing season. While the neonicotinoid treatment favored some bacterial genera known as neonicotinoid biodegraders, there was a decline in the relative abundance of some potentially beneficial soil bacteria in response to the pesticide application. Some of these bacteria, such as the plant growth-promoting rhizobacteria and the bacteria involved in the nitrogen cycle, are vital for plant growth and improve soil fertility. Overall, our results indicate that neonicotinoids have non-target effects on phyllosphere and soil bacterial communities in a soybean-corn agroecosystem. Exploring the interactions among bacteria and other organisms, as well as the bacterial functional responses to the pesticide treatment, may enhance our understanding of these non-target effects and help us adapt agricultural practices to control these impacts.

Keywords: bacterial community structure, bacterial diversity, host-microbe interactions, neonicotinoid seed treatment, pesticide non-target effects, phyllosphere, soil bacterial communities, temporal variation.

1.2 Introduction

The phyllosphere (the aerial surfaces of plants including leaves) and soil are colonized by microbial communities (microbiota), which are of great importance in the regulation of host and ecosystem function. These microbial communities, including beneficial bacteria, play a crucial role in plant growth promotion, decomposition and health control (Vorholt, 2012), as well as in soil fertility, nitrogen fixation, and organic matter production (Doran & Zeiss, 2000; Garbeva et al., 2004). Previous studies have shown that the taxonomic composition of phyllosphere bacteria is associated with host plant species identity (Kembel et al., 2014; Knief et al., 2010; Whipps et al., 2008) and changes predictably during the growing season and as plant ages (Redford & Fierer, 2009; Sugiyama et al., 2014b; Wagner et al., 2016). Host species were found to be a more important driver of variation in phyllosphere bacterial communities than time (Laforest-Lapointe et al., 2016b). Other studies on the composition of soil bacteria have associated community variations with host plant species and growth stage (Wieland et al., 2001), site (Clairmont et al., 2019) and time (Hannula et al., 2019; Tarlera et al., 2008). Host species were also shown to be a stronger driver of variation in soil bacterial communities than host plant growth stage and development time (Wieland et al., 2001).

Bacterial succession refers to the bacterial community variation patterns over time and in response to environmental changes and disturbances (Redford & Fierer, 2009). Comparing temporal versus spatial variation in bacterial community structure, the effects of time (seasonal variability) on bacterial communities is often higher than habitat impacts (Samaritani et al., 2017). Environmental disturbances and perturbations (such as cultivation methods, drought, climate change, and pesticide treatments) can also alter the bacterial community structure and composition (Itoh et al., 2014; Schimel et al., 2007). During the succession process, some bacterial communities may survive by modifying their habitat, increasing their abundance or becoming more resistant or resilient to disturbances (Fierer et al., 2010; Schimel et al., 2007). Hence, if a disturbance is persistent, it can cause long-term changes in bacterial community structure and affect bacterial succession (Fierer et al., 2010). During the last decades, the widespread application of chemical pesticides in agro-ecosystems has influenced many non-target species and their succession patterns (Itoh et al., 2014; Rodríguez-Valdecantos et al., 2017). Pesticides can change the interaction between plants and some bacteria, such as nitrogen-fixing rhizobacteria, which may lead to the inhibition of nitrogen fixation (Fox et al., 2007; Lo, 2010). They can also affect soil fertility and quality by impacting soil bacterial diversity and function and altering their nitrification, denitrification and mineralization of organic matter (Hussain et al., 2009). In this study, to assess the effects of pesticides on the phyllosphere and soil bacterial community structure and intra- and inter-annual succession, we have focused on a class of the most widely used insecticide pesticides, neonicotinoids.

Neonicotinoids (also known as neonics) are a family of systemic and neuro-active insecticides, chemically similar to nicotine, introduced in the late 1980s (Kagabu, 1996; Tomizawa & Casida, 2005). Like nicotine, they interrupt neural transmission in the nervous system by binding to the nicotinic acetylcholine receptors (nAChRs). Because of the fundamental distinctions between the nAChRs of invertebrates and vertebrates, neonicotinoids are selectively more toxic to invertebrates, like insects (Tomizawa et al., 1999; Tomizawa & Casida, 2003). In North America, neonicotinoids have mostly been used as seed treatments to control a variety of foliar and soil early-season insect pests in corn, soybean, wheat and other important crops (Douglas

& Tooker, 2015; Elbert et al., 2008; Samson-Robert et al., 2014). These treatments are most widely applied prophylactically, without any information on the actual presence of the targeted pests. Hence, previous studies have indicated that neonicotinoids often have no significant impact on crop yield (Alford & Krupke, 2018; Cox & Cherney, 2011; Penn & Dale, 2017; Reisig et al., 2012). A recent study that has extensively evaluated yield variations in response to neonicotinoid seed treatment with regards to the abundance and incidence of pest populations has reported that there is no significant difference in crop yield when pest pressure is low, which was the case in most of the sites under study (Labrie et al., 2020). The neonicotinoid compounds are tiny molecules and are highly soluble in water (Bonmatin et al., 2005). Given their systemic nature, plants take them up from the seed covering and translocate them to different tissues and products, including nectar, guttation and pollen (Bonmatin et al., 2005; Girolami et al., 2009; Sur & Stork, 2003). Neonicotinoids may remain active from 20-30 days in soybean (Myers & Hill, 2014) and corn (Alford & Krupke, 2017) to 200 days in winter wheat(Zhang et al., 2016). Plants only absorb about 20% of the seed covering (Alford & Krupke, 2017; Sur & Stork, 2003). The rest of the pesticide persists in soil for up to three years, depending on its active ingredient and the soil properties (e.g. soil type, organic matter content and pH) (Bonmatin et al., 2005; Goulson, 2013). During the last decades, many questions have been raised about the potential impacts of the widespread and prophylactic (Goulson, 2013; Labrie et al., 2020) use of neonicotinoids on non-target organisms. Past studies have shown some negative effects of neonicotinoids on agriculturally beneficial organisms, including beneficial soil invertebrates like earthworms (Pisa et al., 2015), and insect pollinators, particularly honeybees (Bonmatin et al., 2005; Iwasa et al., 2004; Samson-Robert et al., 2014, 2017; Sanchez-Bayo & Goka, 2014). Although neonicotinoids target organisms that possess a nervous system and the nAChRs, some studies have reported that they have non-target impacts on the functions and structure of microbial communities, such as fungal (Moulas et al., 2013) and bacterial structure, abundance and community composition in phyllosphere (Moulas et al., 2013; Zhang et al., 2008, 2009) and soil (Cycoń et al., 2013; Yu et al., 2020). Previous biochemical or culture-based microbiological studies have also confirmed the effects of these insecticides on bacterial respiration, phosphatase activity, and other enzyme activities, including ammonification, nitrification, and denitrification (Ahemad & Khan, 2012; Cycoń & Piotrowska-Seget, 2015; Filimon et al., 2015; Singh & Singh, 2006).

Soybean and corn are two important agricultural crops and are among those that are typically treated by pesticides, including neonicotinoids. In this study, we aim to (1) characterize the drivers of variation in bacterial community structure of soybean and corn phyllosphere and soil and (2) identify the responses of bacterial community composition variation and diversity to neonicotinoid seed treatment in a three-year soybean/corn rotation. We hypothesized that (1) habitat, host species and time will all contribute to variation in bacterial community composition and diversity, and (2) neonicotinoid seed treatment will cause a shift in the bacterial community composition and a decrease in bacterial diversity of both phyllosphere and soil. We address these objectives and hypotheses by quantifying bacterial community structure using bacterial 16S rRNA gene amplicon sequencing in soybean and corn phyllosphere and soil samples collected over three years in Quebec, Canada.

1.3 Materials and Methods

1.3.1 Study Site

We cultivated a three-year rotation of soybean (2016 and 2018) and corn (2017) on the Agriculture and Agri-Food Canada experimental farm in L'Acadie (45°17′38.0"N; 73°20′58.0"W), Quebec, Canada. L'Acadie is located in Canadian hardiness zone 5a. The region is characterized by having a clay loam soil type and a temperate climate. In mid-May of each year, we sowed soybean or corn on a 100 x 30 m field, previously a meadow, that had not received neonicotinoid application during the three years preceding the experiment. Four replicates of each non-neonicotinoid (control) and neonicotinoid-treated plots (100 x 3 m) were established alternately and consisted of four rows each. Two extra neonicotinoid-treated plots surrounded the experimental field. Soybean and corn seeds were coated with three fungicides (difenoconazole, metalaxyl-M and sedaxane) in both control and treated plots. The neonicotinoid-treated seeds were also covered by thiamethoxam at 0.25 mg/seed. The fields were under no-till farming, and glyphosate was applied twice during each growing season (before seeding and one month after it) to control weeds. The corn field was also fertilized with 400 kg/ha NPK (15-15-15) before seeding and 222 kg/ha N (27.5%) one month after seeding. Soil physicochemical properties (e.g. pH, etc.) were constant across the experimental field and did not differ between the growing seasons (Table A.1).

1.3.2 Sample Collection

To study the phyllosphere bacteria (the bacteria collected from the leaf surface in our case), each year we collected 48 samples (two samples per plot at three sampling times during the growing seasons), for a total of 144 samples. The three annual sampling occasions happened in July, August and September. We sampled 50-100 g of healthy mature middle leaves of 6-10 close plants from the two middle rows of each plot. We then stored each sample in a sterile plastic bag and transferred it to the laboratory in a cooler, surrounded by ice packs. We immediately collected the bacterial cells from the leaves by washing them in a 0.85% saline solution and agitating the solution using a stomacher at 250 rpm for 30 sec. We then transferred the solutions to 50-ml tubes, centrifuged them at 4,000 g for 20 minutes and discarded the supernatants. We kept the remaining pellets at -80 °C until use.

To study the soil bacteria, we sampled bulk soil (soil that does not adhere to plant roots) from the upper 12-15 cm layer of soil with a corer (2 cm in diameter) from the soil around the same plants that we sampled for the phyllosphere. For each soil sample, we collected soil from six different spots, in a zigzag pattern and at 10 cm from the plants, and then mixed and pooled them into one 400-500 g sample (Sugiyama et al., 2014b; Gagic et al., 2017). We transferred samples to the laboratory in a cooler and stored at -80 °C until use. Each year, we collected 48 soil samples (two samples per plot at the same three sampling times as phyllosphere), for a total of 144 samples.

1.3.3 DNA extraction

We extracted DNA from the samples of phyllosphere (pellets containing bacterial cells) and soil (directly) using MoBio PowerSoil DNA isolation kit (QIAGEN). Considering the high amount of material to be extracted from each soil sample, we extracted DNA twice, each time from 0.5 g of the same sample, and pooled the extractions together in order to better capture soil bacterial community variation. The rest of the extraction was performed according to the manufacturer's instructions. Then, we measured the concentration and quality of the extracted DNA using Qubit (Thermo Fisher Scientific) and Nanodrop (Thermo Fisher Scientific) prior to storing them at -80 °C.

1.3.4 Bacterial DNA amplification

Following previously described protocols (Kembel et al., 2014; Laforest-Lapointe et al., 2016b; Kim et al., 2018), we amplified the V5-V6 hypervariable regions of the bacterial 16S rRNA gene, using chloroplast-excluding primers (16S primers 799F-1115R (Chelius & Triplett, 2001; Redford & Fierer, 2009)). We added variable length barcodes and Illumina adaptor sequence to the 5' end of the primers. Each PCR reaction (25 μL) contained 1 μL of genomic DNA (1:10 dilution for soil samples), 5 μL 5xHF buffer (Thermo Scientific), 0.5 μL dNTPs (10 mM each), 0.75 μL DMSO, 0.25 μL Phusion Hot Start II polymerase (Thermo Scientific), 1.0 μL of each primer (5 μM), and 15.50 μL double-distilled water. We amplified the bacterial DNA in an Agilent SureCycler 8800 using the following conditions: 98 °C for 30 sec, 35 cycles of 98 °C, 15-sec denaturation; 64 °C, 30-sec annealing, and 72 °C, 30-sec elongation; followed by a final elongation at 72 °C, 10 min. All samples were distributed randomly into several 96-well PCR plates for DNA amplification. Each PCR plate contained one positive and one negative control. Each positive control included *Clavibacter michiganensis*, *Pectobacterium* sp., *E. coli* DHS alpha, *Pantoea stewartii* and *Xanthomonas* sp., while the negative controls were nuclease-free, DEPC-treated and autoclaved water. We also had negative controls of the sampling plastic bags, tubes and the extraction kit. All PCR products were electrophoresed on a 2% agarose gel in 1X TAE buffer, stained with AMRESCO's EZ-Vision dye as loading buffer (VWR Life Science), and visualized by G:BOX gel doc (Syngene).

1.3.5 Normalization, library preparation and sequencing

All PCR products were normalized using SequalPrep PCR Normalization kit (Thermo Fisher Scientific). One library per PCR plate was prepared by pooling all the amplified and normalized DNA. The concentration of each library was determined using Qubit. For each sequencing run, an equimolar concentration of each library was pooled and purified using Ampure XP (Beckman Coulter by Thermo Fisher Scientific), according to the manufacturer's protocol. We used Qubit and Bioanalyzer DNA analysis kit (Agilent) to verify the final concentration and quality of the purified DNA. According to MiSeq Illumina guidance, the 4 nM DNA was denatured using NaOH 0.2 N and then diluted to a 14 pM library. Then, it was PE (paired-end) sequenced on Illumina MiSeq (2 x 300bp), using a 600-cycle MiSeq reagent kit v3, at Agriculture and Agri-Food Canada.

1.3.6 Bioinformatic analyses

We used BBDuk (`http://jgi.doe.gov/data-and-tools/bb-tools/`) to remove Illumina adapters. We also removed barcodes and primers and then demultiplexed the Illumina reads. Afterwards, we applied DADA2 v1.12.1 (Callahan et al., 2016) to remove low-quality sequences, correct the Illumina-sequencing amplicon errors, merge paired-end sequences, eliminate chimeric sequences, and identify amplicon sequence variants (ASVs). We used default parameter settings for all functions except for the following functions: i) in filterAndTrim function, we removed all the sequences with fewer than 50 nucleotides (minLen = 50, instead of 20), ii) in dada function, we set the algorithm to perform pseudo-pooling between samples, and iii) in mergePairs, we set a minimum overlap length of 10 (minOverlap = 10, instead of 12) in order to merge the forward and reverse reads. We finally used the RDP naive Bayesian classifier method implemented in DADA2 with the SILVA 132 rRNA database (Quast et al., 2013; Yilmaz et al., 2014) to annotate the taxonomic identity of ASVs.

1.3.6.1 Sample quality control, decontamination and rarefaction

After verifying the presence and composition of the mock communities in the positive controls, we removed them from the dataset. To minimize sequence artifacts caused by PCR and sequencing errors (Acinas et al., 2005), which may result in spurious ASVs, we performed the following steps of quality filtering and decontamination: (1) removing ASVs that were not taxonomically annotated as belonging to a bacterial phylum (0.78% of all sequences); (2) eliminating the outlier samples (including two of the negative control samples) that had a very different composition from the other samples based on the non-metric multidimensional scaling (NMDS) on Bray-Curtis dissimilarities (Bray & Curtis, 1957); (3) filtering all the samples with less than 1,000 sequences (39 samples, including all the other negative controls, except for the phyllosphere and soil sampling bag controls); (4) removing the contaminating DNA from the bacterial communities using the prevalence method (probability threshold = 0.5) of the decontam package v1.1.2 (Davis et al., 2018) in R v4.0.0 (R Core Team, 2019), which identified 50 ASVs as contaminants based on the most prevalent ASVs in the negative controls; (5) eliminating all the ASVs recognized as chloroplasts or mitochondria (0.15%); (6) excluding the samples with low alpha diversity (Shannon richness < 2, including the soil sampling bag control and one phyllosphere sample); (7) removing the rare ASVs with less than 10 reads (37% of ASVs); and (8) eliminating again the outlier samples detected in the denoised dataset (five samples, including the last remaining negative control, one phyllosphere and four soil samples), which had a highly different composition (based on NMDS on Bray-Curtis dissimilarities) or species richness (based on Shannon diversity) from the other samples of the same habitat. Finally, we selected cutoffs to rarefy samples based on inspection of rarefaction curves for phyllosphere and soil samples, choosing rarefaction cutoffs that approached saturation in the ASV rarefaction curve while keeping as many samples as possible. We first rarefied the soybean and corn phyllosphere and soil samples to 5,000 reads per sample, which excluded 12 samples that contained insufficient numbers of sequences and 699 ASVs. We then made a subset of non-treated (control) samples (119 samples and 13,042 ASVs) to study the soybean and corn phyllosphere and soil bacterial community diversity and composition. We also made a subset of phyllosphere samples (110 samples and 6,695 ASVs) to study the variations in the phyllosphere bacterial community diversity and composition in response to neonicotinoid seed treatment. Since soil samples had more sequences per sample than phyllosphere samples, we rarefied the dataset again, this time to 10,000 reads per sample, which excluded 22 samples that contained insufficient numbers of sequences and 195 ASVs. Therefore, we subset soil samples to study the effects of neonicotinoid seed treatment on their bacterial diversity and composition (132 samples and 13,137 ASVs). Overall, quality control and filtering, decontamination, and rarefaction procedures at 5,000 and 10,000 cutoffs (Figure 1.1) respectively eliminated 41% and 39% of the low-quality ASVs and 20% and 23% of the samples (including all the 15 negative controls). We then analyzed these datasets using different R packages.

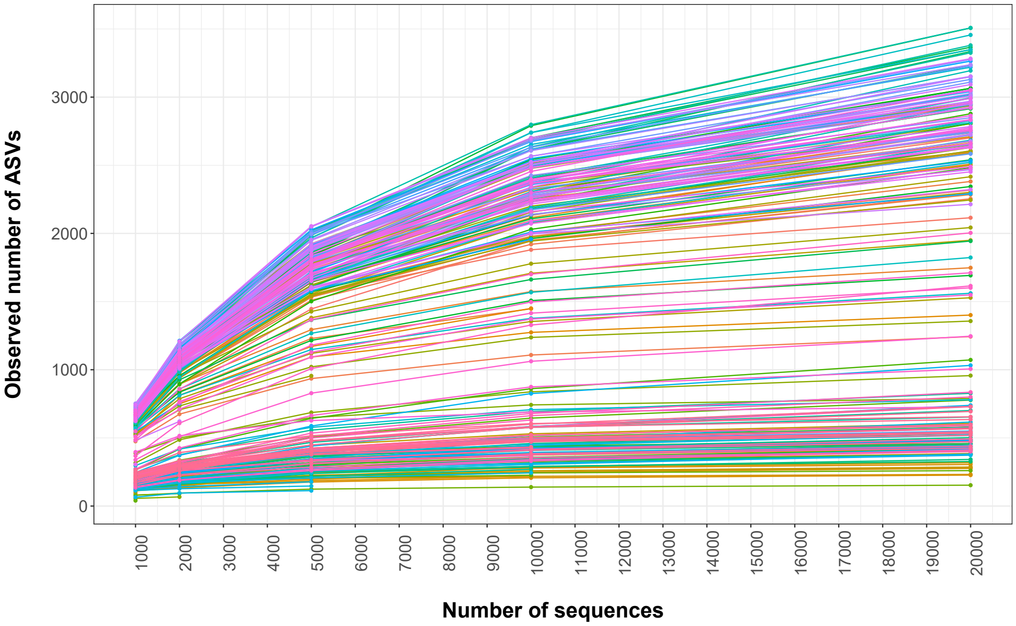

Figure 1.1: Rarefaction curves of the phyllosphere and soil bacterial ASVs. Rarefaction curves are shown for all the phyllosphere and soil samples according to the observed ASVs richness. Each line and color represent one sample. The sequencing coverage (x-axis: number of sequences) is 20,000 reads with cutoffs at 1,000, 2,000, 5,000 and 10,000 reads.

1.3.7 Statistical analyses

1.3.7.1 Characterization of phyllosphere and soil bacterial composition and diversity

To identify the bacterial composition and diversity of the soybean and corn phyllosphere and soil, we analyzed the non-neonicotinoid treated (control) samples that were rarefied to 5,000 reads per sample. This dataset contained 119 samples (including 30 soybean and 21 corn phyllosphere samples, as well as 45 soybean and 23 corn soil samples) with an average of 1,174 ± 65.0 ASVs (mean ± SE) per sample. We conducted permutational multivariate analyses of variance (PERMANOVA) (Anderson, 2001) using the adonis2 function of the vegan package v2.5.7 (Oksanen et al., 2020) in R v4.0.3 (R Core Team, 2019) with 999 permutations first on the whole community dissimilarity matrix to test for the effects of habitats (phyllosphere and soil), host species (soybean and corn), time (month and year), and their interactions on the bacterial composition variation (model: . $\sim$ habitat * host species * month/year), and then on each habitat individually to test for the effects of host species, time and their interactions on the bacterial community composition (model: . $\sim$ host species * month/year).

To assess the bacterial community homogeneity of each habitat and also each host species individually in phyllosphere and soil, we used a multivariate homogeneity test of groups dispersions using the betadisper function of vegan package v2.5.7 (Oksanen et al., 2020) in R v4.0.3 (R Core Team, 2019) and then performed an analysis of variance (ANOVA)-like permutation test with 999 permutations to evaluate the significance of the results.

Furthermore, we used the Shannon index to estimate the soybean and corn phyllosphere and soil bacterial alpha diversity. We conducted the non-parametric Wilcoxon rank-sum test (Wilcoxon, 1945) to compare the Shannon diversity for the following groups: between phyllosphere versus soil samples, and individually in each habitat between soybean versus corn samples, among years, and among months. This test was applied to determine the statistically significant differences of the bacterial ASVs richness among the mentioned groups. We adjusted the P-values using Holm's method (Holm, 1979).

To understand which families drove the variation in bacterial composition across habitats and hosts, we studied the correlations among all the bacterial families of soybean and corn phyllosphere and soil, which had an average relative abundance of more than 0.01, with their habitats and hosts. To achieve this, we used the envfit function of vegan package v2.5.7 (Oksanen et al., 2020) in R v4.0.3 (R Core Team, 2019), which computes the goodness of fit values (R^2) and their significance (with 999 permutations) of the vectors of bacterial families relative abundance onto the principal coordinate analysis (PCoA) ordination (based on Bray-Curtis distances).

1.3.7.2 Effects of neonicotinoid seed treatment on bacterial community composition, diversity and temporal variation

To study the bacterial community variations in response to neonicotinoid seed treatment, we separately analyzed the rarefied phyllosphere (5,000 reads per sample) and soil (10,000 reads per sample) samples. The phyllosphere dataset contained 110 samples (including 67 soybean and 43 corn samples) with an average of 391.1 ± 20.3 ASVs (mean ± SE) per sample, and the soil dataset contained 132 samples (including 85 soybean and 47 corn samples) with an average of 2,257 ± 30.0 ASVs (mean ± SE) per sample. We evaluated the relationships between bacterial communities and their host species, time (year and month) and neonicotinoid seed treatment, using a PERMANOVA with 999 permutations on the community matrix (model: . $\sim$ host species * year * month * neonicotinoid seed treatment) for each habitat individually. We also performed a PCoA (on Bray-Curtis dissimilarities) per habitat to illustrate the composition variation in the bacterial communities. Given the strong effects of host plants on the phyllosphere (Knief et al., 2010; Kembel et al., 2014; Laforest-Lapointe et al., 2016b) and soil (Wieland et al., 2001) bacterial community structure and according to our preliminary results, we also studied the soybean and corn samples individually to understand whether the impacts of neonicotinoid seed treatment on the patterns of bacterial community variation were masked by host species. Thereafter for each crop, we performed a PCoA (based on Bray-Curtis distances) and a PERMANOVA test (model: . $\sim$ year * month * neonicotinoid seed treatment) to explore the phyllosphere and soil bacterial community composition and the drivers of its variation.

We used the Shannon index to determine the phyllosphere and soil bacterial alpha diversity. Then, we conducted the non-parametric Wilcoxon rank-sum test to compare the Shannon diversity between the control versus neonicotinoid-treated samples in each habitat, as well as in soybean and corn separately for each habitat (model: Shannon $\sim$ neonicotinoid seed treatment). For each habitat individually, we used a linear model to evaluate the effects of neonicotinoid application on the bacterial alpha diversity across time (phyllosphere model: Shannon $\sim$ neonicotinoid seed treatment * month; soil model: Shannon $\sim$ neonicotinoid seed treatment * month * year), followed by an ANOVA test to determine the significant interactions. We then used the Wilcoxon rank-sum test, in which we grouped the samples by month (phyllosphere and soil models: Shannon $\sim$ neonicotinoid seed treatment, group by = month) and by year (soil model: Shannon $\sim$ neonicotinoid seed treatment, group by = year) to identify the significance of the interactions suggested by our model. We adjusted the P-values using Holm's method.

1.3.7.3 Effects of neonicotinoid seed treatment on bacterial taxonomic composition

To determine the differentially abundant ASVs and taxa between control and neonicotinoid-treated samples in each habitat, we performed a differential expression analysis of sequence data (DESeq2 (Love et al., 2014)) using the Wald significance test with a local fit type and compared the results by estimating the $\log_2$-fold changes. We analyzed the non-rarefied and non-normalized quality filtered and decontaminated bacterial phyllosphere (118 samples, including 58 control and 60 neonicotinoid-treated samples) and soil samples (137 samples, including 69 control and 68 neonicotinoid-treated samples) separately to identify the differentially abundant ASVs and taxa using the DESeq2 test. We then adjusted the P-values (significance cutoff of 0.05) using the Benjamini-Hochberg false-discovery rate (FDR) method (Hochberg & Benjamini, 1990) to identify the significantly differentially abundant ASVs and taxa between the control and neonicotinoid-treated samples during three years of rotation individually for each habitat.

1.4 Results

1.4.1 Effects of habitat, host species and time on the phyllosphere and soil bacterial communities

In this experiment, the habitat (phyllosphere versus soil) was the strongest driver of bacterial community variation. Habitat alone explained 37.3% of the community variation, while host plant species (soybean versus corn) explained only 3.2%, and their interaction 3.7% (PERMANOVA $P < 0.001$, Table 1.1). Community composition was significantly more homogenous among soil samples than phyllosphere samples (average distance to median 0.42 versus 0.50, ANOVA on multivariate homogeneity of groups dispersions $F = 24.13$, $P < 0.001$) and the phyllosphere communities exhibited less variation in corn than in soybean (average distance to median 0.38 versus 0.48, ANOVA on multivariate homogeneity of groups dispersions $F = 6.20$, $P < 0.05$, Figure 1.2A). Bacterial alpha diversity was significantly higher in soil than in the phyllosphere (Shannon index mean ± SE 7.0 ± 0.02 versus 4.2 ± 0.10, Wilcoxon adjusted $P < 0.0001$). The relative abundance of several bacterial families was strongly associated with soil (such as *Gemmatimonadaceae* and *Solibacteraceae*), soybean phyllosphere (such as *Beijerinckiaceae* and *Rhizobiaceae*) or corn phyllosphere (such as *Sphingomonadaceae* and *Hymenobacteraceae*) ($P < 0.001$, envfit analysis of correlation between PCoA axes and variables, Figure 1.2B; Table A.2).

Time was also a significant determinant of bacterial community variation, particularly in the phyllosphere habitat. Month and year together explained 2.9% of the whole bacterial community composition variation while the interactions between time, habitat and host species explained 7.2% of the variation (PERMANOVA $P <$

0.001, Table 1.1). Time was a much greater driver of community composition variation in the phyllosphere than in soil (15.7% versus 4.6%, PERMANOVA $P < 0.001$, Table 1.1). Alpha diversity varied in time in the phyllosphere but not in soil (Table 1.2). This effect in the phyllosphere was especially obvious between the first and the last year of the rotation where diversity was highest in the last year (Shannon index mean ± SE respectively, 4.0 ± 0.17 versus 4.8 ± 0.20, Wilcoxon adjusted $P < 0.0001$, Table 1.2) but we also observed intra-annual variation in diversity (Table 1.2).

1.4.2 Effects of neonicotinoid seed treatment on bacterial communities

Neonicotinoid seed treatment showed complex effects on the composition of bacterial communities. Neonicotinoid treatment alone explained a small but significant portion of the variation in both the phyllosphere (1.3%) and soil (2.6%) (PERMANOVA $P < 0.01$, Table 1.3). Since the bacterial composition varied greatly among host species and time (Table 1.3, Figure 1.3), the impacts of neonicotinoid seed treatment were partially masked by this variation. Effects of neonicotinoid treatment were especially evident in soils in the middle of the growing season (Figure 1.3C). To uncover neonicotinoid impacts, we analyzed each crop species separately, which revealed a much stronger effect of the neonicotinoid seed treatment on the composition of the phyllosphere communities in corn (5.3%) than in soybean (1.6%) (PERMANOVA $P < 0.001$ and $P < 0.05$ respectively, Table 1.3, Figure 1.4). There was no significant difference in phyllosphere alpha diversity between neonicotinoid treatments overall, but soil bacterial alpha diversity was significantly higher in control versus neonicotinoid-treated samples (Shannon index mean ± SE 7.2 ± 0.02 versus 7.0 ± 0.03, Wilcoxon adjusted $P < 0.001$, Table 1.2).

The overall effect of neonicotinoid seed treatment on the temporal variation of bacterial community composition and alpha diversity was weak. In the phyllosphere, although there was a small significant effect of the interaction between neonicotinoid application and time (month and year) on variation in community composition (1.4%, PERMANOVA $P < 0.05$, Table 1.3), the impacts on inter-annual variation and specific interactions with individual host species were not significant. The interaction of neonicotinoid seed treatment and time was slightly stronger in soil, especially with month (2.4%, PERMANOVA $P < 0.01$, Table 1.3). Uncovering these effects by studying each crop separately revealed that this month-to-month temporal variation in bacterial community structure within a growing season was particularly important in corn (5.6%, PERMANOVA $P < 0.05$, Table 1.3). Similarly, while the interaction between neonicotinoid seed treatment with time had no significant effect on bacterial alpha diversity in the phyllosphere, soil alpha diversity was significantly reduced in the neonicotinoid-treated samples in July and August (interaction between neonicotinoid seed treatment and month: linear regression analysis of Shannon index, F $= 6.27$, ANOVA $P < 0.001$; significant interactions among months and treatment: Shannon index, Wilcoxon $P < 0.001$, Table A.3).

1.4.3 Bacterial taxa impacted by neonicotinoid seed treatment

Neonicotinoid seed treatment led to changes in the relative abundance of some phyllosphere and soil bacterial ASVs. Overall, we detected 34 bacterial ASVs in the phyllosphere and 294 in soil that were significantly differentially abundant between the control and neonicotinoid-treated samples. In the phyllosphere, 22 ASVs (mainly *Bacteroidetes*) were more abundant, and 12 (mainly *Proteobacteria*) were less abundant in response to neonicotinoid seed treatment (Table 1.4). The genera *Hymenobacter*

(13 ASVs) and *Pseudomonas* (4 ASVs) were particularly favored by neonicotinoid treatment, while the genera *Arsenophonus* (4 ASVs) and *Skermanella* (3 ASVs) among others decreased in abundance in neonicotinoid-treated samples (DESeq2 adjusted $P < 0.05$, Figure 1.5A; Table A.3). In soil, 68 ASVs (mainly *Actinobacteria* and *Chloroflexi*) were more abundant in the neonicotinoid-treated samples, while 226 (mainly *Proteobacteria*) were less abundant (Table 1.4). More than 60 genera of soil bacteria were significantly impacted by neonicotinoid treatment (Figure 1.5B; Table A.4). Genera negatively affected by neonicotinoid treatment included some of the beneficial soil bacteria (e.g. *Ammoniphilus*, *Bacillus*, *Bosea*, *Bradyrhizobium*, *Hyphomicrobium*, *Mesorhizobium*, *Microvirga*, *Nitrospira*, *Nitrosospira*, *Rhizobacter* and *Rhodanobacter*) while the genera favored by the neonicotinoid treatment were dominated by Actinobacteria, including genera potentially involved in neonicotinoid degradation (e.g. *Mycobacterium* (Kandil et al., 2015) and *Streptomyces* (Guo et al., 2019)) or other pesticides degradation (e.g. *Arthrobacter* (Tam et al., 1987)).

Table 1.1: Main drivers of the phyllosphere and soil bacterial community composition variation in a three-year soybean/corn rotation. PERMANOVA (Bray-Curtis dissimilarities) determines the contributions of habitat and host plant species and their interactions in the soybean and corn phyllosphere and soil bacterial composition variation in a three-year soybean/corn rotation in L'Acadie, Quebec, Canada. (:) represents the interaction between variables. Significance levels for each variable are given by: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; NS, $P \geq 0.05$.

	Phyllosphere & Soil			**Phyllosphere**			**Soil**		
Variables	**R^2 (%)**	**F**	**Pr(>F)**	**R^2 (%)**	**F**	**Pr(>F)**	**R^2 (%)**	**F**	**Pr(>F)**
Habitat	37.3	100.98	0.001***	-	-	-	-	-	-
Host species	3.2	8.69	<0.001***	18.6	19.62	<0.001***	2.5	1.83	<0.007**
Month/Year	2.9	3.93	<0.001***	15.7	8.28	<0.001***	4.6	1.66	<0.002**
Habitat: Host species	3.7	10.07	<0.001***	-	-	-	-	-	-
Habitat: Month/Year	3.6	4.9	<0.001***	-	-	-	-	-	-
Host species: Month/Year	2.1	2.89	<0.005**	11.4	6.02	<0.001***	NS	NS	NS
Host species: Month: Year	2.5	2.97	<0.005**	14.6	5.14	<0.001***	8	1.94	<0.001***
Habitat: Host species: Month	2.5	3.35	<0.003**	-	-	-	-	-	-
Habitat: Host species: Month: Year	7.2	3.26	<0.001***	-	-	-	-	-	-

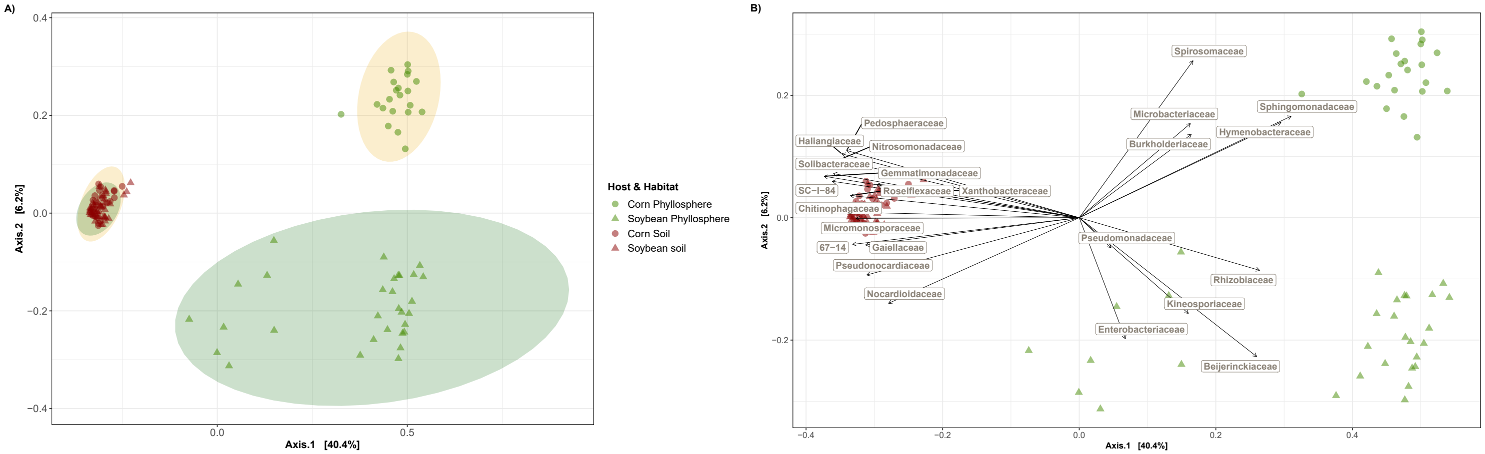

Figure 1.2: Soybean and corn phyllosphere and soil bacterial community diversity and the families who are driving this diversity pattern. Principal coordinate analysis (PCoA) on Bray-Curtis dissimilarities (A) of the bacterial community composition and the correlations between bacterial family abundances and different axes of the PCoA ordination (B) in the phyllosphere and soil bacterial communities in a three-year soybean/corn rotation in L'Acadie, Quebec, Canada. Ordinations show that habitat (red points: soil, green points: phyllosphere) and host species (circle points: corn, triangle points: soybean) explain the bacterial community composition variations. The axes of the ordinations explain 46.6% of the variation in the bacterial community composition. Ellipses (A) are shaded based on host species (yellow for corn and green for soybean samples) and represent a 99% confidence level. Grey arrows (B) indicate the significant correlations ($P < 0.001$, except for the Pseudomonadaceae family) among the bacterial families that had an average relative abundance of more than 0.01 and their habitat and host species. Arrows directions show the correlations among habitats and host species and arrow length indicates the strength of these correlations.

Table 1.2: Bacterial alpha diversity explained by time (year and month) and neonicotinoid seed treatment. Variance in the bacterial alpha diversity (Shannon index) explained by year, month, neonicotinoid seed treatment (NST) in soybean and corn together and individually, interactions between NST and month, and interactions between NST and year. Means and standard errors (SE) of each group are calculated and compared. The significance of their differences is determined using a non-parametric Wilcoxon rank-sum test, and the P-values are adjusted using Holm's method. Significance levels for each variable are given by: **** $P < 0.0001$; *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; NS: $P \geq 0.05$.

		Phyllosphere		**Soil**		
Variables		**Mean ± SE**	**Adjusted *P*-value**	**Mean ± SE**	**Adjusted *P*-value**	**Subset**
Host Species	Soybean	4.4 ± 0.15	NS	7.0 ± 0.03	NS	
	Corn	4.0 ± 0.12		7.0 ± 0.04		
Year	2016	4.0 ± 0.17	NS	7.0 ± 0.05	NS	
	2017	4.0 ± 0.12		7.0 ± 0.04		
	2017	4.0 ± 0.12	<0.01**	7.0 ± 0.04	NS	
	2018	4.8 ± 0.20		7.1 ± 0.02		
	2016	4.0 ± 0.17	<0.0001****	7.0 ± 0.05	NS	
	2018	4.8 ± 0.20		7.1 ± 0.02		
Month	July	4.6 ± 0.30	<0.05*	7.0 ± 0.03	NS	
	August	3.8 ± 0.11		7.0 ± 0.04		
	August	3.8 ± 0.11	<0.001***	7.0 ± 0.04	NS	
	September	4.4 ± 0.86		6.9 ± 0.04		
	July	4.6 ± 0.30	NS	7.0 ± 0.03	<0.05*	
	September	4.4 ± 0.86		6.9 ± 0.04		
Treatment & Host Species	Control	4.2 ± 0.10	NS	7.2 ± 0.02	<0.001***	Soybean & Corn
	NST	4.1 ± 0.08		7.0 ± 0.03		
	Control	4.4 ± 0.15	NS	7.2 ± 0.03	<0.01**	Soybean
	NST	4.2 ± 0.10		7.1 ± 0.03		
	Control	4.0 ± 0.12	NS	7.1 ± 0.05	<0.01**	Corn
	NST	3.9 ± 0.10		7.0 ± 0.05		
Treatment & Month	Control	4.6 ± 0.30	NS	7.2 ± 0.03	<0.001***	July
	NST	4.4 ± 0.20		7.0 ± 0.04		
	Control	3.8 ± 0.11	NS	7.2 ± 0.04	<0.001***	August
	NST	3.8 ± 0.11		7.0 ± 0.05		
	Control	4.4 ± 0.09	NS	7.1 ± 0.05	NS	September
	NST	4.2 ± 0.08		7.1 ± 0.05		
Treatment & Year	Control	-	-	7.1 ± 0.05	NS	2016
	NST	-	-	7.0 ± 0.05		
	Control	-	-	7.1± 0.05	<0.01**	2017
	NST	-	-	6.9 ± 0.05		
	Control	-	-	7.2 ± 0.03	NS	2018
	NST	-	-	7.2 ± 0.03		

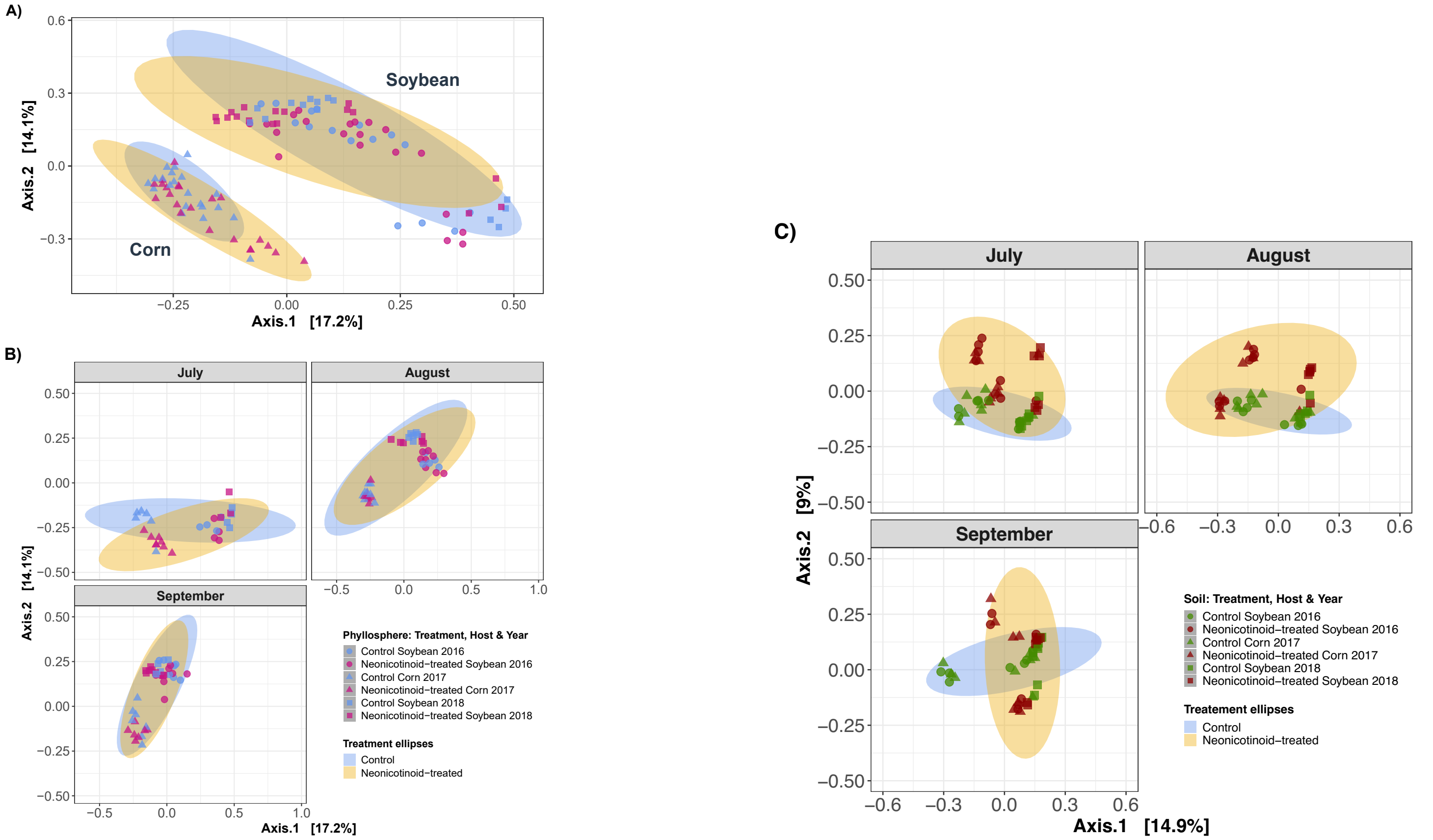

Figure 1.3: Phyllosphere and soil bacterial community composition variations in response to neonicotinoid seed treatment. Principal coordinate analysis (PCoA) on Bray-Curtis dissimilarities demonstrates the composition of phyllosphere (A and B) and soil (C) bacterial community in a three-year soybean (2016: circles and 2018: cubes) and corn (2017: triangles) rotation in L'Acadie, Quebec, Canada. The phyllosphere bacterial community variation among control (blue points) and neonicotinoid-treated (pink points) samples is masked by the effects of host species (A) and time (B). While in soil (C), the bacterial communities vary among control (green points) and neonicotinoid-treated (red points) samples. Ellipses are shaded based on treatment (blue for control and yellow for neonicotinoid-treated samples) and represent a 95% confidence level.

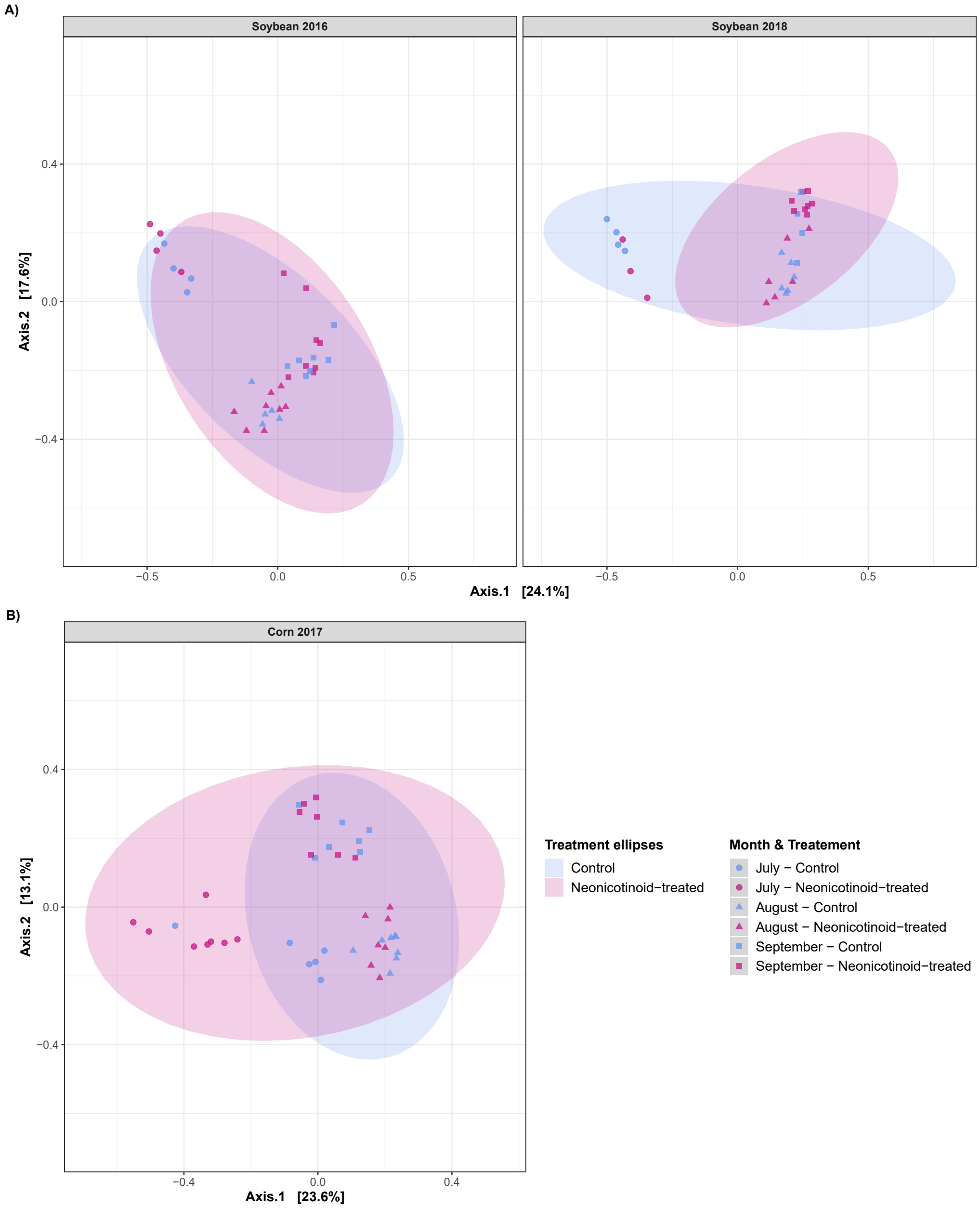

Figure 1.4: Soybean and corn phyllosphere bacterial community composition variations in response to neonicotinoid seed treatment and year. Principal coordinate analysis (PCoA) on Bray-Curtis dissimilarities illustrates the phyllosphere bacterial variation individually for each host species and year of rotation in L'Acadie, Quebec, Canada: A) soybean (left: year 2016; right: year 2018) and B) corn (year 2017). The shapes of the points represent the month and the colors show the treatment. Ellipses are shaded based on treatment (blue for control and pink for neonicotinoid-treated samples) and represent a 95% confidence level.

Table 1.3: Drivers of the phyllosphere and soil bacterial community composition variation in response to neonicotinoid seed treatment in a three-year soybean/corn rotation. PERMANOVA (Bray-Curtis dissimilarities) identifies the proportion of bacterial community composition variation explained by host plant species, time (year and month), neonicotinoid seed treatment (NST) and their interactions in the soybean and corn phyllosphere and soil in response to neonicotinoid seed treatment in a three-year soybean/corn rotation in L'Acadie, Quebec, Canada. (:) represents the interaction between variables. Significance levels for each variable are given by: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; NS, $P \geq 0.05$.

		Phyllosphere						**Soil**					
Variables	**Bray-CurtisDissimilarities**	**Soybean & Corn**		**Soybean**		**Corn**		**Soybean & Corn**		**Soybean**		**Corn**	
Host species	R^2 (%)	14.7		-		-		2.4		-		-	
	F | Pr(>F)	32.9	<0.001***	-	-	-	-	3.61	<0.001***	-	-	-	-
Year	R^2 (%)	7.4		13.1		-		5.7		9.2		-	
	F | Pr(>F)	16.6	<0.001***	16.8	<0.001***	-	-	8.42	<0.001***	8.92	<0.001***	-	-
Month	R^2 (%)	15.2		28.3		30		2.6		3.2		5.8	
	F | Pr(>F)	16.96	<0.001***	18.27	<0.001***	9.25	<0.001***	1.89	<0.001***	1.55	<0.012*	1.39	<0.036*
NST	R^2 (%)	1.3		1.6		5.3		2.6		3.4		3.7	
	F | Pr(>F)	2.81	<0.002**	2.12	<0.021*	3.3	<0.001***	3.82	<0.001***	3.33	<0.001***	1.78	<0.017*
NST:Host species	R^2 (%)	1.2		-		-		NS		-		-	
	F | Pr(>F)	2.56	<0.002**	-	-	-	-	NS	NS	-	-	-	-
NST: Year	R^2 (%)	0.8		NS		-		1.1		1.8		-	
	F | Pr(>F)	1.73	<0.043*	NS	NS	-	-	1.63	<0.030*	1.73	<0.014*	-	-
NST: Month	R^2 (%)	NS		NS		NS		2.4		NS		5.6	
	F | Pr(>F)	NS	NS	NS	NS	NS	NS	1.8	<0.002**	NS	NS	1.34	<0.048*
NST: Month:Host species	R^2 (%)	1.4		-		-		NS		-		-	
	F | Pr(>F)	1.55	<0.028*	-	-	-	-	NS	NS	-	-	-	-
NST: Year:Month	R^2 (%)	1.4		NS		-		NS		NS		-	
	F | Pr(>F)	1.57	<0.026*	NS	NS	-	-	NS	NS	NS	NS	-	-

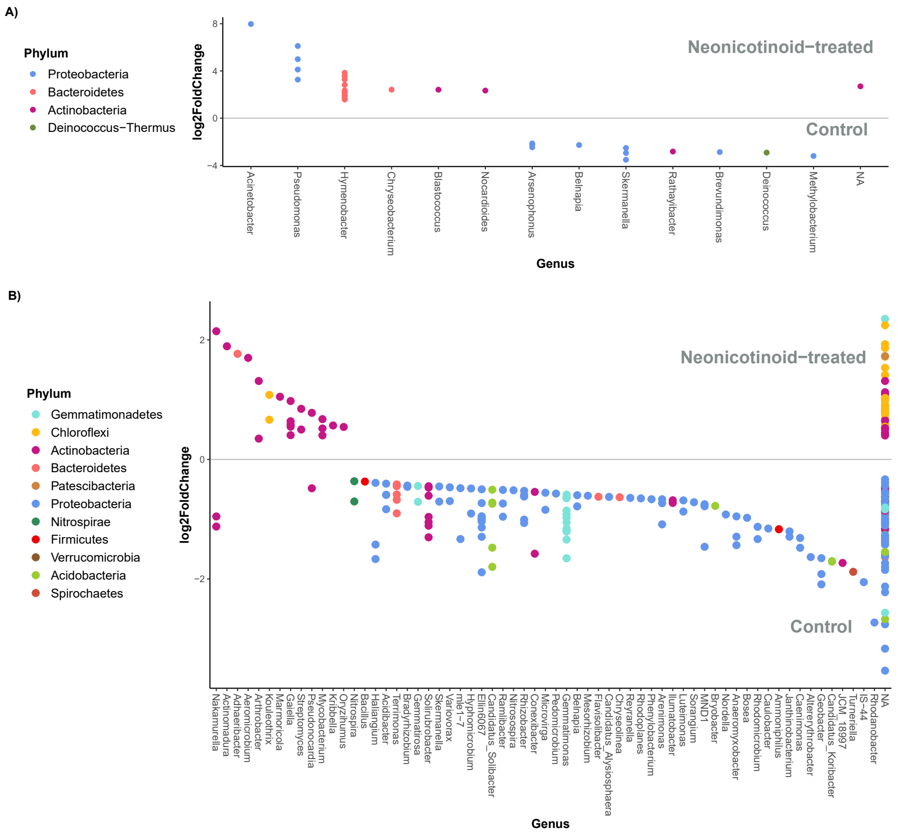

Figure 1.5: Phyllosphere and soil bacterial taxa (phyla and genera) associated with control and neonicotinoid seed treatment. Differential expression analysis of sequence data (DESeq2) illustrates the bacterial ASVs that are significantly differentially abundant (adjusted $P < 0.05$) between control and neonicotinoid-treated samples of soybean and corn phyllosphere (A) and soil (B) in a three-year rotation in L'Acadie. Each point represents one ASV related to a genus on the x-axis, and its color shows the phylum it belongs to. The ASVs on the top of each graph ($\log_2$FoldChange > 0) are associated with the neonicotinoid-treated samples, while the others ($\log_2$FoldChange < 0) are related to the controls.

Table 1.4: Phyllosphere and soil bacterial phyla associated with control and neonicotinoid seed treatment. Differential expression analysis of sequence data (DESeq2) identified the bacterial phyla of the ASVs that are significantly differentially abundant (adjusted $P < 0.05$) between control and neonicotinoid-treated samples of soybean and corn phyllosphere and soil in a three-year rotation in L'Acadie.

		Number of ASVs associated with treatment	
Habitat	**Phylum**	**Control**	**Neonicotinoid-treated**
Phyllosphere	*Actinobacteria*	1	3
	Bacteroidetes	0	14
	Deinococcus-Thermus	1	0
	Proteobacteria	10	5
Soil	*Acidobacteria*	11	0
	Actinobacteria	27	31
	Bacteroidetes	11	1
	Chloroflexi	3	33
	Firmicutes	2	0
	Gemmatimonadetes	27	2
	Nitrospirae	2	0
	Patescibacteria	0	1
	Proteobacteria	139	0
	Spirochaetes	1	0
	Verrucomicrobia	3	0

1.5 Discussion

Our findings indicate that habitat (soil versus phyllosphere), host species (soy versus corn), time, and their interactions are all strong drivers of bacterial composition variation in a soybean and corn agroecosystem. While this result is perhaps not surprising given that previous studies have identified these factors as important drivers of phyllosphere (Knief et al., 2010; Kembel et al., 2014; Laforest-Lapointe et al., 2016a) and soil bacterial communities (Wieland et al., 2001; Tarlera et al., 2008; Hannula et al., 2019), our results suggest that complex interactions among these factors drive overall community composition and diversity. In particular, we have shown a role for temporal variation, alone and in interaction with habitat and host species, as an important driver of bacterial community composition variation, especially in the phyllosphere. While succession of microbial communities in the phyllosphere has been documented previously (Redford & Fierer, 2009; Wagner et al., 2016; Manching et al., 2017), here we have shown that even in a rotation of annual crops, the patterns of bacterial succession within and among years are an important driver of community structure.

We have shown that neonicotinoid seed treatments have a non-target impact on bacterial community structure and diversity in a soybean/corn agroecosystem, in particular on the taxonomic composition of soil bacterial communities over the growing season. Phyllosphere and soil bacteria exhibit different patterns of community composition, alpha diversity and temporal variation throughout the growing season and in response to neonicotinoid application. In the phyllosphere, host plant species and time are stronger drivers of bacterial community variation than neonicotinoid seed treatment; however, neonicotinoids interact with these parameters to influence the phyllosphere bacterial community composition. Overall, soil bacteria exhibited stronger changes in community composition and a significant decline in bacterial alpha diversity in response to neonicotinoid treatment, while phyllosphere bacteria responses to neonicotinoids were weaker. Our results complement previous lab-based studies of neonicotinoid effects on bacterial communities (Cai et al., 2016;

Zhang et al., 2018; Yu et al., 2020), providing some of the first field-based evidence that neonicotinoids impact bacterial diversity in agroecosystems.

Overall, soil bacterial communities were more affected by neonicotinoid pesticide treatment than phyllosphere bacterial communities. Neonicotinoid effects on soil bacterial community composition and diversity varied greatly in time, with the impacts of neonicotinoid application on the soil bacterial community composition and alpha diversity most pronounced in the middle of the growing season. We suggest that this could be explained by the fact that neonicotinoids' active period is much shorter in plants (Myers & Hill, 2014; Alford & Krupke, 2017) than in soils, where they potentially persist for months or years (Goulson, 2013; Bonmatin et al., 2015). Despite the reported accumulation potential of neonicotinoids in soils over time (Wood & Goulson, 2017), we did not observe any significant inter-annual difference in bacterial diversity among years in interaction with the pesticide treatment, perhaps due to degradation or leaching of the neonicotinoids (Banerjee et al., 2008; Kurwadkar et al., 2013).

We also observed that the more homogenous the bacterial community composition is, the more it is altered by the neonicotinoid application (soil more than phyllosphere and corn phyllosphere more than soybean phyllosphere). We need further studies to determine if the homogeneity of the bacterial communities resulted in less resilience in response to perturbations or if less variability within groups allowed us to notice more changes in the communities.

In addition to community-wide responses of bacteria to the neonicotinoid treatment, numerous bacterial taxa increased or decreased in relative abundance in response to neonicotinoids. Bacterial taxa that were favored by the pesticide treatment include several genera that are known to be potentially involved in neonicotinoid degradation (e.g. *Hymenobacter* (Guo et al., 2020), *Mycobacterium* (Kandil et al., 2015), *Pseudomonas* (Pandey et al., 2009), and *Streptomyces* (Guo et al., 2019)). In soils, there was a decline in the relative abundance of several ASVs from *Proteobacteria* and *Gemmatimonadetes* phyla and an increase in some ASVs from *Chloroflexi* and *Actinobacteria*, a result partially in accordance with a previous study that reported a decrease in the relative abundance of *Gemmatimonadetes* and *OD1* phyla and an increase in the relative abundance of the *Chloroflexi* and *Nitrospirae* phyla in response to the neonicotinoid treatments (Yu et al., 2020).

Neonicotinoid seed treatment led to decreases in the relative abundance of several potentially beneficial soil bacteria, including the plant growth-promoting rhizobacteria (PGPR) that are capable of developing a symbiotic association with host plants (e.g. *Bacillus*, *Bosea*, *Mesorhizobium* and *Rhizobacter* (Podile & Krishna Kishore, 2006)), nitrogen-fixing bacteria (e.g. *Bradyrhizobium* and *Microvirga* (Kumar et al., 2015)), and other bacteria involved in the nitrogen cycle (e.g. *Ammoniphilus*, *Hyphomicrobium*, *Nitrospira*, *Nitrosospira* and *Rhodanobacter* (Pitombo et al., 2016)). While plant growth and yield variations in response to the pesticide application were not determined in our research, a recent study conducted in the same bioclimatic conditions indicated no significant impact on yield in the absence of the targeted pests (Labrie et al., 2020). However, although we did not measure the effects of neonicotinoid treatments on ecosystem processes such as nitrification, our results suggest a potential mechanism for the negative effects of neonicotinoids on nitrification that have been observed in previous studies (Filimon et al., 2015; Zhang et al., 2018).

Given that invertebrates are the main target of neonicotinoids, we suggest that the effects of this pesticide on bacterial communities could be related to the trophic interactions between bacteria and the invertebrates (e.g. free-living nematodes and microarthropods) affected by neonicotinoids. This insecticide may indirectly alter the bacterial community composition by affecting the top-down regulation of these communities through reducing the higher trophic levels that feed on bacteria (Staley et al., 2015; Thakur & Geisen, 2019a). Future research to evaluate the effects of neonicotinoids on these eukaryotic microbial communities, the trans-kingdom and trophic interactions between them and bacterial communities, and especially the prey-predator dynamics, as well as gene expression and functional variations of microbial communities, will improve our understanding of the mechanisms driving the microbial community variations in response to the pesticide application.

1.6 Conclusion

To date, there have been few studies that have evaluated the impacts of neonicotinoid seed treatments on phyllosphere and soil bacterial communities. To our knowledge, this study is the first with an experimental design that represents real farming conditions in a crop rotation. Despite the fact that neonicotinoids target invertebrates, we observed their non-target impacts on bacterial communities of the phyllosphere and soil, especially the beneficial bacteria that are crucial for plant growth and health and soil fertility and quality. Future studies to identify the genomic and physiological features associated with tolerance of neonicotinoids will be required to understand the mechanistic reasons for these associations. Investigating the biological and trophic interactions among bacteria and other micro- and macro-organisms that are affected by pesticides will help us to better understand the non-target effects of pesticides on microbial diversity and how to control them with better agricultural practices.

1.7 Data and code availability statement

We have deposited the raw sequences at the NCBI Sequence Read Archive (SRA accession number PRJNA662376). Scripts to perform the analyses of the current study are available in the following GitHub repository: `https://github.com/memoll/acadie_16s`.

1.8 Acknowledgements

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CHAPTER II

NEONICOTINOID SEED TREATMENTS INFLUENCE SOIL NEMATODE TAXONOMIC COMPOSITION AND THE SOIL MICROBIAL CO-OCCURRENCE NETWORKS

(Article in preparation)

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2.1 Abstract

Neonicotinoids, a family of systemic insecticides, are widely used to control early-season and foliar-feeding pests. Some studies have revealed a non-target impact of neonicotinoids on pollinators and other invertebrates, but few studies investigated their impacts on soil microbiota. Given the crucial role of soil microbial communities in agroecosystem regulation and their contribution to soil fertility, it is critical to understand their structure and changes in response to disturbances such as pesticide application. Among these communities, free-living nematodes have the potential to indicate the ecological changes in soil caused by environmental stress. These beneficial terrestrial invertebrates have a key role in forming and modulating soil microbial composition and function by feeding on other soil microorganisms or interacting with them. In this study, we used 18S rRNA gene amplicon sequencing to characterize the effects of neonicotinoids on soil nematode communities in a three-year soybean/corn crop rotation in Quebec, Canada. We also analyzed the co-occurrence networks of nematodes and bacteria to explore their relationships and quantify the changes in microbial co-occurrence patterns in soil exposed to neonicotinoid pesticides. We found that neonicotinoid seed treatment, host species and year significantly explained variation in nematode community composition. Neonicotinoid application also affected some nematode families from different trophic groups. For example, we observed the absence of the bacterivorous family Monhysteridae and a decrease in the total relative abundance of the omnivorous family Dorylaimidae in neonicotinoid-treated samples. We did not detect any significant changes in the soil food web indices based on faunal analysis. However, there was a decrease in the omnivore's metabolic footprint in the neonicotinoid-treated samples, which indicates the presence of stress in the ecosystem. Our results also showed that neonicotinoids altered the patterns of nematode-bacteria co-occurrence. However, it is not clear if the impacts of neonicotinoid seed treatment on bacterial co-occurrence networks were direct or were mediated by their impacts on nematodes that feed on bacteria. Further studies will be needed to assess the effects of neonicotinoids on microbial gene expression and functional activities to gain a more profound insight into the mechanism of neonicotinoid impacts on nematodes and the role of nematodes in the food web and microbial network variation in soil exposed to neonicotinoids.

Keywords: co-occurrence relationships, nematode community composition, neonicotinoid seed treatment, network analysis, soil food web, trophic groups

2.2 Introduction

In modern agriculture, pesticides are broadly used to prevent crop losses due to diseases, weeds, or insect pests. Neonicotinoids are a class of systemic, neuro-active insecticides discovered in the late 1980s (Kagabu, 1996; Tomizawa & Casida, 2005). These pesticides have similar chemical structures to nicotine and act on nicotinic acetylcholine receptors (nAChRs). They disrupt the central nervous system's neurotransmission, particularly in invertebrates (e.g., arthropods, annelids, nematodes, etc.) that are selectively more sensitive to these insecticides (Tomizawa & Casida, 2005). In field crops, neonicotinoids are usually used prophylactically as seed coverings to control various early-season insect pests (Goulson, 2013; Samson-Robert et al., 2017; Labrie et al., 2020). Previous studies have reported their non-target negative impacts on agriculturally beneficial organisms, for instance, insect pollinators such as honeybees (Iwasa et al., 2004; Samson-Robert et al., 2014; Bonmatin et al., 2015) and butterflies (Pisa et al., 2015; Whitehorn et al., 2018), and terrestrial invertebrates like earthworms (Pisa et al., 2015; Qi et al., 2018). However, there are only a few studies on the non-target effects of neonicotinoids on the composition and function of beneficial microbial communities, such as bacteria (Cycoń et al., 2013; Li et al., 2018; Yu et al., 2020; Parizadeh et al., 2021), fungi (Li et al., 2018) and free-living nematodes (Hopewell et al., 2017; Bradford et al., 2020). This study evaluates the effects of neonicotinoid seed treatments on soil nematodes and nematode-bacteria co-occurrence networks.

In sustainable agriculture, it is crucial to maintain soil quality and health, which is directly tied to the diversity and dynamics of microbial communities inhabiting it (Bender et al., 2016; de Vries & Wallenstein, 2017). These communities often vary quickly in response to small environmental changes due to stress and perturbations such as pesticide application, making them potential biological indicators to monitor and evaluate soil quality and ecological functions (Kennedy & Stubbs, 2006; Sharma et al., 2010). Among soil microbes, eukaryotic microbes and mesofauna, especially free-living nematodes, are excellent biological indicators of ecological changes in soil (Bongers, 1990; Bongers & Ferris, 1999) because of their temporal stability compared to more dynamic microbial communities such as bacteria (Nannipieri et al., 1990; Neher, 2001). Nematodes are one of the most abundant soil invertebrates. Free-living nematodes are among the main consumers of soil microbiota (Nannipieri et al., 1990; Neher, 2001). They play a key role in shaping soil microbial communities, influencing soil functions and soil food web stability (Ferris, 2010a). However, our knowledge about the effects of neonicotinoids on these ecologically essential invertebrates is still limited to mostly lab-based studies on single species. A previous study observed some non-target adverse neurological effects of neonicotinoids on the model organism nematode *Caenorhabditis elegans* (e.g., their impacts on the chemosensory ability of nematodes) (Hopewell et al., 2017). Another recent paper reported a decrease in the overall growth and egg-laying of *C. elegans* exposed to neonicotinoids (Bradford et al., 2020).

Studying community diversity and compositional variation is vital in understanding the effects of pesticide application on microbial community structure. However, these communities are constantly in interaction with each other. Thus, assessing the changes in their co-occurrence patterns may offer a more in-depth insight into community stability and dynamics (Allesina & Tang, 2012; García-Callejas et al., 2018). For years, ecologists have used ecological networks to investigate patterns of co-occurrence relationships among organisms in different ecosystems and model their variation in response to stress and environmental disturbances (Ulanowicz, 2004; Bascompte, 2009). Our previous study demonstrated that neonicotinoids alter soil bacterial taxonomic composition and community structure (Parizadeh et al., 2021). Since neonicotinoids are selectively more toxic to invertebrates, we hypothesized that the bacterial community response to neonicotinoids might be mediated by this pesticide's effects on their relationships with the higher trophic levels that feed on them (e.g., microarthropods and free-living nematodes). Co-occurrence networks of microbial communities help us identify the shifts in microbial co-occurrence patterns caused by neonicotinoid application, determine the keystone species in the ecosystem, and better understand the ecological processes that drive the complexity, stability, and resilience of the networks (Williams et al., 2014). Previous studies have shown that some pesticides reduce the complexity of soil bacterial networks (Fournier et al., 2020; Zhang et al., 2021a,b), but in general, there is a lack of empirical data on the impacts of neonicotinoids and other pesticides on the co-occurrence patterns of soil microbial networks, especially at different trophic levels.

In this study, we use amplicon sequencing to (1) characterize communities of soil free-living nematodes, (2) quantify variation in the composition and trophic functions of nematodes, and (3) assess the changes in nematode-bacteria co-occurrence patterns in response to neonicotinoid seed treatment in a three-year soybean/corn crop rotation. We hypothesized that neonicotinoid seed treatment (1) affects the community composition of soil free-living nematodes, (2) decreases soil nematode diversity, (3) modulates soil nematode taxonomic composition and trophic functions, and (4) influences the co-occurrence patterns of bacteria and the free-living nematodes that feed on them. To address these objectives, we used 18S rRNA gene amplicon sequencing to quantify soil nematode communities in a three-year soybean/corn crop rotation in Quebec, Canada. We also used bacterial 16S rRNA gene sequences from our previous study (Parizadeh et al., 2021) collected from the same field and with the same conditions to evaluate the co-occurrence networks among nematode and bacterial families.

2.3 Materials and Methods

2.3.1 Study Site

In a three-year crop rotation, we planted soybean and corn on an experimental farm at Agriculture and Agri-Food Canada in L'Acadie, Quebec, Canada (45°17′38.0"N; 73°20′58.0"W). This site has a temperate climate and clay loam soil and is located in the Canadian hardiness zone 5a. Prior to the experiment, the field was a meadow and had not been treated by any type of neonicotinoids during the past three years. Each year in mid-May, we cultivated four replicates of non-neonicotinoid-treated (control) and neonicotinoid-treated soybean (2016 and 2018) or corn (2017) seeds. Each plot was 100 x 3 m and contained four rows. Two extra neonicotinoid-treated plots surrounded the experimental field. All seeds were covered by three fungicides (difenoconazole, metalaxyl-M and sedaxane). The neonicotinoid-treated seeds were also coated with 0.25 mg/seed thiamethoxam. The fields were under no-tilling, and weeds were controlled by glyphosate (before and one month after seeding). On the corn field, we also applied 400 kg/ha NPK fertilizer (15-15-15) before seeding and 222 kg/ha N fertilizer (27.5%) one month after seeding of the crops. Soil physicochemical properties (e.g., pH, etc.) did not differ across the field nor between the growing seasons (see more details in our previous study (Parizadeh et al., 2021)).

2.3.2 Soil Sample Collection

We retrieved 48 soil samples during each growing season, two samples per plot at three sampling times (in July, August and September), a total of 144 samples. We used a 2-cm diameter corer to take soil samples from the upper 12-15 cm layer of the bulk soil (soil that does not adhere to plant roots). For each sample, we collected soil from six different spots in a zigzag pattern, at 10 cm around 6-10 close plants, and then pooled them into one sample (400-500 g). We then transferred the samples to the laboratory in a cooler and stored half of them at -80°C for DNA extraction.

2.3.3 DNA extraction

We used the MoBio/QIAGEN PowerSoil DNA isolation kit to extract DNA from 0.5 g of each soil sample. To increase the possibility of better capturing the soil microbial variation, we extracted DNA twice from each sample and then pooled them. We performed the rest of the extraction according to the manufacturer's instructions. We then used Qubit (Thermo Fisher Scientific) and Nanodrop (Thermo Fisher Scientific) to quantify and qualify the extracted DNA before storing them at -80°C.

2.3.4 DNA Amplification, library preparation, normalization and purification

To target the soil nematodes and bacterial communities, we amplified the 18S and 16S rRNA genes, respectively, using an Agilent SureCycler 8,800. Each PCR reaction (25 μL) contained 1 μL (1:10 diluted genomic DNA), 5 μL 5xHF buffer (Thermo Scientific), 0.5 μL dNTPs (10 mM each), 0.75 μL DMSO, 0.25 μL Phusion Hot

Start II polymerase (Thermo Scientific), 1.0 μL of each of the two specific primers of the target gene amplification (5 mM), and 15.50 μL double-distilled water. To amplify the 18S rRNA genes, we performed a two-step semi-nested PCR following Sapkota's protocol (Sapkota & Nicolaisen, 2015). We first pre-amplified the DNA templates with the suggested primers by the protocol, NemF and 18Sr2b. NemF is a primer designed to target nematodes and other metazoans and to exclude plant and fungal DNA regarding the mismatches at the 3' end (Sapkota & Nicolaisen, 2015). The amplification started with an initial DNA denaturation at 94°C for 5 min, followed by 28 cycles at 94°C, 30-sec denaturation; 53°C, 30-sec annealing, and 72°C, 1 min elongation; followed by 10 min of final elongation at 72°C. To generate smaller amplicons that were appropriate for sequencing, we diluted the PCR products (1:10) and used 1.0 μL of each of them as a template in the second step of the PCR, which was performed using NF1 (forward) and 18Sr2b (reverse) primers (Porazinska et al., 2009, 2010; Creer et al., 2010). The second amplification included 5 min of initial DNA denaturation at 94°C followed by 22 cycles of 94°C, 30-sec denaturation; 58°C, 30-sec annealing, and 72°C, 1 min elongation; ended by a final elongation at 72°C, 10 min. For the bacteria, we used the chloroplast-excluding primers (16S primers 799F- 1115R (Chelius & Triplett, 2001; Redford et al., 2010)) to amplify the V5-V6 hypervariable regions of the bacterial 16S rRNA gene-based on the former protocols (Kembel et al., 2014; Laforest-Lapointe et al., 2016b; Kim et al., 2018). We amplified the bacterial DNA using the following conditions: 98 °C for 30 sec, 35 cycles of 98 °C, 15-sec denaturation; 64 °C, 30-sec annealing, and 72 °C, 30-sec elongation; followed by a final elongation at 72 °C, 10 min. Variable-length barcodes and Illumina adaptor sequences were added to the 5' end of the 16S rRNA gene primers and the second step of 18S rRNA gene amplification. We randomly distributed all samples into several 96-well PCR plates, such that the 18S and 16S rRNA gene primers were not on the same plate. Each PCR plate also contained one positive and one negative control. Each positive control in the 18S rRNA amplification plates contained *Ditylenchus dipsaci*, *Globodera rostochiensis*, *Meloidogyne hapla*, *Pratylenchus crenatus* and *Pratylenchus penetrans*. The positive controls of the 16S rRNA amplification plates included *Clavibacter michiganensis*, *Pectobacterium* sp., *E. coli* DHS alpha, *Pantoea stewartii* and *Xanthomonas* sp. We used nuclease-free, DEPC-treated and autoclaved water as PCR plate negative control. We also included negative extraction controls for the sampling bag, 50-ml tube, and the extraction kit. After the DNA amplification, we electrophoresed all PCR products on a 2% agarose gel in 1X TAE buffer, stained them with AMRESCO's EZ-Vision dye as a loading buffer (VWR Life Science), and then visualized them by G:BOX gel doc (Syngene).

2.3.5 Normalization, library preparation and sequencing

We used the SequalPrep PCR Normalization kit (Thermo Fisher Scientific) to normalize the PCR products. We pooled all the amplified, normalized DNA and prepared one library per PCR plate. We then determined the concentration of each library using Qubit. For each sequencing run, we pooled and purified an equimolar concentration of each library using Ampure XP (Beckman Coulter by Thermo Fisher Scientific) and based on the manufacturer's protocol. We measured the final concentration and quality of the purified DNA using Qubit and Bioanalyzer DNA analysis kit (Agilent). We denatured the 4 nM DNA using NaOH 0.2 N and then diluted it to a 14 pM library, according to MiSeq Illumina guidance. Lastly, we sequenced the final DNA samples on Illumina MiSeq at Agriculture and Agri-Food Canada using a 600-cycle (2 x 300bp paired-end) MiSeq reagent kit v3.

2.3.6 Bioinformatic analyses

2.3.6.1 Taxonomic annotation

We used BBDuk v35.85 (`http://jgi.doe.gov/data-and-tools/bb-tools/`) to filter the Illumina adapters. Then, we removed barcodes and primers and demultiplexed the Illumina reads. We applied the DADA2 pipeline (Callahan et al., 2016) to denoise and remove low-quality sequences, join the paired-end sequences, remove the chimeric sequences, and identify amplicon sequence variants (ASVs). To analyze the 18S and 16S rRNA gene data, we respectively used the denoise-paired function of DADA2 wrapped by the QIIME2 v2019.7.0 plugin (Bolyen et al., 2019) and the DADA2 package v1.12.1 (Callahan et al., 2016) in R v4.0.0 (R Core Team, 2019). To annotate the taxonomic identity of the ASVs, we assigned them to species-level taxonomy using a custom trained 18S rRNA naive Bayesian classifier (Bokulich et al., 2018) trained on the 99% SILVA 132 database in QIIME2 for the nematode ASVs, and the RDP naive Bayesian classifier method implemented in DADA2 package and the SILVA 132 rRNA database for the bacterial ASVs. We used default parameter settings for all functions in these analyses, except for some modifications for the 16S rRNA gene data analysis (see more details in our previous study (Parizadeh et al., 2021)).

2.3.6.2 Quality filtering and rarefaction

After evaluating the mock community composition in the positive controls of each dataset, we removed these samples. We then performed the following steps of sequence quality filtering to minimize the sequence artifacts resulted from PCR and sequencing procedures (Acinas et al., 2005): (1) Our 18S rRNA primers were designed to capture nematodes and other metazoan sequences and exclude those of fungi and plants. To study soil nematode communities, we subset the Nematoda phylum from the 18S rRNA dataset (30.7% of total relative abundance), which eliminated 12 samples including four negative controls. We also removed the ASVs that were not annotated to any bacterial phylum from the 16S rRNA dataset to keep only characterized bacteria phyla (more than 99.9% of total relative abundance); (2) we then excluded the samples with fewer than 1,000 sequences from both datasets (18 nematode samples, including the four remaining negative controls, and 17 bacteria samples, including 13 negative controls); (3) from the bacteria dataset, we removed the bacterial DNA contaminants characterized by the prevalence method (26 ASVs, probability threshold = 0.5) of the decontam package v1.1.2 (Davis et al., 2018) in R, and the chloroplast and mitochondria ASVs (0.07% of ASVs); (4) we also filtered the rare ASVs with fewer than 10 reads from both datasets (excluding 25% of remaining nematode ASVs and 40% of remaining bacteria ASVs); (5) We cleaned the nematode dataset from a few ASVs that were likely mis-annotated as a nematode (eight ASVs assigned as the Bilateria genus) or were an animal parasitic nematode (one ASV assigned as the Eucoleus genus); (6) finally, we eliminated outlier samples (three nematode samples and four bacteria samples including the last remaining negative control) that had a highly different composition, based on the non-metric multidimensional scaling (NMDS) on Bray-Curtis dissimilarities (Bray & Curtis, 1957) and Shannon diversity from the other samples.

We then investigated the ASV rarefaction curves (Figure C.1) to choose rarefaction cutoffs that reach saturation in the rarefaction curves while keeping the most samples possible. To study the soil microbial community composition, we first rarefied the nematode sequences to 1,000 reads per sample (Figure C.1A). We also rarefied the bacteria dataset to 10,000 reads per sample (Figure C.1B). These cutoffs excluded no nematode samples and five bacteria samples that did not have enough sequences, as well as 20 nematode ASVs and 185 bacterial ASVs. Our final denoised and rarefied datasets consisted of 119 nematode samples (including 58 control and 61 neonicotinoid-treated samples) with an average of 25.1± 0.6 observed ASV richness (mean ± SE) per sample, and 132 bacteria samples (including 67 control and 65 neonicotinoid-treated samples) with an average of 2,255 ± 29.8 observed ASV richness (mean ± SE) per sample. We then used R to analyze these datasets.

2.3.7 Statistical analyses

2.3.7.1 Effects of neonicotinoid seed treatment on free-living nematode community composition and diversity

To determine the variation in the soil nematode community structure in response to neonicotinoid seed treatment, we analyzed the rarefied nematode dataset. We first performed a permutational analysis of variance (PERMANOVA) with 999 permutations on the community matrix (model: . $\sim$ neonicotinoid seed treatment * host species * year * month) using the adonis2 function of the vegan package v2.5.7 (Oksanen et al., 2020) in R v4.0.3 (R Core Team, 2019) to assess the relationships between nematode communities and neonicotinoid seed treatment, host species, time (year and month), and their interactions. To display the composition variation in the nematode communities, we performed principal coordinate analysis (PCoA) ordinations (based on Bray-Curtis dissimilarities). We used the Shannon index to estimate the nematode alpha diversity and applied the non-parametric Wilcoxon rank-sum test (Wilcoxon, 1945) to determine if the nematode alpha diversity statistically significantly varied among the control and neonicotinoid-treated samples.

2.3.7.2 Effects of neonicotinoid seed treatment on nematodes taxonomic composition and functions

To evaluate soil nematode taxonomic composition and functional variation, we first assigned the nematode families to trophic functional groups and classified them into the following categories: bacterivores, fungivores, herbivores, omnivores and predators, according to previous studies (Yeates et al., 1993; Ferris, 1999; Cesarz et al., 2015). For the families that were associated with more than one trophic group based on the references, we chose the group that was assigned to their identified genera. We used the rarefied nematode dataset (119 nematode samples, including 58 control and 61 neonicotinoid-treated samples), agglomerated at the family level, to calculate the relative abundance of each nematode trophic group and family in control and neonicotinoid-treated samples. We then compared the total relative abundance of each nematode trophic group, as well as each family between control and neonicotinoid-treated samples using the non-parametric Wilcoxon rank-sum test and adjusted the P-values using Holm's method to control the bias caused by false-positive results. After that, to assess nematode community and trophic group composition and function variation in response to neonicotinoid seed treatment, we performed the nematode faunal analysis using the R-based NINJA (Nematode INdicator Joint Analysis) tool (Sieriebriennikov et al., 2014). NINJA uses the relative weighted abundance of nematode families and their trophic groups to calculate the nematode community-level indices of the soil food web that serve as ecological indicators of environmental disturbances. For this purpose, we first ordered the nematode families based on their life strategies on a colonizer–persister (cp) scale, ranging from cp1 (early colonizers with short life cycles, resistant to perturbations) to cp5 (persisters in undisturbed environments with long life cycles, sensitive to perturbations) (Bongers, 1990; Ferris et al., 2001). We then computed the following typically used soil food web indices: (1) maturity index: representing the proportion of various cp groups in nematode communities. A stable undisturbed soil food web shows a high number of persisters and so a high maturity index value; (2) enrichment index: representing the responsiveness of the primary decomposers, bacterivorous and fungivorous nematodes; (3) structure index: indicating the sensitivity of the nematode trophic groups to perturbations; (4) metabolic footprint of each trophic group: indicating the nematode's contribution in ecosystem services and functions based on its carbon utilization and energy flow (Bongers & Bongers, 1998; Ferris et al., 2001; Ferris, 2010b). Finally, we applied ANOVA tests to monitor the significant overall and temporal changes between control and neonicotinoid-treated samples.

2.3.7.3 Effects of neonicotinoid seed treatment on the co-occurrence of nematodes and bacterial families

We used network analysis to quantify the co-occurrence of microbial communities and identify the impacts of neonicotinoid application on the inter-kingdom co-occurrence of soil nematodes and bacterial families. To achieve this, we used the rarefied datasets agglomerated at the family level and kept only the samples that passed the quality filtering and rarefaction steps in both datasets. Thus, out of 119 nematode and 132 bacteria samples, we ended up analyzing 109 samples, which included 28 nematode and 199 bacterial families in total. We then subset control and neonicotinoid-treated samples (respectively, 54 and 55 samples) and filtered families that were present in fewer than five samples. We produced one correlation matrix per treatment based on the co-occurrence and relative abundance of nematode and bacterial families by applying the sparCC algorithm (Friedman & Alm, 2012) using the SpiecEasi package v1.1.0 (Kurtz et al., 2015) in R v4.0.3 (R Core Team, 2019) as a measure of co-occurrence, where positive and negative correlations respectively indicate positive and negative co-occurrence relationships, and examined the statistical significance of the correlations using bootstrapped estimates with 999 permutations. Significant correlations were defined as the pairs of families with an absolute correlation coefficient threshold more than 0.3 and adjusted P-value (Benjamini & Hochberg, 1995) less than 0.01 that had occurred together at least once (109 families in control and 106 families in neonicotinoid-treated samples). For each treatment, we constructed one ecological network of significant correlations, with nodes representing microbial families and edges (links between nodes) representing the correlations between them (Albert & Barabási, 2002), using the igraph package v1.2.5 (Csardi et al., 2006) in R v4.0.3 (R Core Team, 2019). To determine whether the observed networks were significantly different from a network structure expected by chance, we created null models of random networks by randomly generating communities with the same number of nodes (microbial families) but randomizing the links among nodes. Thus, for each treatment, we simulated null models of 999 random networks by randomizing the co-occurrence of pairs of microbial families while keeping the same number of families and their co-occurrences (i.e., nodes and edges of the network). To assess whether neonicotinoid application induced changes in the nematode-bacteria co-occurrence networks, we calculated the following network topological properties for the whole network and for individual network nodes (i.e., families) using the functions provided by the igraph package v1.2.5 (Csardi et al., 2006): (i) network-level metrics: (1) motifs: subgraphs representing patterns of interactions between families

(Milo et al., 2002) and (2) modularity: the degree of division of a network into modules containing co-occurring taxa (Newman, 2006); and (ii) network node metrics: (1) betweenness centrality: the number of times a node serves as a bridge within the shortest path of the other nodes in a network (Freeman, 1977). The nodes (microbial families) with the highest betweenness centrality might be identified as keystone microbial families (Agler et al., 2016), and (2) coreness: a measure to determine if each node belongs to a more or less densely connected part of the network (Batagelj & Zaversnik, 2003). We used a Z-test that compared the observed value of each metric to the distribution of the randomly generated metrics from null models to investigate whether the network topological properties varied significantly among the observed and random networks. We assessed the Z-score (distance of the metric value from null model mean divided by null model standard deviation), rank (of the observed metric comparing to 999 null models) and a two-tailed P-value (the observed metric rank divided by the total number of random networks) for each network property against the null models.

2.4 Results

2.4.1 Nematode community composition variation in response to neonicotinoid seed treatment

Neonicotinoid seed treatment significantly affected nematode community composition (2.0% of compositional variation explained, PERMANOVA $P < 0.001$, Table 2.1, Figure 2.1). Host species and year also significantly explained some variation in community composition (2.6% and 3.0%, PERMANOVA $P < 0.001$, Table 2.1, Figure 2.1). However, the interactions among these parameters had no significant effect on the communities. Finally, nematode alpha diversity was not significantly different between the control and neonicotinoid-treated samples (Shannon index mean ± SE 2.3 ± 0.05 vs. 2.3 ± 0.06, P-value = 0.94).

2.4.2 Impacts of neonicotinoid seed treatment on nematode taxonomic composition and trophic functions

Neonicotinoid seed treatment had significant effects on the taxonomic composition of soil nematodes by modulating the relative abundance of certain families from different trophic groups. Bacterivores were the dominant trophic group in both control and neonicotinoid-treated samples (respectively 52.4% and 51.1% of total relative abundance, Figure 2.2A). Three bacterivorous families - Rhabditidae, Alaimidae and Cephalobidae - were respectively the most abundant nematode families in control samples (respectively 16.4%, 15.4% and 9.3% of total relative abundance). Neonicotinoid-treated samples were also dominated by the same three families (Alaimidae 23.7%, Rhabditidae 12.1% and Cephalobidae 9.9% of total relative abundance, Figure 2.2B). Overall, in terms of nematode trophic functional groups, there was no significant difference between control and neonicotinoid-treated samples (Figure 2.2A). However, out of 28 identified nematode families, the relative abundance of two families was significantly higher in control than neonicotinoid-treated samples: (1) the bacterivorous family Monhysteridae was only present in control samples (total relative abundance of 0.3%, Wilcoxon adjusted $P < 0.05$, Figure 2.2B) and (2) the omnivorous family Dorylaimidae had a significantly higher total relative abundance in control than neonicotinoid-treated samples (total relative abundance of 2.5% vs. 1.1% , Wilcoxon adjusted $P < 0.05$, Figure 2.2B). Overall, the nematode maturity index slightly increased in the neonicotinoid-treated samples compared to

Table 2.1: Drivers of the soil nematode community composition variation in response to neonicotinoid seed treatment in a three-year soybean/corn rotation in l'Acadie, Quebec, Canada. Results are based on a PERMANOVA on Bray-Curtis dissimilarities among samples to quantify variation in community composition (R^2) explained by each factor. (:) represents the interaction between variables. Significance levels for each variable are given by: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; NS, $P \geq 0.05$.

Variables	R^2 (%)	F	Pr(>F)
Neonicotinoid seed treatment	2.0	2.5	<0.001***
Host species	2.6	3.1	<0.001***
Year	3.0	3.6	<0.001***
Month	NS	NS	NS
Neonicotinoid seed treatment : Host species	NS	NS	NS
Neonicotinoid seed treatment : Year	NS	NS	NS
Neonicotinoid seed treatment : Month	NS	NS	NS
Neonicotinoid seed treatment : Host species : Month	NS	NS	NS
Neonicotinoid seed treatment : Year: Month	NS	NS	NS

control (mean ± SD 2.84 ± 0.52 vs. 2.54 ± 0.51, $P < 0.05$) but no temporal variation with years or months within each year was observed ($P > 0.05$). Conversely, the omnivore's metabolic footprint reduced in the neonicotinoid-treated samples compared to control (mean ± SD 0.07± 0.11 vs. 0.13 ± 0.08, $P < 0.05$) while no difference was found among the other trophic groups. The nematode faunal analysis did not detect any overall or inter-annual impact of neonicotinoid seed treatment on the enrichment and structure indices of the soil food web. According to the standard faunal profile (Ferris et al., 2001), soil nematode families were highly enriched and structured in both control and neonicotinoid-treated samples (Figure C.2).

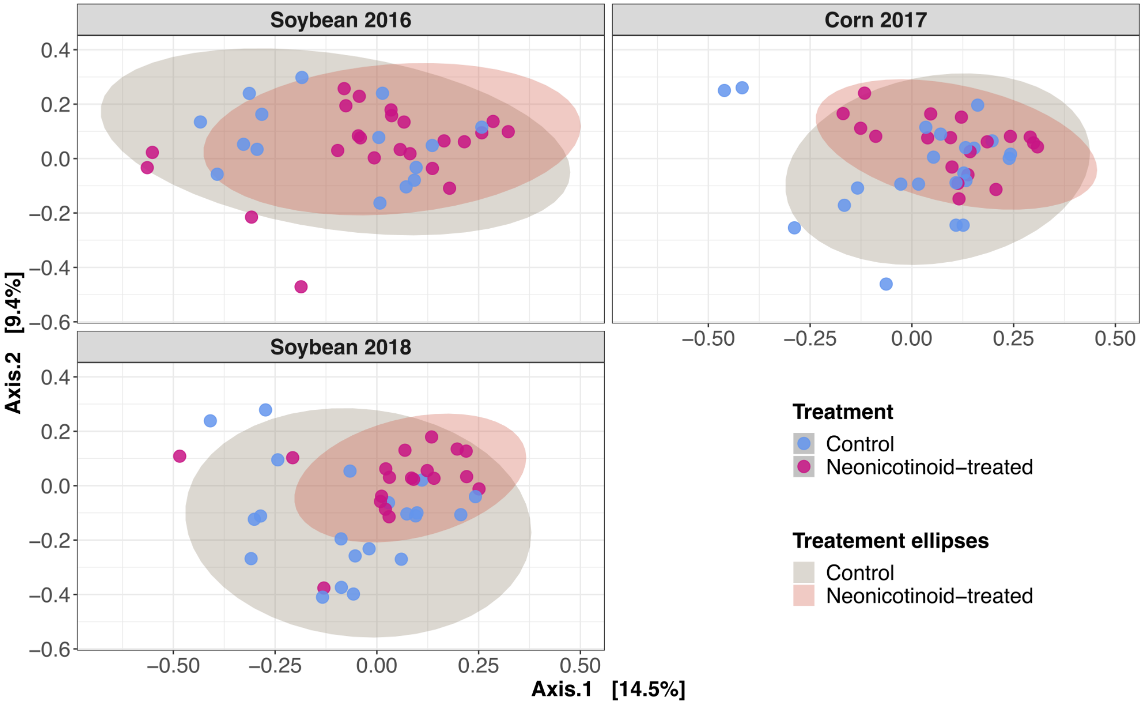

Figure 2.1: Soil nematode community composition variation in response to neonicotinoid seed treatment. Principal coordinate analysis (PCoA) on Bray-Curtis dissimilarities demonstrates the composition variation of soil nematode communities between control (n = 58) and neonicotinoid-treated (n = 61) samples in a three-year soybean (2016 and 2018) and corn (2017) rotation in L'Acadie, Quebec, Canada. Nematode communities vary among control (blue points) and neonicotinoid-treated (pink points) samples. Ellipses are shaded based on treatment (grey for control and red for neonicotinoid-treated samples) and represent a 95% confidence interval.

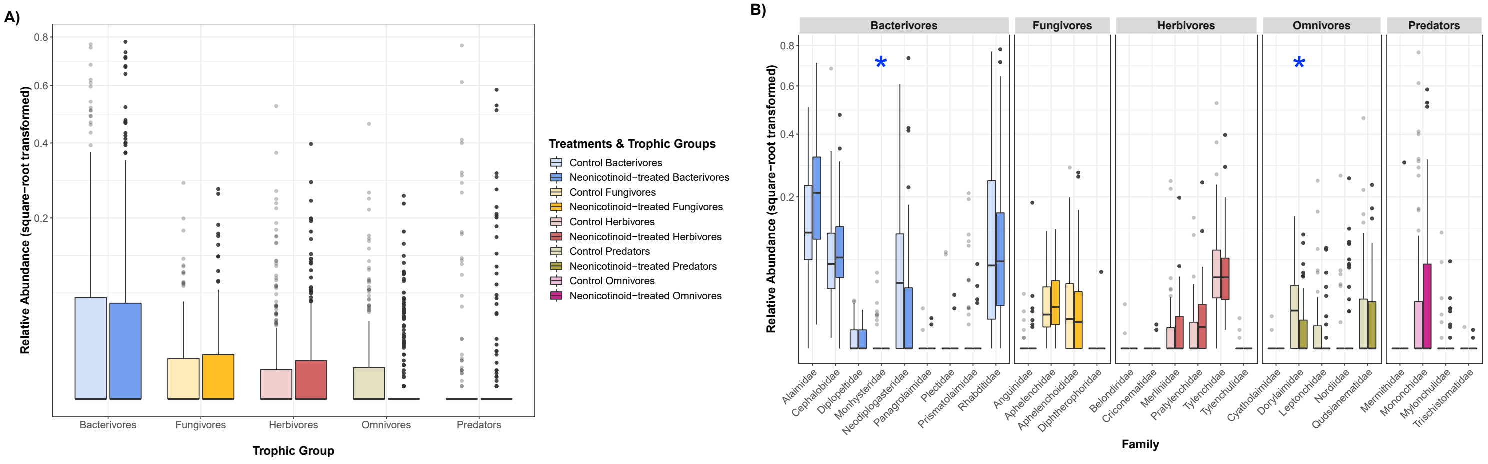

Figure 2.2: Changes in soil nematode families and their relative abundance in response to neonicotinoid seed treatment. Changes in the total relative abundance of nematode families based on their trophic groups (A) and the relative abundance of each nematode family (B) in response to neonicotinoid seed treatment in a three-year soybean/corn rotation in L'Acadie, Quebec, Canada. Box and whisker plots show the distribution of the nematodes in each trophic group (A) or family (B) based on their relative abundance (shown as square root transformed on the y-axis). Each color indicates one trophic group (blue: bacterivores, yellow: fungivores, red: herbivores, green: omnivores, and pink: predators). Boxes with lighter colors represent control, while darker boxes show the neonicotinoid-treated samples. In each box, the lower and upper hinges indicate the first and third quartiles, and the line in the middle shows the median. Lower and upper whiskers respectively extend from the hinge to the minimum and maximum data points, at most 1.5-times the interquartile of the hinge. Each point represents one soil sample. Stars indicate the groups whose relative abundance was significantly different between control and neonicotinoid-treated samples based on P-values from a Wilcoxon rank-sum test. The y-axis is scaled using square roots to better visualize the groups with very low relative abundance. NAs have been considered in calculating relative abundance but have not been shown. † Significance levels for each variable are given by: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; NS, $P \geq 0.05$.

2.4.3 Neonicotinoid seed treatment effects on nematode-bacteria co-occurrence networks

We observed notable differences in microbial networks between control and neonicotinoid-treated soil samples following co-occurrence network analysis. We detected 391 significant non-zero correlations in control samples, including 259 positive and 132 negative correlations, and 1,110 significant non-zero correlations in neonicotinoid-treated samples (2.8 times more than control), including 579 positive and 531 negative correlations ($|\text{correlation coefficient}| > 0.3$, BH-adjusted $P < 0.01$). The inter-kingdom co-occurrence relationships among nematodes and bacteria were affected by neonicotinoid seed treatment. In the control network, we observed three dominant bacterivorous nematode families (Cephalobidae, Neodiplogasteridae and Rhabditidae) showing significant positive co-occurrence relationships and two dominant bacterivorous nematode families (Alaimidae and Neodiplogasteridae) showing significant negative co-occurrence relationships with several bacterial families (Figure 2.3, Table 2.2). However, no significant co-occurrence between bacterivorous nematodes and bacteria was detected in the neonicotinoid-treated network. Interestingly, some positive and negative inter-kingdom correlations between non-predator-prey pairs were also observed in the control network, mostly including herbivorous nematodes with bacterial families, which were lost in the neonicotinoid-treated network (Table 2.2). Overall, co-occurrence of bacterial families with different nematode trophic groups, including bacterivorous, fungivorous and herbivorous nematode families (mostly from the cp1-3 nematodes) was observed in the control network, while co-occurrence between omnivorous and predator nematode families (mostly cp4 nematodes) were detected in the neonicotinoid-treated network (Table 2.2).

Neonicotinoid seed treatment affected the structure and taxonomic composition of the co-occurrence networks. As mentioned earlier, our co-occurrence networks had almost the same number of nodes (microbial families; 109 in control vs. 106 in the neonicotinoid-treated network). However, the number of co-occurrence relationships in the networks were more than doubled in response to neonicotinoid application (391 edges in control vs. 1,110 edges in neonicotinoid-treated network, Figure 2.3, Table 2.3). These networks consisted of nine nematode families and 100 bacterial families from 14 phyla co-occurring in control samples (Figure 2.3A) versus five nematode families and 101 bacterial families from 13 phyla co-occurring in neonicotinoid-treated samples (Figure 2.3B). Null model analysis suggested that all measured network metrics in both control and neonicotinoid-treated networks were significantly different from random networks. In terms of network topological properties, while the number of network motifs was significantly higher than random null models for both networks and network modularity was significantly higher than random null models in control network and lower in neonicotinoid-treated network as evidenced by the difference of their Z-scores, the control network had less number of motifs and a higher modularity value than the neonicotinoid-treated network (observed motifs 3,110 vs. 22,726, Z-scores 10.21 and 29.32, P-value < 0.05; observed modularity 0.46 vs. 0.09, Z-scores 10.42 and -4.34 , P-value < 0.05; Figure C.3, Table 2.3). Moreover, while the betweenness centrality and coreness of nodes in both networks were also significantly different than those of random networks based on their Z-score differences, the control network nodes showed much higher average and maximum betweenness centrality values and lower average and maximum coreness values than neonicotinoid-treated network nodes (observed betweenness centrality average 240.4 vs. 57.26, maximum 480.8 vs. 272.9, Z-scores 6.66 and 13.69, P-values < 0.05; observed coreness average 4.2 vs. 13.6, maximum 8.0 vs. 24.0, various Z-scores,

P-values < 0.05; Table B.1). Neonicotinoid seed treatment also led to shifts in the taxonomic identity of the microbial families with the highest values for different network metrics. *Rhodobacteraceae* (*Proteobacteria* phylum) in the control network and *Gaiellaceae* (*Actinobacteria* phylum) in the neonicotinoid-treated network showed the highest betweenness centralities (Table B.1). The core microbial families, those with the highest coreness values associated with each network, were dominated by the families from the *Actinobacteria* phylum in both co-occurrence networks (Figure 2.3, Table B.1). We identified 11 bacterial families, all from the *Actinobacteria* phylum, with the highest coreness values in both networks, while 24 bacterial families from different bacterial phyla and dominated by *Proteobacteria* and *Actinobacteria* phyla showed the same highest coreness value only in the neonicotinoid-treated network (Table B.1). None of the nematode families were among the microbial families with the highest observed network node metrics (Table B.1).

2.5 Discussion

Neonicotinoid seed treatment had a significant impact on soil nematode community composition in a soybean-corn agroecosystem. The effect of neonicotinoids was similar in magnitude to the variation explained by host species (corn vs. soybean) or year. These results corroborate other studies showing non-target impacts of neonicotinoids on different organisms, including nematodes (Seres et al., 2016), fungi (Hannula et al., 2019) and bacterial communities (Wieland et al., 2001; Tarlera et al., 2008; Hannula et al., 2019; Parizadeh et al., 2021). However, there was no significant change in the nematode alpha diversity due to neonicotinoid seed treatment, indicating that neonicotinoid application changed the relative abundance and contribution of certain nematode families in soil without reducing the total number of species present.

Table 2.2: Interkingdom co-occurrences of nematodes families from different trophic groups and bacterial families in control and neonicotinoid-treated soil samples in a three-year soybean/corn rotation in L'Acadie, Quebec, Canada. Trophic groups (Yeates et al., 1993) and cp classes (c –colonizers, p –persisters) (Bongers, 1990) of the nematode families co-occurred with bacterial communities are presented. Correlations are calculated using the SparCC algorithm and only the significant positive and negative correlations ($|\text{correlation coefficient}| > 0.3$, BH-adjusted $P < 0.01$) are presented, marked by (✓) and (-) respectively.

Trophic Group	Nematode Families	cp	Correlations in Control Network		Correlations in Neonicotinoid-treated Network	
			Positive	Negative	Positive	Negative
Bacterivore	Alaimidae	4	-	✓	-	-
	Cephalobidae	2	✓	-	-	-
	Neodiplogasteridae	1	✓	✓	-	-
Fungivore	Aphelenchoididae	2	-	✓	-	-
Herbivore	Merliniidae	3	-	✓	-	-
	Pratylenchidae	3	✓	-	-	-
	Tylenchidae	2	✓	✓	✓	-
Omnivore	Dorylaimidae	4	-	✓	-	-
	Nordiidae	4	-	-	✓	-
Predator	Mononchidae	4	-	-	-	✓

Table 2.3: Topological properties of co-occurrence networks and their respective null models obtained from microbial communities co-occurrence in control and neonicotinoid-treated soil samples in a three-year soybean/corn rotation in l'Acadie, Quebec, Canada.

		Co-occurrence network	Null model			
Metrics	Treatments	Observed	Z-score	P-value	Mean	Standard deviation
Motifs	Control	3110	10.21	< 0.05	2650.68	44.98
	Neonicotinoid-treated	22726	29.32	< 0.05	19947.60	94.77
Modularity	Control	0.46	10.42	< 0.05	0.29	0.02
	Neonicotinoid-treated	0.09	- 4.34	< 0.05	0.14	0.01
Nodes	Control	109				
	Neonicotinoid-treated	106				
Edges	Control	391				
	Neonicotinoid-treated	1110				

Our results suggest that some bacterivorous (Monhysteridae) and omnivorous (Dorylaimidae) nematode families may be more responsive and sensitive to neonicotinoid seed treatment. Many studies have used the Dorylaimidae family as an indicator of environmental disturbance because of its sensitivity to different stresses, including pesticide application (Thomas, 1978; Sohlenius & Sandor, 1989; Gomes et al., 2003). On the other hand, based on the faunal analysis of the soil food web, neither the nematode enrichment index nor the structure index was affected by neonicotinoid application. However, even with similar soil food web indices, the difference in nematode community composition could lead to functional consequences. For example, we observed a decrease in the omnivore's metabolic footprint in response to neonicotinoid seed treatment, indicating less contribution of this trophic group in ecosystem services and functions in the neonicotinoid-treated samples. A recent study on the impacts of insecticide application and pest management practices on the soil nematodes also showed no significant changes in nematode species richness but some effects on the metabolic footprint of specific trophic groups (Yang et al., 2020). Fiscus and Neher (2002) have studied in detail the direct and indirect effects of physical and chemical agricultural disturbances on soil nematodes and revealed that they differ greatly among genera (Fiscus & Neher, 2002). Surprisingly, the maturity index, a key index to monitor the ecological effect of disturbance on nematode communities and soil food web stability, was slightly higher in the neonicotinoid-treated samples. This is probably explained by the relative importance of the Alaimidae in our dataset. This family is a relatively high cp value nematode (cp4) and has been reported to be positively sensitive to chemical disturbance (Neher, 2001). Overall, we suggest that the soil ecosystem of our field was already in a relatively stable, enriched and structured status, with low to moderate disturbance, as evidenced by the results of the faunal analysis. The fact that the field was a meadow for the past three years before the experiment may explain the stability of the soil food web. Taken together, these results suggest that the effect of neonicotinoid application on soil nematodes could be specific to certain families and trophic groups and thus not evident when looking at community-wide measures such as taxon richness. Further research on nematode functions is needed to better assess their variation in response to neonicotinoid seed treatment.

Ecological network analysis indicated that neonicotinoid seed treatment modulated nematode-bacteria co-occurrence patterns. Despite the larger number of co-occurrence relationships of microbial families in neonicotinoid-treated than control network, these relationships were dominated by co-occurrences among bacterial families, and there were far fewer inter-kingdom co-occurrences among nematodes and bacteria in the neonicotinoid-treated network. A shift in the trophic group of the nematodes co-occurred with bacteria from bacterivorous nematodes in the control network to an omnivorous nematode in the neonicotinoid-treated network might be related to the variation in the taxonomic composition of the bacterial families in each of these networks. It has been hypothesized that measures of network structure such as network modularity are related to the sensitivity and resilience of networks faced with environmental stress and networks with lower modularity are more sensitive to stress and more likely to spread disturbance across the network (Kharrazi et al., 2020). The lower network modularity observed in the neonicotinoid-treated network could suggest that this network has lower stability and resilience to stress than the control network. Conversely, a recent study reported a decrease in the number of nodes and edges and network complexity with increasing neonicotinoid concentrations (Zhang et al., 2021b). However, this study used much higher neonicotinoid concentrations and different soil types and physicochemical properties (Zhang et al., 2021b) compared to our study. Our results have generated hypotheses about nematode and bacterial interactions that will need to be followed up by future studies that directly characterize interactions among nematodes and bacteria and measure the stability and resilience of networks.

Although we observed shifts in nematode-bacteria co-occurrence patterns in soil exposed to neonicotinoid pesticide, there was no adequate evidence to confirm whether the variation in bacterial communities was related to the impacts of neonicotinoids on nematodes, as higher trophic groups that feed on bacteria or the direct effects of neonicotinoids on bacteria that can also impact nematodes. Thus, it is still not clear if the non-target effects of neonicotinoid seed treatment on bacterial community variation depend on the top-down effects of nematode predation or the other higher trophic groups that feed on bacteria, such as microarthropods (Staley et al., 2015; Thakur & Geisen, 2019b). Another scenario, also suggested by a past study (Bradford et al., 2020), is that bottom-up effects of neonicotinoids on bacteria that in turn influence the nematodes by limiting the availability of bacteria as their food resources are what drive the patterns we observe. Further lab-based studies to examine how neonicotinoid seed treatment affects the behavioral interaction between mock bacterial communities and single species of nematodes are needed to investigate this matter. In addition to shifts in overall network structure, we found that several bacterial families mostly from the *Proteobacteria* and *Actinobacteria* phyla have the highest coreness values in the neonicotinoid-treated network but not in the control network. Our previous study showed that the bacteria favored by neonicotinoid seed treatment were dominated by *Actinobacteria*, including genera that are potentially involved in the biodegradation of neonicotinoids and those that were negatively affected by neonicotinoid seed treatment were dominated by *Proteobacteria*, including the plant growth-promoting rhizobacteria (PGPR) (Parizadeh et al., 2021). The effects of neonicotinoid seed treatment on these keystone bacteria could in turn influence the other members of the network given their core position within the network of ecological interactions (Kitsak et al., 2010). Future research to evaluate the role of other beneficial eukaryotic microbial communities, such as microarthropods, in the microbial co-occurrence networks will also be required to improve our understanding of the impacts of pesticides on microbial trophic relationships.

2.6 Conclusions

Despite the essential role of nematodes as bioindicators of soil quality and ecological functions, there have been only a few studies that focused on evaluating the effects of neonicotinoids on them, which were lab-based studies conducted on single species. As far as we know, the present study is the first experimental design that simulates real farming conditions while quantifying neonicotinoid effects on nematode communities. This work advances our understanding of the impacts of neonicotinoid seed treatment on soil nematode communities and the nematode-bacteria co-occurrence networks. We observed that neonicotinoids influence soil nematode community compositions and some families from different trophic groups, as well as nematode-bacteria co-occurrence networks. Future research to evaluate the role of other beneficial eukaryotic microbial communities, such as microarthropods, in the microbial co-occurrence networks and to investigate the effects of neonicotinoid seed treatment on top-down or bottom-up regulations of microbial communities will be required to improve our understanding of the impacts of pesticides on microbial trophic relationships.

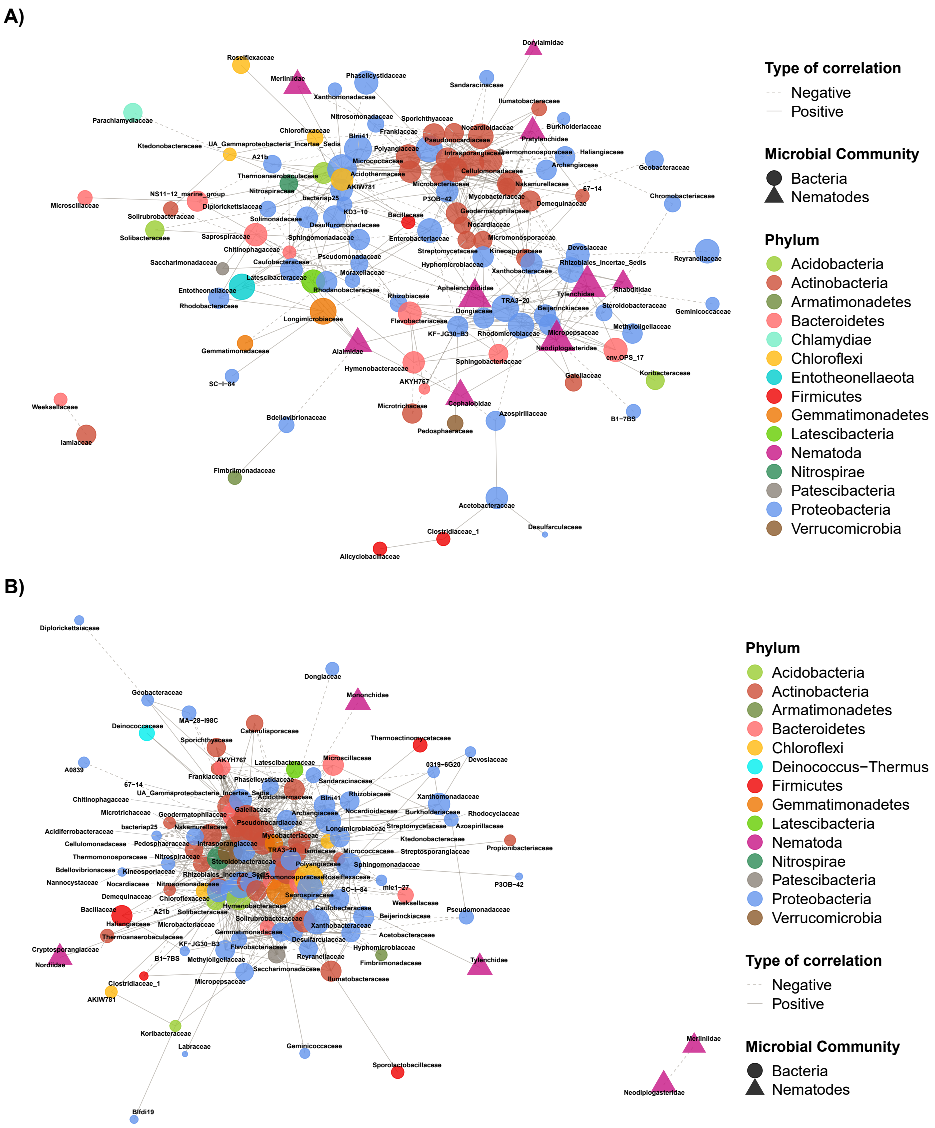

Figure 2.3: Inter-kingdom nematode-bacteria co-occurrence network analysis. Co-occurrence networks among nematode (triangles) and bacterial communities (circles) in control (A, n = 54) and neonicotinoid-treated (B, n = 55) soil samples in a three-year soybean/corn rotation in L'Acadie, Quebec, Canada. Each node represents one microbial family. Node size corresponds to microbial relative abundance and its color shows the microbial phylum. Only nodes with absolute correlationsgreater than 0.30 and BH-adjusted P-value less than 0.01 based on the SparCC algorithm are represented. Edges linking two nodes represent positive (solid lines) and negative (dashed lines) co-occurrences. Unknown or unidentified families are labeled with their corresponding order.

2.7 Acknowledgements

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CHAPTER III

EFFECTS OF NEONICOTINOID SEED TREATMENTS ON SOIL MICROBIAL GENE EXPRESSION VARY WITH TIME IN AN AGRICULTURAL ECOSYSTEM

(Article in preparation)

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3.1 Abstract

Neonicotinoids, a class of systemic insecticides, have been widely used for decades against various insect pests. Past studies have reported non-target effects of neonicotinoids on some beneficial macro- and micro-organisms. Given the crucial role that the soil microbiota plays in sustaining soil fertility, it is critical to understand how microbial taxonomic composition and gene expression respond to neonicotinoid exposure. To date, few studies have focused on this question, and these studies have evaluated the shifts in soil microbial taxonomic composition or used soil biochemical analyses to assess the changes in microbial functions. In this study, we have applied a metatranscriptomic approach to quantify the variability in soil microbial gene expression in a two-year soybean/corn crop rotation in Quebec, Canada. We identified weak and temporally inconsistent effects of neonicotinoid application on soil microbial gene expression, as well as a strong temporal variation in soil microbial gene expression among months and years. Neonicotinoid seed treatment altered the expression of a small number of microbial genes, including genes associated with heat shock proteins, regulatory functions, metabolic processes, photosynthesis and DNA repair. These changes in gene expression varied during the growing season and between years. Overall, the composition of soil microbial expressed genes seems to be more resilient and less affected by neonicotinoid application than soil microbial taxonomic composition. Our study is among the first to document the effects of neonicotinoid seed treatment on microbial gene expression and highlights the strong temporal variability of soil microbial gene expression and its responses to neonicotinoid seed treatments.

Keywords: metatranscriptomics, microbial functional categories, microbial composition and diversity, microbial gene expression, neonicotinoid seed treatment, temporal variability.

3.2 Introduction

Soil quality is frequently used as an indicator of environmental health in sustainable agriculture (Sharma et al., 2010). It refers to the capacity of soil to function in order to sustain biological productivity and maintain or improve environmental quality and the health of humans, plants, animals, and other living organisms (Doran et al., 1997). Soil microbial diversity, composition and functions are important indicators to monitor and evaluate soil quality (van Bruggen et al., 2006; Sharma et al., 2010). Ecological disturbances caused by environmental stress and perturbations such as pesticide application have been shown to influence microbial community structure and functional diversity (Itoh et al., 2014; Arora & Sahni, 2016). To better understand the effects of these disturbances on soil microbiome, it is crucial to study microbial functional activities and gene expression (Nannipieri et al., 2003). Past studies have reported effects of some pesticides on soil microbial functional activities such as microbial biomass enzyme activities and biochemical reactions, including carbon or nitrogen mineralization, nitrogen fixation, nitrification, and denitrification (Hussain et al., 2009; Arora & Sahni, 2016). However, to date, a systematic evaluation of the effects of pesticide application on community-wide soil microbial gene expression is lacking. Here we address this lack of knowledge by measuring the effects of neonicotinoid application and temporal variation on soil microbial gene expression in a soybean-corn agroecosystem in Quebec.

Neonicotinoids are a widely used family of systemic neuro-active insecticides that are chemically similar to nicotine. They were introduced to the world in the late 1980s (Kagabu, 1996; Tomizawa & Casida, 2005) and today, they are used prophylactically in the form of seed treatments against a variety of insect pests (Goulson, 2013; Samson-Robert et al., 2017; Labrie et al., 2020). Past studies have shown the non-target effects of these pesticides on beneficial insect pollinators such as honeybees and butterflies and soil invertebrates such as earthworms (Iwasa et al., 2004; Samson-Robert et al., 2014; Bonmatin et al., 2015; Pisa et al., 2015; Qi et al., 2018; Whitehorn et al., 2018). Neonicotinoids are supposed to be selectively more toxic to invertebrates because of the fundamental distinctions between their nicotinic acetylcholine receptors (nAChRs) compared to vertebrates (Tomizawa & Casida, 2005). However, non-target impacts of these pesticides on the taxonomic composition of soil microbial communities have been documented, including shifts in the abundance of diverse taxa, such as a decrease in bacteria genera involved in nitrification and an increase in bacteria genera related to neonicotinoid biodegradation (Cycoń et al., 2013; Hopewell et al., 2017; Li et al., 2018; Zhang et al., 2018; Bradford et al., 2020; Yu et al., 2020; Parizadeh et al., 2021). An increase in the abundance of the genes coding for the cytochrome P450 enzyme family has been reported in response to neonicotinoid exposure, based on soil microbial amplicon and metagenomic sequencing (Wu et al., 2021; Yu et al., 2021). Previous studies have indicated that this family of detoxifying enzymes is also overexpressed in the insects resistant to this pesticide and is involved in neonicotinoid biodegradation (Bass et al., 2011; Xie et al., 2012; Chen et al., 2019). Another study has reported that nitrogen-fixing and nitrifying bacteria are very sensitive to neonicotinoids (Cycoń & Piotrowska-Seget, 2015). Studies on the effects of neonicotinoids on gene expression in different plant species have shown a variety of responses, including a decrease in the expression of cell wall synthesis-related genes, which may lead to a lower resistance to cell-content feeder insects, and an increase in the expression of (1) photosynthesis-related genes, which may prolong the energy production period, (2) pathogenesis-related genes, and (3) stress tolerance-related genes (for example genes involved in tolerance to drought and cold) (Elbert et al., 2008; Cataneo et al., 2010; Larsen & Falk, 2013; Wulff et al., 2019). However, these changes are not consistent and their mechanisms are unknown (Magalhaes et al., 2009; Seagraves & Lundgren, 2012).

To our knowledge, none of these past studies have quantified community-wide changes in soil microbial gene expression in response to neonicotinoid seed treatment; rather, they have focused on the expression of one or a few genes at a time. Similarly, biochemical studies have shown that neonicotinoids can have non-target impacts on soil microbial functional activities and biochemical processes, such as a decline in soil respiration, nitrification and the activity of nitrite and nitrate reductase enzyme, as well as an inhibition in metabolic processes resulting in a decrease in enzymatic activity (Cycon et al., 2005; Singh & Singh, 2005; Cycoń & Piotrowska-Seget, 2015; Filimon et al., 2015). But, these studies have focused on one or a few indicators of microbial function. Thus, while there is evidence for changes in individual measures of microbial functional activities, we are not aware of studies that have used transcriptomic or metatranscriptomic approaches to quantify community-wide changes in soil microbial gene expression in response to neonicotinoid seed treatment.

In this study, we used metatranscriptomics to evaluate the effects of neonicotinoid seed treatment on soil microbial gene expression. Metatranscriptomics (also known as RNA-seq) identifies the genes that are actually being expressed in a given environment and can help to better study the active functions and the adaptations of microbial communities to environmental changes and stress (Bailly et al., 2007; Brotto et al., 2018; Ma et al., 2019). In this study, our specific objectives were to (1) characterize soil microbial gene expression, including bacterial and eukaryotic expressed genes, in a two-year soybean/corn crop rotation using metatranscriptomic sequencing, and (2) assess the effects of neonicotinoid seed treatment on soil microbial gene expression in this agroecosystem. We hypothesized that (1) neonicotinoid seed treatment and time affect soil microbial gene expression and (2) the expression of pesticide degradation-related genes increases, while the expression of nitrification-related genes decreases in response to neonicotinoid seed treatment. To address our objectives and hypotheses, we studied soil microbial gene expression using a metatranscriptomic approach in a two-year soybean/corn crop rotation in Quebec, Canada.

3.3 Materials and Methods

3.3.1 Study Site

The study was conducted in an experimental farm in Agriculture and Agri-Food Canada, located in L'Acadie (45°17′38.0"N; 73°C20′58.0"W), Quebec, Canada. L'Acadie is in the Canadian hardiness zone 5a and has a temperate climate and clay loam soil. In a two-year crop rotation system, we planted soybean (2016) and corn (2017) in mid-May, in 100 x 3 m plots with four replicates of non-neonicotinoid-treated (control) and neonicotinoid-treated seeds. There were four rows in each plot and the field was surrounded by two extra neonicotinoid-treated plots. All seeds were coated by three fungicides (difenoconazole, metalaxyl-M and sedaxane), in addition to 0.25 mg/seed thiamethoxam for the neonicotinoid-treated seeds. For three years before the experiment, the field had not been treated by any type of neonicotinoids and was a no-till meadow. We used glyphosate before and one month after seeding to control weeds, and in the corn field we also used 400 kg/ha NPK fertilizer (15-15-15) before seeding and 222 kg/ha N fertilizer (27.5%) one month after seeding. There were no significant differences in soil physicochemical properties (e.g., pH, etc.) across the field, nor between months or years (see more details in our previous study (Parizadeh et al., 2021)).

3.3.2 Soil Sample Collection

Each year, we retrieved 32 soil samples, two samples per plot at two sampling times (in June and September), for a total of 64 samples. For each sample, we used a sterile 2-cm diameter corer to collect soil from the upper 12-15 cm layer of the bulk soil (soil that does not adhere to plant roots) from six different spots at 10 cm around 6-10 close plants in a zigzag pattern and pooled them into one 400-500 g sample. Samples were immediately transferred to the laboratory in a cooler and kept at -80°C for RNA extraction.

3.3.3 RNA extraction

We extracted RNA using the MoBio/QIAGEN RNeasy PowerSoil Total RNA Kit from 2 g of each soil sample according to the manufacturer's instructions. To better capture the soil microbial functional variation, we extracted RNA twice from each sample and pooled them into one. We also pooled the extracted RNA of the two samples collected from the same plot (each replicate). Before and after pooling, total extracted RNA was quantified using a NanoDrop Spectrophotometer ND-1000 (NanoDrop Technologies, Inc.), and its integrity was assessed using RNA 6000 Nano LabChip Kit in microcapillary electrophoresis (Agilent 2100 Bioanalyzer, Agilent Technologies). Samples were then stored at -80°C until sequencing.

3.3.4 Library preparation and metatranscriptomic sequencing

RNA samples with an RNA integrity number (RIN) $\geq$ 8.0 were sent to Genome Québec (Montreal, Quebec, Canada) for metatranscriptomic sequencing. To increase the number of sequenced mRNAs, ribosomal RNA (rRNA) was depleted from 250 ng of total RNA using Illumina Ribo-Zero rRNA Removal Kits Bacteria. Residual RNA was cleaned up using the Agencourt RNACleanTM XP Kit (Beckman Coulter) and eluted in water. The second round of ribo-depletion was done using Illumina Ribo-Zero rRNA Removal Kits (Yeast). Residual RNA was again cleaned up using the Agencourt RNACleanTM XP Kit (Beckman Coulter) and eluted in water. Complementary DNA (cDNA) synthesis was achieved with the NEBNext RNA First-Strand Synthesis and NEBNext Ultra Directional RNA Second Strand Synthesis Modules (New England BioLabs). The remaining steps of library preparation were done using the NEBNext Ultra II DNA Library Prep Kit for Illumina (New England BioLabs). Adapters and PCR primers from New England BioLabs were employed. Libraries were quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Life Technologies) and the Kapa Illumina GA with Revised Primers-SYBR Fast Universal kit (Kapa Biosystems). The average fragment size (313 bp, including adapters) was determined using a LabChip GX instrument (PerkinElmer). RNA samples were finally paired-end sequenced on four lanes (eight samples per lane) on Illumina HiSeq at the Genome Québec facility (Montreal, Quebec, Canada).

3.3.5 Bioinformatic analyses, quality filtering and rarefaction

We processed the metatranscriptomic data according to the standalone metatranscriptome analysis (SAMSA2) pipeline (Westreich et al., 2018). We first merged the paired-end reads to make contigs using PEAR v0.9.5 (Zhang et al., 2014). Then, we applied Trimmomatic v0.32 (Bolger et al., 2014) (parameters: PE -phred33, SLIDINGWINDOW:4:15 and MINLEN:99) on the merged metatranscriptomes to remove adaptor contamination and low-quality sequences. Physical depletion of rRNA using the ribo-depletion kits usually eliminates about 80% of ribosomal RNA (Westreich et al., 2018). To remove the rest of the rRNA, we performed a bioinformatic ribo-depletion using SortMeRNA v2.0 (Kopylova et al., 2012). For gene annotation, we used DIAMOND aligner v2.0.4 (Buchfink et al., 2015, 2021) to BLAST the metatranscriptomes against the SEED Subsystems hierarchical database (Overbeek et al., 2014) and the NCBI's RefSeq bacterial genomes and eukaryotic genomes databases (Tatusova et al., 2014). We used the python scripts provided by SAMSA2 to (1) group the identified SEED genes into a four-level hierarchy of subsystems (a set of genes that are associated with each other and perform a particular biological process together), (2) aggregate the large results of annotations into summarized tables of microbial genes, and (3) calculate the metatranscriptome abundance counts for further analyses. In order to minimize the possible technical artifacts caused by the number of reads, PCR, library preparation or sequencing, we performed the following steps of data cleaning: (1) given the lack of standard labeling of genes in databases, we inspected the names of the 100 most abundant genes in each annotated dataset and gave a unique name to the same genes that were labeled differently and then combined the duplicate genes, as follows: (i) in the RefSeq-based annotations of bacteria, we replaced "DNA-directed RNA polymerase subunit beta′" with "DNA-directed RNA polymerase subunit beta", (ii) in the RefSeq-based annotations of eukaryotes, we substituted "′Cold-shock′ DNA-binding domain containing protein" by "cold-shock DNA-binding domain-containing protein", and (iii) in the level 4 of SEED-based hierarchical annotations, we changed “DNA-directed RNA polymerase beta′ subunit (EC 2.7.7.6)" to "DNA-directed RNA polymerase beta subunit (EC 2.7.7.6)"; (2) then, we explored samples to verify if there are any outlier samples with a very different composition of microbial expressed genes based on Shannon diversity and the non-metric multidimensional scaling (NMDS) on Bray-Curtis dissimilarities (Bray & Curtis, 1957); (3) we removed the rare expressed genes with fewer than five reads in the entire metatranscriptome from the RefSeq-based annotation results (respectively, 37.5% and 36.0% of the total number of bacterial and eukaryotic expressed genes); (4) we also filtered all the expressed genes annotated as hypothetical proteins (1.0% of the remaining SEED-based hierarchical expressed genes, 0.1% of the remaining RefSeq-based bacterial expressed genes and 36.7% of the remaining RefSeq-based eukaryotic expressed genes), and (5) then we rarefied samples based on their rarefaction curves (Figure E.1) to approximately the lowest number of reads per sample in SEED-based hierarchical annotations (1,430,000 reads per sample and keeping all the samples and remaining expressed genes) and RefSeq-based annotations (1,800,000 and 260,000 reads per sample of the RefSeq-based bacterial and eukaryotic annotated datasets, respectively, which resulted in keeping all samples and 98.5% of the remaining expressed genes). Finally, we used R to analyze these datasets.

3.3.6 Statistical analyses

3.3.6.1 Soil SEED hierarchical microbial and RefSeq bacterial and eukaryotic functional profiling

To profile the microbial functional categories and their hierarchical levels of the soil samples collected from a two-year rotation of soybean and corn, we quantified the richness of functional categories of expressed genes (number of functional categories per sample) in SEED-based hierarchical and RefSeq-based annotated data. We also determined the ten most abundant microbial functional categories at different levels of SEED hierarchy, as well as the ten most abundant RefSeq bacterial and eukaryotic functional categories according to the total relative abundance of the annotated metatranscriptomes.

3.3.6.2 Effects of neonicotinoid seed treatment on the composition and diversity of soil microbial expressed genes

To study the impacts of neonicotinoid seed treatment on microbial gene expression variation, we first examined the relationships between microbial expressed genes and neonicotinoid seed treatment and time (year and month). To achieve this, we performed a permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001) with 999 permutations on each of the annotated datasets separately using the adonis2 function of the vegan package v2.5.7 (Oksanen et al., 2020) in R v4.0.3 (R Core Team, 2019) (model: . ∼ year/month * neonicotinoid seed treatment). We also conducted a principal coordinate analysis (PCoA) ordination based on Bray-Curtis dissimilarities on each annotated dataset to visualize the variation in microbial gene expression across the soil samples in response to neonicotinoid seed treatment. Finally, we evaluated the impacts of neonicotinoid seed treatment and time (year and month) on the alpha diversity of SEED-based hierarchical microbial expressed genes and RefSeq-based microbial expressed genes using the Shannon index. For each dataset, we examined the significance of differences in alpha diversity of expressed genes between control and neonicotinoid-treated samples using the non-parametric Wilcoxon rank-sum test (Wilcoxon, 1945).

3.3.6.3 Effects of neonicotinoid seed treatment on differential gene expression in soil microbiome

We performed a differential expression analysis of sequence data using DESeq2 (Love et al., 2014) individually on each annotated dataset to identify microbial expressed genes that differed in abundance between all the control and neonicotinoid-treated samples, and between the control and neonicotinoid-treated samples from each sampling time during the growing season (June and September) and from each year (2016 and 2017) to study the temporal effects of neonicotinoid seed treatment on microbial gene expression, as well as between each sampling time and year regardless of the treatment to study the temporal changes of microbial gene expression. We conducted these analyses on the non-rarefied and non-normalized quality filtered and denoised data. We used the $\log_2$-fold changes in gene expression levels to identify genes that were differentially expressed in control versus neonicotinoid-treated samples, between months, and between years, and the Wald test with a local fit type to test the significance of the gene expression differences. Finally, we adjusted the P-values by applying the Benjamini-Hochberg false-discovery rate (FDR) method (Hochberg & Benjamini, 1990) to correct for multiple testing. We chose a significance cutoff of adjusted P-values < 0.05 to identify significantly differentially expressed genes between control and neonicotinoid-treated samples or across time.

3.4 Results

3.4.1 Soil microbial profiling based on SEED hierarchical microbial functional and RefSeq bacterial and eukaryotic functional categories

We detected an average (mean ± SE) of 4,878 ± 4 SEED hierarchical functional categories (level 4) per sample, 22,902 ± 162 RefSeq bacterial functional categories per sample, and 9,899 ± 206 RefSeq eukaryotic functional categories per sample. The SEED-based hierarchical annotation results indicated that 50.5% of the total relative abundance of microbial expressed genes at level 1 of the SEED hierarchy belonged to the ten most abundant microbial functional categories at this level (Table 3.1A and Figure E.2). The majority of the most abundant level 4 SEED hierarchy functional categories were similar to the ten most abundant bacterial and eukaryotic RefSeq-based functional categories, including genes related to chaperone GroEL, chaperone DnaK, DNA-directed RNA polymerase beta subunit, elongation factor G and elongation factor T (Table 3.1B and Figure E.2). The ten most abundant functional categories respectively accounted for 21.7%, 10.0% and 18.1% of the total relative abundance of, respectively, SEED hierarchical microbial (level 4), RefSeq bacterial and eukaryotic expressed genes (Table 3.1B and Figure E.2).

3.4.2 Effects of neonicotinoid seed treatment on the composition and diversity of soil microbial expressed genes

Neonicotinoid seed treatment had no significant effect on the overall composition and diversity of soil microbial expressed genes (based on PERMANOVA and Wilcoxon rank-sum test on Shannon index). However, time (year and month) was an important driver of variation in the composition and diversity of soil microbial expressed genes.

Table 3.1: Ten most abundant soil SEED hierarchical functional categories (levels 1-3: A and level 4: B), RefSeq bacterial eukaryotic functional categories (B) in a two-year soybean/corn crop rotation in L'Acadie, Quebec, Canada.

A)

Functional Databases	**Functional Categories**	**Relative Abundance (%)**
SEED Hierarchical Profile (Level 1)	Protein biosynthesis	13.20
	No hierarchy / NA	9.67
	Transcription	5.44
	Protein folding	5.29
	Clustering-based subsystems	4.46
	Central carbohydrate metabolism	3.56
	Protein degradation	2.50
	Resistance to antibiotics and toxic compounds	2.38
	Lysine, threonine, methionine, and cysteine	2.04
	Heat shock	1.93
SEED Hierarchical Profile (Level 2)	No hierarchy / NA	25.30
	Protein Metabolism	21.60
	Carbohydrates	9.38
	Amino Acids and Derivatives	6.77
	RNA Metabolism	6.74
	Stress Response	5.33
	Respiration	3.83
	Cofactors, Vitamins, Prosthetic Groups, Pigments	3.25
	Virulence, Disease and Defense	2.54
	Clustering-based subsystems	2.15
SEED Hierarchical Profile (Level 3)	No hierarchy / NA	9.57
	Ribosome LSU bacterial	4.60
	GroEL GroES	4.42
	Ribosome SSU bacterial	3.60
	RNA polymerase bacterial	3.02
	Translation elongation factors bacterial	1.98
	Heat shock dnaK gene cluster extended	1.93
	Proteolysis in bacteria, ATP-dependent	1.90
	Transcription initiation, bacterial sigma factors	1.63
	Ton and Tol transport systems	1.42

B)

SEED Hierarchy (Level 4)	Relative Abundance (%)	RefSeq Bacteria	Relative Abundance (%)	RefSeq Eukaryotes	Relative Abundance (%)
No hierarchy / NA	9.57	Molecular chaperone GroEL	2.59	Heat shock protein 60, mitochondrial precursor	4.33
Heat shock protein 60 family chaperone GroEL	3.80	DNA-directed RNA polymerase subunit beta	2.00	Heat shock protein 78, mitochondrial precursor	2.12
DNA-directed RNA polymerase beta subunit (EC 2.7.7.6)	2.57	Molecular chaperone DnaK	0.96	Putative chaperonin GroL	1.73
Chaperone protein DnaK	1.21	ABC transporter ATP-binding protein	0.91	Cold-shock DNA-binding domain-containing protein	1.66
Translation elongation factor Tu	0.87	MFS transporter	0.65	Chaperonin homolog Hsp-60, mitochondrial	1.53
RNA polymerase sigma factor RpoD	0.80	Elongation factor G	0.64	Elongation factor Tu, mitochondrial precursor	1.53
Translation elongation factor G	0.76	Endopeptidase La	0.60	Chaperonin Hsp-60	1.45
ATP-dependent protease La (EC 3.4.21.53) Type I	0.75	ABC transporter substrate-binding protein	0.57	Heat shock 60kD protein 1	1.40
SSU ribosomal protein S1p	0.71	DNA-binding response regulator	0.56	Chaperone protein DnaK	1.30
Cell division protein FtsH (EC 3.4.24.-)	0.65	Elongation factor Tu	0.54	Chaperonin homolog HSP60, mitochondrial precursor, partial	1.00

Year and month together explained significant variation in gene expression at level 4 of SEED hierarchical functional categories (25.07%), RefSeq bacterial functional categories (21.33%) and RefSeq eukaryotic functional categories (10.90%) (PERMANOVA $P < 0.001$, Table 3.2 and Figure 3.1).

Additionally, while the alpha diversity of microbial functional categories of expressed genes was not affected by year, it was significantly higher in June than September in SEED hierarchical functional categories (Shannon index mean ± SE 6.57 ± 0.02 versus 6.46 ± 0.01, Wilcoxon P-value < 0.0001), RefSeq bacterial functional categories (Shannon index mean ± SE 7.70 ± 0.02 versus 7.58 ± 0.01, Wilcoxon P-value < 0.0001) and RefSeq eukaryotic functional categories (Shannon index mean ± SE 7.14 ± 0.06 versus 6.87 ± 0.06, Wilcoxon P-value < 0.001).

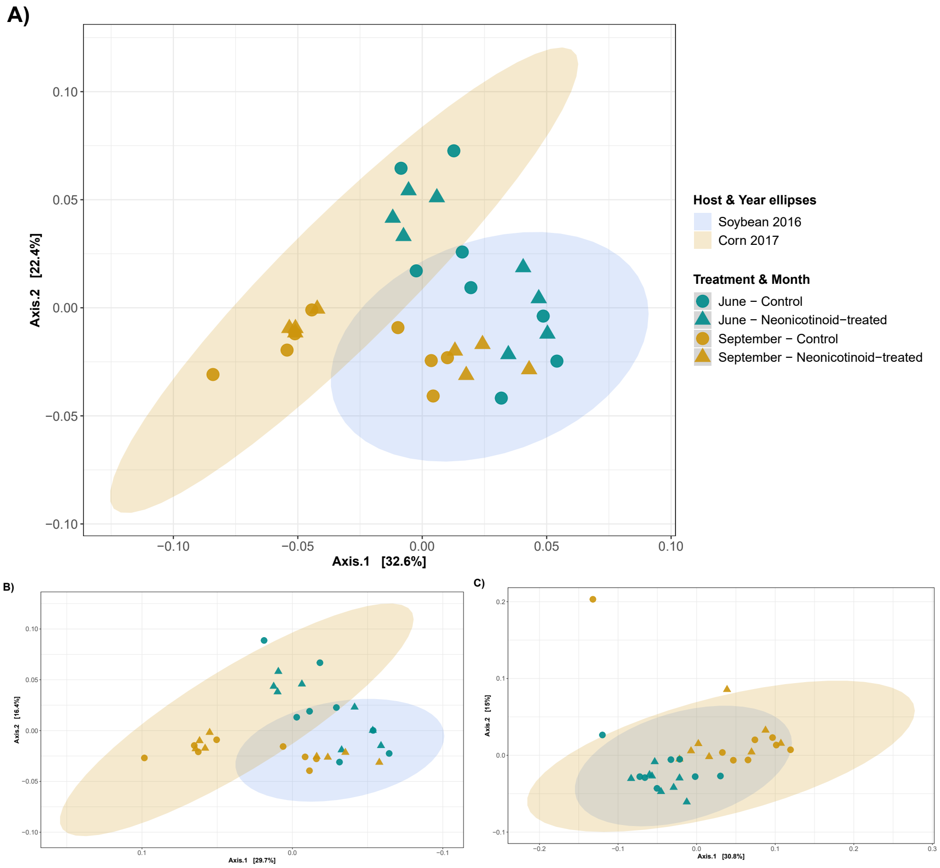

Figure 3.1: Composition variation of soil microbial expressed genes in response to neonicotinoid seed treatment and time. Principal coordinate analysis (PCoA) on Bray-Curtis dissimilarities illustrates the composition variation of soil SEED hierarchical microbial (level4; A), RefSeq bacterial (B) and RefSeq eukaryotic (C) expressed genes between control (n = 16) and neonicotinoid-treated (n = 16) samples two-year soybean (2016) and corn (2017) rotation in L'Acadie, Quebec, Canada. Microbial gene expression varies among months (June: green points and September: yellow points) in control (circle) and neonicotinoid-treated (triangle) samples. Ellipses are shaded based on host species and year of cultivation (blue for 2016 soybean samples and yellow for 2017 corn samples) and represent a 99% confidence level.

Table 3.2: Drivers of the soil microbial gene expression variation in response to neonicotinoid seed treatment and time and their interactions in a two-year soybean/corn rotation in l'Acadie, Quebec, Canada (PERMANOVA based on Bray-Curtis dissimilarities). (:) represents the interaction between variables and (/) represents the nested interaction between variables. Significance levels for each variable are given by: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; NS, $P \geq 0.05$.

	SEED Hierarchical Gene Expression			**RefSeq Bacterial Gene Expression**			**REfSeq Eukaryotic Gene Expression**		
Variables	**R^2 (%)**	**F**	**Pr(>F)**	**R^2 (%)**	**F**	**Pr(>F)**	**R^2 (%)**	**F**	**Pr(>F)**
Year/Month	25.07	14.64	0.001***	21.33	10.86	0.001***	10.90	4.65	0.001***
Neonicotinoid seed treatment	1.91	1.11	NS	2.13	1.08	NS	1.87	0.80	NS
Year/Month : Neonicotinoid seed treatment	NS	NS	NS	NS	NS	NS	NS	NS	NS

3.4.3 Effects of neonicotinoid seed treatment on differential gene expression in soil microbiome

Analysis of differential expression of genes identified no significant effect of neonicotinoid seed treatment on gene expression of all samples from both sampling times and both years of rotation together (DESeq2 adjusted $P < 0.05$). However, looking individually at each year of rotation, neonicotinoid seed treatment led to significantly increased expression of two SEED hierarchical functional categories (level 4: phycobilisome core-membrane linker polypeptide and excinuclease ABC subunit A paralog in greater Bacteroides group) in 2016, when the field was planted with soybean, and decreased expression of one SEED hierarchical functional category (level 4: inner membrane protein CreD) in 2017, in the corn field (DESeq2 adjusted $P < 0.05$, Table 3.3). In 2016, the expression of some RefSeq bacterial functional categories also significantly decreased (chaperone protein ClpB and heat-shock protein IbpA) or increased (protochlorophyllide oxidoreductase) in neonicotinoid-treated samples (DESeq2 adjusted $P < 0.05$, Table 3.3). Finally, for each sampling time, the expression of genes from a few RefSeq bacterial functional categories decreased in June (phosphonate C-P lyase system protein PhnG and beta-aspartyl-peptidase) and in September (chaperone protein ClpB) in response to neonicotinoid seed treatment (DESeq2 adjusted $P < 0.05$, Table 3.3).

While there were relatively few changes in gene expression as a result of neonicotinoid seed treatment, the expression of many soil microbial genes was impacted by time (DESeq2 adjusted $P < 0.05$). Among the SEED hierarchical functional categories (level 4), the expression of 910 genes significantly increased and 903 genes significantly decreased in 2017 versus 2016, and the expression of 516 versus 540 genes significantly increased and decreased in September versus June (DESeq2 adjusted $P < 0.05$, Tables D.1 and D.2). For example, a gene that encodes the glutathione-regulated potassium-efflux system ancillary protein KefG was significantly overexpressed in 2016 compared to 2017, as well as in September compared to June (DESeq2 adjusted $P < 0.05$, Tables D.1 and D.2). Among the RefSeq bacterial functional categories, the expression of 2250 and 2561 genes significantly increased and decreased in 2017 versus 2016, and the expression of 1256 versus 1860 genes significantly increased and decreased in September versus June (DESeq2 adjusted $P < 0.05$, Tables D.3 and D.4). For example, genes that encode avidin, hydroxyacid oxidoreductase and nitrogenase molybdenum-iron protein alpha chain were overexpressed in September compared to June, and also in 2016 compared to 2017 while the expression of a gene coding for pesticidal proteins significantly increased in 2017 versus 2016 (DESeq2 adjusted $P < 0.05$, Tables D.3 and D.4). Finally, among the RefSeq eukaryotic functional categories, the expression of 554 and 614 genes significantly increased and decreased in 2017 versus 2016, and the expression of 322 versus 339 genes significantly increased and decreased in September versus June (DESeq2 adjusted $P < 0.05$, Tables D.5 and D.6). For instance, a gene that encodes Kunitz

Table 3.3: Soil SEED hierarchical microbial (level 4), RefSeq bacterial and eukaryotic expressed genes associated with control and neonicotinoid seed treatment at different times. Soil microbial genes that are significantly differentially expressed (adjusted $P < 0.05$) among different times and between control and neonicotinoid-treated samples in a two-year soybean/corn rotation in L'Acadie, Quebec, Canada identified by Differential expression analysis of sequence data (DESeq2).

Condition	**Variable**	**Gene Expression**					
		SEED Hierarchy (Level 4 | Level 1)	**$\log_2$-fold Change**	**RefSeq Bacteria**	**$\log_2$-fold Change**	**RefSeq Eukaryotes**	**$\log_2$-fold Change**
Treatment	Control	-	-	-	-	-	-
	Neonicotinoid-treated	-	-	-	-	-	-
Treatment in June	Control	-	-	Phosphonate C-P lyase system protein PhnG	- 17.04	-	-
				Beta-aspartyl-peptidase	- 3.41		
	Neonicotinoid-treated	-	-	-	-	-	-
Treatment in September	Control	-	-	Chaperone protein ClpB	-2.34	-	-
	Neonicotinoid-treated	-	-	-	-	-	-
Treatment in 2016	Control	-	-	Chaperone protein ClpB	- 2.62	-	-
				Heat-shock protein IbpA	- 2.20		
	Neonicotinoid-treated	Phycobilisome core-membrane linker polypeptide | Light-harvesting complexes	3.60	Protochlorophyllide oxidoreductase	2.05	-	-
		Excinuclease ABC subunit A paralog in greater Bacteroides group | DNA repair	0.61				
Treatment in 2017	Control	Inner membrane protein CreD | Bacteriocins, ribosomally synthesized antibacterial peptides	- 0.85	-	-	-	-
	Neonicotinoid-treated	-	-	-	-	-	-

trypsin inhibitor precursor was overexpressed in September compared to June and in 2016 compared to 2017. In addition, the expression of a gene that encodes alpha-amylase inhibitor/lipid transfer/seed storage family protein precursor increased in June versus September, and the expression of another gene encoding nematode resistance protein-like HSPRO2 increased in 2016 versus 2017 (DESeq2 adjusted $P < 0.05$, Tables D.5 and D.6). Finally, based on all three microbial annotated datasets, the expression of several heat shock protein-related genes (such as heat shock protein 60, protein IbpA, chaperone protein ClpB, chaperone GroEL and chaperone GroES) increased in September, whereas the expression of the cold shock protein-related genes (such as cold shock proteins CapB, CspA and CspD) increased in June (DESeq2 adjusted $P < 0.05$, Tables D.2, D.4 and D.6).

3.5 Discussion

Neonicotinoid seed treatment had weak and temporally variable effects on soil microbial gene expression in a soybean-corn agroecosystem. Conversely, time was a strong driver of the composition and diversity of soil microbial expressed genes, as expected and similar to its important effects on soil microbial taxonomic composition and diversity (Hannula et al., 2019; Parizadeh et al., 2021). Time had a very strong effect on the expression of numerous soil microbial genes. Among them, several genes associated with cold shock protein were overexpressed in June, whereas many genes related to heat shock protein were overexpressed in September, suggesting that temporal variation in gene expression is related to changes in environmental conditions and in particular to temperature. A few previous studies have also shown the temporal changes of soil microbial functional activities and biochemical processes in response to different agrochemical treatments, including fertilizer or pesticide application (Mentzer et al., 2006; Wu et al., 2017). Our results thus suggest that while gene expression in soil microbial communities is highly variable in time, these communities are either highly resistant or resilient to changes in gene expression in response to neonicotinoid seed treatment. This can be due to functional redundancy in the identity of expressed genes, despite the major variation in the taxonomic composition of these microbial communities that we have previously observed (Parizadeh et al., 2021). Past studies have suggested that various co-occurred microbial communities may be functionally redundant. Therefore, changes in microbial taxonomic composition and diversity, especially when the community is diverse, do not necessarily affect ecosystem function (Walker, 1992; Fetzer et al., 2015). There is thus an open question whether gene expression in soil microbial communities exhibits the pattern of functional redundancy as documented in other ecosystems (Burke et al., 2011; Gosalbes et al., 2011; Steffen et al., 2012; Franzosa et al., 2014; Grządziel, 2017; Lajoie et al., 2020).

Our findings indicate that the expression of some genes related to heat shock protein, metabolic processes (i.e., phosphonate break down and enzyme catalysis), and regulatory functions (i.e., respiration) decreased, while the expression of several genes related to DNA repair increased, at different time-spans in the neonicotinoid-treated samples compared to control samples. This suggests a temporally variable interaction between neonicotinoids and environmental stressors. We detected a decline in the expression of the genes related to metabolic processes, such as phosphonate C-P lyase system protein PhnG related, a gene implicated in phosphonate break down, and beta-aspartyl-peptidase, which is an enzyme catalyzer, in the neonicotinoid-treated samples. This is in accordance with previous biochemical studies showing changes in soil microbial metabolic processes in response to neonicotinoid application (Cycon et al., 2005; Singh & Singh, 2005; Cycoń & Piotrowska-Seget, 2015; Filimon et al., 2015). The observed decrease in the expression of genes such as CreD, which plays a crucial role in regulatory functions including respiration (Zamorano et al., 2014; Huang et al., 2015), in the samples exposed to neonicotinoid treatment at some times also agrees with the findings of past biochemical studies showing negative effects of neonicotinoids on soil bacterial respiration (Cycoń & Piotrowska-Seget, 2015; Pitombo et al., 2016; Yu et al., 2020). Finally, an increase in the expression of genes related to DNA repair (genes encoding excinuclease ABC (subunit A)) in response to neonicotinoid seed treatment at some time points suggests that neonicotinoids may induce DNA damage in microbial cells.

Overall, despite our hypothesis that the expression of pesticide degradation-related genes would increase and the expression of nitrification-related genes decrease in response to neonicotinoid seed treatment, and previous observations of soil microbial taxonomic and physiochemical changes due to neonicotinoid application (Singh & Singh, 2006; Cycoń & Piotrowska-Seget, 2015; Filimon et al., 2015; Zhang et al., 2018; Parizadeh et al., 2021), we did not detect any significant shifts in the expression of genes related to biodegradation of neonicotinoids or any decline in the expression of the genes associated with nitrification. We suggest several possible explanations for this finding: First, as mentioned previously, strong temporal changes in the expression of soil microbial genes may have masked subtle effects of neonicotinoid seed treatments on gene expression. Secondly, changes in gene expression in response to neonicotinoid seed treatment may have been short-lived, and thus the gradual changes in microbial gene expression are not captured by our sampling interval. However, this seems unlikely since we sampled both early and late in the growing season. Finally, it is possible that soil microbial communities are functionally resistant or resilient, leading to few changes in gene expression in response to neonicotinoid seed treatment. Compared to measures of soil microbial community taxonomic structure (Parizadeh et al., 2021), soil microbial gene expression seems to be less sensitive to the stress imposed by neonicotinoid application. This is probably due to the functional resilience and redundancy of microbial communities (Swift et al., 2008), and it is in line with the findings of previous studies showing less variability in microbial gene expression than taxonomic composition (Burke et al., 2011; Gosalbes et al., 2011; Franzosa et al., 2014; Staley et al., 2014; Lajoie et al., 2020). Further validation of these findings using metabolomic analysis to quantify microbial metabolites and determine changes in microbiome metabolism in response to neonicotinoid seed treatment may help us improve our understanding of soil microbial functional dynamics and make our findings more reproducible and applicable.

Our findings are based on only two years of soybean/corn crop rotation, which makes it impossible for us to distinguish the effects of host species versus time. We did not measure environmental changes during the growing season, neither did we quantify the homogeneity of neonicotinoid concentrations across the treated samples. The changes in neonicotinoid concentration in soil over time and among samples due to their consumption and biodegradation of neonicotinoids, the potential for an increase in the residuals of neonicotinoid and degradation products towards the end of the season and the the accumulation of these products in soil over the years of rotation, and finally the changes in temperature, humidity and other environmental factors during the experience may also partially explain the effects of time on the microbial gene expression variation, and future studies will be required to distinguish among the impacts of these factors. Thus, overall we can only conclude that some combination of host species and time had important impacts on microbial communities.

The present results are based on microbial annotations against the SEED Subsystems hierarchical database and the NCBI's RefSeq bacterial genomes and eukaryotic genomes databases. These databases are popular and reliable; however, due to a lack of standard labeling of genes, a future challenge will be to improve microbial genome databases, in particular for diverse ecosystems such as soils for which there are relatively few reference genomes and databases available and for which many gene functions remain unknown. Technological advancements such as long-read sequencing and an assembly-based approach to transcriptomics should also advance our understanding of the gene expression in large microbial eukaryotic genomes.

3.6 Conclusions

In this study, we used metatranscriptomics of soil microbial communities to demonstrate high temporal variability but relatively weak and temporally variable effects of neonicotinoid seed treatment on soil microbial gene expression in a soybean-corn agroecosystem. In different time-spans, genes related to heat shock protein, regulatory functions (such as soil respiration) and metabolic processes (such as phosphonate breakdown and enzyme catalysis) were underexpressed in response to neonicotinoid seed treatment, whereas genes related to photosynthesis and DNA repair were overexpressed in response to neonicotinoid seed treatment. Our results demonstrate the crucial role of time and temporal changes in shaping soil microbial gene expression. To our knowledge, our study provides the first example of the impacts of neonicotinoid seed treatment on community-wide soil microbial gene expression in an experimental design representing real farming conditions. Overall, metatranscriptomic studies offer real-time and in-depth insight into the biologically active microbiomes and how microbial gene expression responds to neonicotinoid seed treatment.

3.7 Acknowledgements

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CONCLUSION

Despite the crucial role of the phyllosphere and soil microbial communities in protecting plant crops and soil quality and regulating ecosystem functions, our knowledge of the non-target effects of the environmental stress caused by pesticides on these communities in agroecosystems is still limited. Understanding the variation in microbial diversity, composition and functions in communities exposed to pesticides provide new insights into pest-management practices and pesticide application strategies. This may help us in identifying and limiting the use of molecules that greatly influence microbial taxonomic composition and functions. It can also lead us to determine the most efficient forms and time of pesticide application instead of methods like seed treatment that may affect more non-target species and also in order to avoid using pesticides during critical crop growth stages, such as root colonization. My thesis research advances our understanding of the non-target impacts of the widely used pesticides of the neonicotinoid family on community-wide variation in the phyllosphere and soil microbiome in a soybean-corn agroecosystem. To our knowledge, this is the first example of an experimental design that simulates real farming conditions to assess how agricultural microbiomes in a crop rotation respond to neonicotinoid seed treatment. It also offers new key information on the contribution of important drivers of microbial community variation, such as plant host species and time and their possible interactions with neonicotinoid seed treatments.

4.0 Global conclusions

In this thesis, I have shown that neonicotinoid seed treatment affected the phyllosphere and soil bacterial community, soil nematode community taxonomic composition, and the co-occurrence patterns among soil nematode and bacterial families. However, host species and time were often stronger drivers of microbial community composition variation than neonicotinoid seed treatment. In terms of gene expression, the effects of neonicotinoid seed treatment on soil microbial gene expression varied with time. Overall, this thesis offers a more profound understanding of the variability in the phyllosphere and soil microbial community composition and real-time insight into the soil microbial gene expression in response to neonicotinoid seed treatment in a soybean-corn agroecosystem. It also gives us an indication of the role of host species and time as strong drivers of microbial community variation in an agroecosystem.

The main objective of this thesis was to improve our knowledge of the impacts of neonicotinoid seed treatment on microbial community composition, diversity, patterns of co-occurrence, and gene expression. To achieve this goal, the first objective was to characterize the phyllosphere and soil microbial taxonomic composition and gene expression. The second objective was to determine the drivers of microbial taxonomic composition and gene expression variation. Finally, the third objective was to identify the shifts in microbial community composition and diversity, taxonomic composition, co-occurrence patterns and gene expression in response to neonicotinoid seed treatment. Therefore, in the first chapter, we presented the bacterial communities of the phyllosphere and soil, the contribution of habitat (phyllosphere vs. soil), host species (soybean vs. corn) and time (years and months) in the bacterial community variation, and the effects of neonicotinoid seed treatment on the bacterial community diversity and taxonomic composition in a three-year soybean/corn crop rotation. Then, in the second chapter, we demonstrated the nematode communities of soil, the contribution of host species (soybean vs. corn) and time (years and months) in the nematode community variation, and the effects of neonicotinoid seed treatment on the nematode community diversity, taxonomic composition and trophic groups in a three-year soybean/corn crop rotation. We also illustrated the changes in the patterns of the soil food web and co-occurrence networks among soil bacterial and nematode families influenced by neonicotinoid seed treatment. Finally, in the third chapter, we focused on the soil microbial expressed genes, the proportion of variability in microbial gene expression explained by time (years and months), and the effects of neonicotinoid seed treatment on the composition and diversity of soil expressed genes in a two-year soybean/corn crop rotation. To address these objectives, I performed a conceptual review of literature, a field-based study design and sample collection, several meta-analyses, faunal analysis and ecological co-occurrence network analysis. I applied diverse next-generation technologies, including amplicon sequencing of bacterial 16S rRNA gene and nematode 18S rRNA gene to characterize the taxonomic composition of the present microbial communities and metatranscriptomic sequencing to identify active and expressed microbial genes. I describe the distinct contributions of the research presented here to advances in microbiome research in agroecosystems and sustainable agriculture in the following paragraphs.

4.1 Neonicotinoid seed treatment impacted the phyllosphere and especially soil bacterial community taxonomic composition

Even though invertebrates are the main targets of neonicotinoids, we observed non-target effects of neonicotinoid seed treatment on phyllosphere and soil bacterial communities and taxonomic composition in a soybean-corn agroecosystem. Among the drivers of bacterial community variation under study, habitat was the strongest driver of community variation. Time and host species, alone and in interaction with each other, were also important determinants of bacterial community composition, especially in the phyllosphere. The importance of these factors masked the relatively subtle effects of neonicotinoids on phyllosphere bacterial communities. On the other hand, neonicotinoid seed treatment significantly affected soil bacterial community composition and alpha diversity. These effects were as strong as host species and temporal changes during the growing season. Moreover, the taxonomic composition of bacterial communities in the phyllosphere and particularly in soil varied significantly in response to neonicotinoid seed treatment. While the relative abundance of some bacterial genera related to neonicotinoid biodegradation increased, we observed a decrease in the relative abundance of several potentially beneficial soil bacteria genera, such as plant growth-promoting rhizobacteria that can develop a symbiotic association with host plants, nitrogen-fixing bacteria and other bacteria involved in the nitrogen cycle.

4.2 Neonicotinoid seed treatment affected certain soil nematode families and the structure and taxonomic composition of the co-occurrence networks among bacterial and nematode families

Neonicotinoid seed treatment significantly impacted the soil nematode community composition but did not affect the nematode alpha diversity. In terms of taxonomic composition, it impacted the relative abundance and contribution of certain families of nematodes while the total number of species did not reduce. There was a decrease in the relative abundance of an omnivorous nematode family (Dorylaimidae) in the neonicotinoid-treated samples, which is known as an indicator of environmental disturbance.

We did not detect any significant changes in the soil food web indices based on nematode faunal analysis. However, neonicotinoid seed treatment led to a decrease in the omnivore's metabolic footprint, indicating stress in the ecosystem networks. While no bacterivorous nematode co-occurred with bacterial families in the neonicotinoid-treated network, some bacterivorous families were positively or negatively correlated with bacterial families in the control network. One of the principal challenges remaining in the study of the effects of neonicotinoid seed treatment on the inter-kingdom relationships among soil microbial communities is to understand whether bacterial communities are affected by neonicotinoids through their impacts on higher trophic groups that feed on them, such as nematodes (top-down effects), or the impacts of neonicotinoids on bacterial communities influence nematodes that feed on bacteria (bottom-up effects). The first hypothesis is that since nematodes are invertebrates and so among the primary targets of neonicotinoids and also play an important role in shaping bacterial communities, the variation that we observed in soil bacterial taxonomic composition in neonicotinoid-treated samples and co-occurrence networks in the first two chapters, might be associated with the responsiveness and sensitivity of some nematode trophic groups and families to neonicotinoid exposure and controlled by the top-down effects of nematode predation and interactions with bacteria. Based on previous studies, especially in a relatively stable ecosystem, bacterivorous nematodes may show a preference for some bacterial species more than others and avoid feeding on certain bacteria depending on the concentration of the metabolites that they produce or other bacterial traits, such as growth rate and cell size (Bargmann et al., 1993; Weber & Traunspurger, 2013; Liu et al., 2018). Thus, losing the bacterivorous nematodes co-occurred with bacteria in response to neonicotinoids might have given an opportunity of survival to those bacteria that were prey to nematodes in the control network. The second hypothesis is that the bacterial taxonomic composition variation in response to neonicotinoid seed treatment, as observed in the first chapter, may explain the bottom-up control of the nematode-bacteria co-occurrence networks and the variation in co-occurrence patterns in the samples exposed to neonicotinoids because of the changes in nematode predation behavior based on both their food preference and the availability of food resource, such as different bacteria. The last hypothesis is that some species of both nematodes and bacteria might have mutually impacted the predation behavior of one another. Thus, the effects of neonicotinoids on these specific species led to the observed variation in their co-occurrence patterns. To address these questions, further lab-based studies at lower taxonomic levels than family, such as genus or species, are required to examine how neonicotinoid seed treatment affects the behavioral interactions among mock bacterial communities and single species of nematodes.

4.3 Effects of neonicotinoid seed treatment on the soil microbial gene expression varied with time

Despite the critical role of microbial communities in the maintenance of soil quality and regulation of ecosystem functions, our knowledge on community-wide microbial functional variation in response to environmental stress induced by pesticide application is very limited. To evaluate the responses of soil microbial communities to pesticides, such as neonicotinoids, in addition to identifying the microbial communities that are present in the ecosystem and the impacts of neonicotinoids on their taxonomic composition, it is crucial to assess which microbial communities are active and how microbial gene expression is affected by pesticide application. The work presented here shows that neonicotinoid seed treatment had weak and temporally variable effects on the expression of soil microbial genes. Soil microbial gene expression seemed to be more resilient to the stress caused by neonicotinoid application than its taxonomic composition, based on a comparison with the results from the two previous chapters. We also observed that time was a strong driver of variation in soil microbial gene expression. Neonicotinoid seed treatment induced no significant shift in expression of the genes related to biodegradation of neonicotinoids or no decline in the expression of the genes associated with nitrification. However, the expression of some genes associated with heat shock protein, regulatory functions and metabolic processes decreased while the expression of the genes related to photosynthesis and DNA repair increased at different time points in response to neonicotinoid seed treatment.

4.4 Limits and challenges

Although sequencing amplicons of rRNA gene and metatranscriptomics provide estimates of the relative abundance of different microbial taxa and genes in a given sample, they do not deliver any information on how the total amounts of microbial taxa and genes vary across samples. Using quantitative PCR, also known as qPCR, or synthetic spike-in standards (Tkacz et al., 2018) to quantify the absolute abundance of taxa and genes may offer complementary information to better understand responses of microbial communities to neonicotinoid exposure, especially if the total microbial biomass is significantly different between control and treated samples. Since we have not measured microbial biomass and cell counts, we do not have that information. This is a common shortcoming in studies using sequencing to quantify microbial communities, and moving forward, it will be important to try to quantify how both the absolute and relative abundance of taxa and genes vary across communities. Additionally, our results are restricted by limitations in the reference databases used to identify taxa and genes. To date, a vast majority of the phyllosphere and soil 16S rRNA genes cannot be annotated to lower taxonomic levels than phylum by the available reference databases. For gene annotations, in particular, there is no standard method of labeling genes in most of the reliable public genome databases, making it difficult to annotate community-wide gene expression in transcriptomic and metatranscriptomic studies such as ours. Using multiple databases and annotated data cleaning were some of the ways that helped us to address these limitations. Nevertheless, data curation practices prior to annotation are one of the challenges to improving annotation results in the future. And finally, it is important to note that there are many metatranscriptomic analysis pipelines with different approaches, making it challenging to select the proper, efficient and accurate approach to use when analyzing these types of data. While we used an analysis pipeline and methodology that is the de facto standard in the field, it should be acknowledged that different methodological approaches could give slightly different answers. As for the reference databases, it will be important in the future to evaluate the impact of analysis and annotation methods on the results of these types of analyses.

Moreover, our soil samples have been collected from bulk soil at only 10 cm from the plants to make sure that soil was affected by neonicotinoids and root exudates. However, since we aimed to sample the same plants and the soil around them several times during the growing season, we were unable to quantify the rhizosphere communities. We are aware of the fact that since neonicotinoids are systemic pesticides and spread through the plant system and into the roots, they might affect the rhizosphere microbial communities more than those in bulk soil due to the higher concentration of neonicotinoids in the soil close to the root. A future study to address this question is required.

A final important caveat to mention is the fact that we did not measure neonicotinoid concentration in the soil samples, nor did we directly measure indicators of agroecosystem function such as crop yield and health. With respect to neonicotinoid concentrations, our study was designed in a way that minimized the possibility of contaminating the control blocks with neonicotinoids. However, we did not quantify the homogeneity of neonicotinoid concentrations across the treated samples. Given the fact that neonicotinoids are used and degraded in soil, their concentration varies over time and space (among samples). Thus, at the beginning of the growing season, the concentration of neonicotinoids was higher, whereas towards the end of the season, probably decreased, and the residuals of neonicotinoid and degradation products increased. These products may also accumulate in the soil over the years. This may partially explain the effects of time on the microbial taxonomic composition and gene expression variation and should be tested in further studies.

Similarly, for direct measures of agroecosystem productivity and function, while it was beyond the scope of this study, it would be interesting in future studies to directly measure the effects of neonicotinoid application simultaneously for both measures of microbial community structure and function as well as ecosystem-level measures of function and host plants and agroecosystem health and productivity, in order to identify the potential mechanisms of neonicotinoid impacts on microbes and crop plants.

4.5 Future directions

Further studies on this subject should aim to the following objectives:

1. Examining the interactions among bacterial communities and their predators from higher trophic groups, including nematodes and arthropods, to test for the effects of neonicotinoid seed treatment on top-down or bottom-up regulations of these microbial communities; as mentioned above, lab-based studies to test for the impacts of neonicotinoid seed treatment on the behavioral interactions among mock bacterial communities and single species of nematodes may improve our understanding of the mechanisms that drive the variation in microbial community and co-occurrence patterns in response to neonicotinoid seed treatment.

2. Bringing this study design to other study sites with different environmental conditions and soil types and also performing a long-term crop rotation; our

results indicated that time is a relatively strong driver of microbial community variation in a soybean-corn agroecosystem. Besides, time was in interaction with neonicotinoid seed treatment in affecting bacterial community composition and microbial gene expression. Previous studies have also shown the effects of site and time on the variability of microbial communities (Knief et al., 2010; Laforest-Lapointe et al., 2016b; Clairmont et al., 2019; Hannula et al., 2019). Performing this experiment in different sites, as well as a long-term crop rotation will help us to assess the reproducibility of the present findings and evaluate the spatial and long-term temporal changes of microbial communities exposed to neonicotinoids. In addition, in our metatranscriptomic approach, we only had two years of soybean/corn crop rotation, and thus, our model could not test whether a proportion of the soil microbial gene expression variation that was explained by time, was actually affected by host species. Performing a long-term rotation will allow us to investigate the effects of host species and their interactions with neonicotinoid seed treatment on the microbial expressed genes over time.

3. Quantifying the absolute abundance of microbial taxa and expressed genes; an increase in the microbial community relative abundance may not always lead to an increase in their absolute abundance and can change depending on the microbial cell count and biomass variation among samples (Azarbad et al., 2021). As mentioned earlier, we can use qPCR to measure the absolute abundance of microbial taxa and expressed genes. This will provide us with complementary information to better understand the microbial community variation in response to neonicotinoid seed treatment and make our results more applicable and reproducible.

4. Reaping the benefits of advances in sequencing technology and data analysis, such as long-read sequencing and an assembly-based analyzing approach; among various pipelines to process metatranscriptomic data, we applied SAMSA2, a de facto standard pipeline in the field an assembly-free approach., in which we typically align short sequence reads to the reference database. However, long-read sequencing technologies and assembly-based approaches that assemble sequence reads into longer contigs before alignment may improve our knowledge of microbial gene expression variation, especially about large microbial eukaryotic genomes.

5. Performing metabolomics analysis to identify soil microbial metabolites and quantify the effects of neonicotinoid seed treatment on microbial metabolism variation using spectrophotometry technology. This approach will also offer complementary information on microbial functional dynamics and changes in response to pesticide application.

APPENDIX A

SUPPLEMENTARY TABLES - CHAPTER I

Table A.1: Soil physicochemical properties. For each plot, physicochemical properties of soil were measured every year, twice (2016 and 2017) or three times (2018) during the growing seasons.

Plot No.	Sample Type	Treat -ment	pH (w)	pH (Ca Cl2)	pH (SMP)	EC (uS/ cm)	CEC (cmol/ kg)	OM (%)	N (%)	NH_3 -N (mg/ kg)	NO -N (mg/ kg)	K (mg/ kg)	Ca (mg/ kg)	Mn (mg/ kg)	Fe (mg/ kg)	Mg (mg/ kg)	Cu (mg/ kg)	Zn (mg/ kg)	P (mg/ kg)	Al (mg/ kg)	Sand (%)	Silt (%)	Clay (%)
1	Soy June 2016	Neonic	6.40	5.80	7.00	114	12	4.4	0.13	1	16	151	2356	36	277	169	1.5	1.3	54	868	32	36	33
2		Control	6.20	5.65	6.95	122	12	5.3	0.15	2	19	88	2432	14	264	227	1.5	1.4	60	789	23	39	39
3		Neonic	6.55	6.05	7.05	179	13	5.6	0.15	1	12	140	3211	12	274	224	1.7	1.3	54	801	21	37	41
4		Control	6.30	5.70	7.00	115	13	5.5	0.17	2	18	193	2847	15	302	215	1.7	1.3	57	832	21	38	41
5		Neonic	6.35	5.70	7.00	86	13	5.4	0.15	1	9	156	2864	13	323	223	1.6	1.0	46	837	16	43	41
6		Control	6.10	5.65	7.05	102	12	5.6	0.16	4	14	176	2349	14	361	244	1.6	1.2	47	1045	34	29	37
7		Neonic	6.25	5.70	7.10	112	9	4.4	0.14	2	18	135	1864	29	276	144	1.4	1.2	55	964	33	34	33
8		Control	6.25	5.70	7.05	104	12	5.0	0.15	3	12	168	2390	15	392	270	1.7	1.2	44	1044	22	38	41
1	Soy Sep 2016	Neonic	6.35	5.70	7.35	54	8	4.4	0.13	3	5	165	2216	31	228	159	1.5	1.4	53	685	32	37	31
2		Control	6.10	5.55	7.35	56	9	4.5	0.14	3	9	148	2522	13	263	239	1.7	1.4	51	729	21	40	40
3		Neonic	6.15	5.60	7.35	60	9	5.4	0.15	3	7	148	2658	13	299	223	2.1	1.6	54	752	19	40	40
4		Control	6.20	5.60	7.30	59	10	5.1	0.14	4	7	176	2727	14	305	223	1.9	1.7	59	769	22	39	40
5		Neonic	6.25	5.65	7.35	55	9	5.0	0.15	6	6	155	2727	13	330	229	1.7	1.3	52	787	20	40	40
6		Control	6.10	5.50	7.30	55	10	4.9	0.15	3	5	161	2624	11	346	237	1.7	1.5	50	821	19	41	40
7		Neonic	6.10	5.60	7.40	53	7	4.0	0.13	3	4	74	2060	23	199	104	1.1	1.2	47	566	30	37	32
8		Control	6.20	5.60	7.35	56	9	5.8	0.13	4	5	173	2584	13	356	254	1.7	1.3	42	751	19	40	41
1	Corn June 2017	Neonic	5.95	5.50	7.10	177	11	5.0	0.16	8	37	171	2309	12	287	214	1.0	0.8	60	944	28	31	41
2		Control	5.85	5.50	7.10	330	12	5.4	0.18	26	78	226	2672	9	333	257	1.0	0.8	99	1006	29	25	45
3		Neonic	6.05	5.60	7.05	228	12	5.2	0.16	6	41	175	2569	10	300	228	1.1	0.8	71	894	27	29	45
4		Control	6.05	5.65	7.10	258	11	5.0	0.17	16	49	210	2322	10	287	196	1.0	0.9	91	840	24	35	41
5		Neonic	6.00	5.50	7.05	184	12	5.5	0.16	11	34	162	2445	8	329	245	1.0	0.6	46	929	22	34	44
6		Control	5.90	5.50	7.10	232	11	5.1	0.17	10	42	159	2342	9	310	208	1.0	0.8	56	848	24	34	43
7		Neonic	5.90	5.50	7.10	166	10	4.7	0.15	5	32	168	2115	11	270	172	0.9	0.7	63	862	26	34	40
8		Control	6.00	5.60	7.10	227	12	5.3	0.15	8	41	159	2617	8	354	275	1.1	0.7	49	926	23	33	44
1	Corn Sep 2017	Neonic	6.20	5.55	7.10	56	11	4.6	0.16	6	3	136	2391	10	257	215	1.0	0.7	53	884	27	33	40
2		Control	6.15	5.50	7.10	56	11	5.2	0.18	5	3	133	2561	6	263	231	1.0	0.8	49	829	23	32	45
3		Neonic	6.25	5.60	7.10	54	11	4.7	0.16	5	3	145	2540	7	256	225	1.0	0.8	61	813	23	33	44
4		Control	6.30	5.65	7.10	55	11	4.7	0.17	4	3	132	2458	8	288	181	1.0	0.7	70	897	27	33	41

Table A.1 continued from previous page

Plot No.	Sample Type	Treat -ment	pH (w)	pH (Ca Cl2)	pH (SMP)	EC (uS/ cm)	CEC (cmol/ kg)	OM (%)	N (%)	NH_3 -N (mg/ kg)	NO_3 -N (mg/ kg)	K (mg/ kg)	Ca (mg/ kg)	Mn (mg/ kg)	Fe (mg/ kg)	Mg (mg/ kg)	Cu (mg/ kg)	Zn (mg/ kg)	P (mg/ kg)	Al (mg/ kg)	Sand (%)	Silt (%)	Clay (%)
5		Neonic	6.15	5.50	7.10	53	11	5.3	0.17	5	2	137	2467	5	296	240	1.0	0.6	47	844	23	32	45
6		Control	6.25	5.60	7.10	51	11	4.9	0.16	4	2	122	2380	6	273	207	0.9	0.7	49	778	27	29	44
7		Neonic	6.20	5.55	7.10	50	10	4.5	0.16	4	2	121	2090	8	250	167	1.0	0.8	56	790	36	24	40
8		Control	6.25	5.60	7.10	49	11	4.8	0.16	6	1	139	2555	6	319	276	0.9	0.6	62	905	23	33	44
1	Soy May 2018	Neonic	6.05	5.50	7.05	88	11	4.2	0.13	4	5	145	2046	28	234	159	2	1	57	874	29	34	37
2		Control	6.00	5.55	6.95	75	13	4.9	0.15	4	6	146	2492	13	248	237	2	1	44	792	20	34	46
3		Neonic	6.00	5.55	7.00	80	13	5.0	0.16	6	7	173	2519	15	285	229	2	2	60	914	17	36	47
4		Control	6.25	5.80	7.10	85	10	4.3	0.14	4	7	147	2145	27	249	145	2	2	68	857	31	34	35
5		Neonic	5.90	5.40	6.95	84	13	4.9	0.15	5	6	178	2348	13	313	244	2	1	50	924	18	38	44
6		Control	5.95	5.45	6.95	71	12	5.2	0.15	5	5	148	2230	16	300	201	2	1	57	891	20	37	43
7		Neonic	5.95	5.45	7.00	71	11	4.1	0.14	5	5	147	1836	26	255	140	1	1	57	891	24	40	36
8		Control	6.00	5.55	6.95	73	13	4.8	0.15	5	5	154	2417	17	330	268	2	1	46	932	18	37	45
1	Soy July 2018	Neonic	5.95	5.55	6.95	72	11	3.9	0.13	2	13	169	1943	32	240	167	2	1	68	938	30	41	29
2		Control	6.05	5.60	7.05	69	12	4.9	0.14	3	9	123	2467	16	263	239	2	2	45	924	21	36	43
3		Neonic	6.10	5.65	7.05	67	12	4.9	0.15	3	8	158	2478	14	269	228	2	1	54	888	20	35	45
4		Control	6.25	5.80	7.00	82	11	4.3	0.13	2	12	150	2099	26	242	150	2	1	58	851	29	35	36
5		Neonic	6.15	5.45	6.95	58	13	5.3	0.14	2	8	164	2363	11	320	250	2	1	47	974	18	38	44
6		Control	6.05	5.55	6.95	60	12	4.9	0.14	3	6	137	2256	15	293	226	2	1	54	917	21	36	43
7		Neonic	6.00	5.50	7.00	66	11	3.9	0.14	2	10	166	1847	26	269	151	2	1	62	930	30	34	36
8		Control	5.95	5.65	7.00	64	13	4.7	0.14	3	6	140	2415	14	322	269	2	1	46	958	19	37	44
1	Soy Sep 2018	Neonic	6.20	5.50	7.20	45	9	3.6	0.11	3	1	121	2061	35	284	162	3	2	61	948	32	33	35
2		Control	6.40	5.70	7.15	57	11	4.9	0.13	4	1	113	2489	17	301	223	3	2	57	865	23	36	41
3		Neonic	6.20	5.65	7	49	11	5.0	0.15	3	3	141	2530	16	312	215	3	2	70	877	23	36	42
4		Control	6.40	5.75	7.20	49	9	4.3	0.13	3	1	110	2137	24	249	135	3	2	69	770	31	36	33
5		Neonic	6.20	5.55	7.15	53	11	4.6	0.14	3	1	169	2606	16	402	247	3	2	62	1005	26	29	44
6		Control	6.25	5.60	7.20	46	10	4.9	0.13	4	4	135	2404	20	361	235	3	2	60	944	21	38	41
7		Neonic	6.15	5.50	7.25	46	8	3.8	0.12	3	2	136	1831	37	353	151	2	2	69	962	33	31	37
8		Control	6.30	5.65	7.15	43	11	5.1	0.13	4	1	139	2484	15	366	263	3	2	52	947	23	35	43

Table A.2: Correlation between bacterial families relative abundances and their habitats and host species. Envfit analysis of correlations between bacterial families of soybean and corn phyllosphere and soil with an average relative abundance of more than 0.01 and the axes of PCoA ordination (on Bray-Curtis dissimilarities). (✓) and (-) respectively show whether the bacterial family is significantly correlated with the soybean or corn phyllosphere or soil or not. Significance levels for each variable are given by: *** P 0.001; ** P 0.01; * P 0.05; NS: $P \geq 0.05$.

	Phyllosphere		Soil					
Family	Soybean	Corn	Soybean	Corn	Axis1	Axis2	R2	Pr(>r)
67-14	-	-	✓	✓	-0.99149	-0.13021	69.9	0.001***
Beijerinckiaceae	✓	-	-	-	0.75308	-0.65793	74.4	0.001***
Burkholderiaceae	-	✓	-	-	0.76944	0.63872	28.4	0.001***
Chitinophagaceae	-	-	✓	✓	-0.99964	0.02693	73.4	0.001***
Enterobacteriaceae	✓	-	-	-	0.32299	-0.9464	27.4	0.001***
Gaiellaceae	-	-	✓	✓	-0.99002	-0.14092	62.5	0.001***
Gemmatimonadaceae	-	-	✓	✓	-0.98396	0.17841	90.1	0.001***
Haliangiaceae	-	-	✓	✓	-0.95773	0.28768	82.4	0.001***
Hymenobacteraceae	-	✓	-	-	0.8844	0.46673	69.7	0.001***
Kineosporiaceae	✓	-	-	-	0.71447	-0.69967	31.3	0.001***
Microbacteriaceae	-	✓	-	-	0.72647	0.6872	31.4	0.001***
Micromonosporaceae	-	-	✓	✓	-0.99999	-0.00341	66.8	0.001***
Nitrosomonadaceae	-	-	✓	✓	-0.9655	0.26041	82.7	0.001***
Nocardioidaceae	-	-	✓	✓	-0.89403	-0.448	61	0.001***
Pedosphaeraceae	-	-	✓	✓	-0.95167	0.30713	80	0.001***
Pseudomonadaceae	-	-	-	-	0.68831	-0.72542	NS	NS
Pseudonocardiaceae	-	-	✓	✓	-0.95761	-0.28808	66	0.001***
Rhizobiaceae	✓	-	-	-	0.95086	-0.30963	48.2	0.001***
Roseiflexaceae	-	-	✓	✓	-0.99425	0.10712	71	0.001***
SC-I-84	-	-	✓	✓	-0.98672	0.16245	84	0.001***
Solibacteraceae	-	-	✓	✓	-0.98054	0.1963	84.2	0.001***
Sphingomonadaceae	-	✓	-	-	0.88175	0.47172	77.4	0.001***
Spirosomaceae	-	✓	-	-	0.54447	0.83878	58.5	0.001***
Xanthobacteraceae	-	-	✓	✓	-0.98372	0.17972	57	0.001***

Table A.3: Significantly differentially bacterial ASVs of phyllosphere associated with control and neonicotinoid seed treatment. Differential expression analysis on sequence data (DESeq2) detects the bacterial ASVs that are significantly differentially abundant (adjusted $P < 0.05$) between soybean and corn phyllosphere control and neonicotinoid-treated samples in a three-year rotation in L'Acadie. The ASVs with a positive $\log_2$FoldChange are associated with the neonicotinoid-treated samples, while the ones with a negative $\log_2$FoldChange are related to the controls.

ASV	$\log_2$FoldChange	padj	Phylum	Class	Order	Family	Genus
ASV14394	7.974218	0.000446	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter
ASV37099	2.822354	0.000446	Bacteroidetes	Bacteroidia	Cytophagales	Hymenobacteraceae	Hymenobacter
ASV37265	3.845118	0.000446	Bacteroidetes	Bacteroidia	Cytophagales	Hymenobacteraceae	Hymenobacter
ASV26187	-2.271686	0.0044252	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	Belnapia
ASV37399	1.581144	0.0097775	Bacteroidetes	Bacteroidia	Cytophagales	Hymenobacteraceae	Hymenobacter
ASV37336	2.352606	0.0116842	Bacteroidetes	Bacteroidia	Cytophagales	Hymenobacteraceae	Hymenobacter
ASV14254	3.265717	0.0169992	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
ASV14054	-2.464085	0.0197485	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Arsenophonus
ASV14235	4.1213	0.0197485	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
ASV37433	1.868017	0.0197485	Bacteroidetes	Bacteroidia	Cytophagales	Hymenobacteraceae	Hymenobacter
ASV3710	2.413512	0.020783	Actinobacteria	Actinobacteria	Frankiales	Geodermatophilaceae	Blastococcus
ASV37100	3.281765	0.020783	Bacteroidetes	Bacteroidia	Cytophagales	Hymenobacteraceae	Hymenobacter
ASV29983	-2.870354	0.0218608	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Brevundimonas
ASV14188	6.113562	0.023042	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
ASV2289	2.340008	0.0240228	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Nocardioides
ASV14189	4.996532	0.0243181	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
ASV27677	-2.520411	0.0243181	Proteobacteria	Alphaproteobacteria	Azospirillales	Azospirillaceae	Skermanella
ASV37446	1.984539	0.0243181	Bacteroidetes	Bacteroidia	Cytophagales	Hymenobacteraceae	Hymenobacter
ASV37665	2.417444	0.0243181	Bacteroidetes	Bacteroidia	Flavobacteriales	Weeksellaceae	Chryseobacterium
ASV37361	1.718623	0.0271058	Bacteroidetes	Bacteroidia	Cytophagales	Hymenobacteraceae	Hymenobacter
ASV37391	2.29827	0.0271058	Bacteroidetes	Bacteroidia	Cytophagales	Hymenobacteraceae	Hymenobacter
ASV27613	-3.517223	0.0274748	Proteobacteria	Alphaproteobacteria	Azospirillales	Azospirillaceae	Skermanella
ASV2791	-2.821215	0.0316961	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Rathayibacter
ASV14050	-2.135488	0.0346683	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Arsenophonus
ASV14057	-2.198312	0.0346683	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Arsenophonus
ASV37341	1.95928	0.0346683	Bacteroidetes	Bacteroidia	Cytophagales	Hymenobacteraceae	Hymenobacter
ASV14051	-2.236104	0.038565	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Arsenophonus
ASV37117	3.561363	0.038565	Bacteroidetes	Bacteroidia	Cytophagales	Hymenobacteraceae	Hymenobacter
ASV37342	2.09291	0.0411024	Bacteroidetes	Bacteroidia	Cytophagales	Hymenobacteraceae	Hymenobacter
ASV27644	-2.947756	0.0425147	Proteobacteria	Alphaproteobacteria	Azospirillales	Azospirillaceae	Skermanella

Table A.3 continued from previous page

ASV	$\log_2$FoldChange	padj	Phylum	Class	Order	Family	Genus
ASV957	2.698637	0.043203	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	NA
ASV42557	-3.201353	0.043203	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylobacterium
ASV37362	2.169321	0.044052	Bacteroidetes	Bacteroidia	Cytophagales	Hymenobacteraceae	Hymenobacter
ASV13047	-2.912545	0.0469482	Deinococcus-Thermus	Deinococci	Deinococcales	Deinococcaceae	Deinococcus

Table A.4: Significantly differentially soil bacterial ASVs associated with control and neonicotinoid seed treatment. Differential expression analysis on sequence data (DESeq2) identifies the ASVs that are significantly differentially abundant (adjusted $P < 0.05$) between the control and neonicotinoid-treated samples of soybean and corn soil bacteria in a three-year rotation in L'Acadie. The ASVs favored by neonicotinoid-treated samples have a positive $\log_2$FoldChange, while the ASVs associated with control have a negative $\log_2$FoldChange.

ASV	$\log_2$FoldChange	padj	Phylum	Class	Order	Family	Genus
ASV7846	1.9279854	0.0000028	Chloroflexi	Chloroflexia	Chloroflexales	Roseiflexaceae	NA
ASV7118	0.8469585	0.0000034	Chloroflexi	Chloroflexia	Chloroflexales	Roseiflexaceae	NA
ASV8617	0.5961708	0.0000034	Chloroflexi	Dehalococcoidia	S085	NA	NA
ASV5909	-0.7105277	0.0000202	Actinobacteria	Thermoleophilia	Solirubrobacterales	67-14	NA
ASV18787	-1.3319079	0.0000202	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	mle1-7
ASV43935	-1.0629096	0.0000202	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Rhizobacter
ASV30527	-0.8320296	0.0001206	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	NA
ASV19338	-0.9822895	0.0001777	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	SC-I-84	NA
ASV30805	-0.9191404	0.0001777	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	Gemmatimonas
ASV18868	-1.0188547	0.0002163	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	Ellin6067
ASV7608	0.8591203	0.0002445	Chloroflexi	Chloroflexia	Chloroflexales	Roseiflexaceae	NA
ASV30887	-1.154677	0.0002826	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	Gemmatimonas
ASV42805	-1.1268944	0.0002826	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhodomicrobiaceae	Rhodomicrobium
ASV16323	-2.7580422	0.0002991	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae	NA
ASV30317	-0.8389655	0.0002991	Proteobacteria	Deltaproteobacteria	RCP2-54	NA	NA
ASV44431	-1.0498529	0.0002993	Actinobacteria	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae	Solirubrobacter
ASV18845	-1.1388545	0.0004176	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	Ellin6067
ASV44580	-0.7691647	0.0004641	Actinobacteria	Thermoleophilia	Solirubrobacterales	NA	NA
ASV7263	0.9195598	0.000522	Chloroflexi	Chloroflexia	Chloroflexales	Roseiflexaceae	NA
ASV5133	-0.9214405	0.0007116	Actinobacteria	Thermoleophilia	Gaiellales	NA	NA
ASV23233	-0.7030717	0.0007116	Nitrospirae	Nitrospira	Nitrospirales	Nitrospiraceae	Nitrospira
ASV27613	-0.70512	0.0007156	Proteobacteria	Alphaproteobacteria	Azospirillales	Azospirillaceae	Skermanella
ASV19267	-2.2206159	0.0008282	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	A21b	NA
ASV7271	0.6149805	0.0008636	Chloroflexi	Chloroflexia	Chloroflexales	Roseiflexaceae	NA
ASV35384	-0.5894458	0.0008795	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	NA
ASV7734	0.6648916	0.0009304	Chloroflexi	Chloroflexia	Chloroflexales	Roseiflexaceae	Kouleothrix
ASV18893	-1.002218	0.0009304	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	Ellin6067
ASV21906	-1.6659302	0.0009304	Proteobacteria	Deltaproteobacteria	Myxococcales	Haliangiaceae	Haliangium
ASV31015	-0.6637544	0.0009304	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	NA
ASV4759	-0.6833093	0.0009898	Actinobacteria	Acidimicrobiia	Microtrichales	Ilumatobacteraceae	Ilumatobacter
ASV19592	-1.9174724	0.0009898	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	Geobacter
ASV43108	-0.4739943	0.0009898	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	NA

Table A.4 continued from previous page

ASV	$\log_2$FoldChange	padj	Phylum	Class	Order	Family	Genus
ASV16560	-0.633763	0.0010182	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	SC-I-84	NA
ASV18954	-0.9807376	0.0010182	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	Ellin6067
ASV17953	-0.9579936	0.0010919	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Ramlibacter
ASV3402	1.8934102	0.0012221	Actinobacteria	Actinobacteria	Streptosporangiales	Thermomonosporaceae	Actinomadura
ASV4499	-0.6041671	0.0012221	Actinobacteria	Acidimicrobiia	IMCC26256	NA	NA
ASV34389	-0.9018388	0.0012221	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Terrimonas
ASV7479	0.4775328	0.0014114	Chloroflexi	Chloroflexia	Chloroflexales	Roseiflexaceae	NA
ASV17790	-1.2926162	0.0014114	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium
ASV27988	-0.6428812	0.0014114	Proteobacteria	Alphaproteobacteria	Reyranellales	Reyranellaceae	Reyranella
ASV30768	-0.5880331	0.0014114	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	Gemmatimonas
ASV31192	-0.6055414	0.0014114	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	NA
ASV43384	-0.4373139	0.0014114	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Bradyrhizobium
ASV16481	-0.6190261	0.0015296	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	SC-I-84	NA
ASV7570	1.4105565	0.0019467	Chloroflexi	Chloroflexia	Chloroflexales	Roseiflexaceae	NA
ASV2930	2.1451653	0.0019962	Actinobacteria	Actinobacteria	Frankiales	Nakamurellaceae	Nakamurella
ASV3966	-0.5671525	0.0019962	Actinobacteria	Acidimicrobiia	IMCC26256	NA	NA
ASV19303	-1.4127104	0.0020647	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	SC-I-84	NA
ASV29925	-1.1562574	0.0020647	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter
ASV43386	-0.4582436	0.0021407	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Bradyrhizobium
ASV37017	1.7669239	0.0021444	Bacteroidetes	Bacteroidia	Cytophagales	Hymenobacteraceae	Adhaeribacter
ASV17923	-1.3136793	0.0021767	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Caenimonas
ASV2639	0.543634	0.0021939	Actinobacteria	Actinobacteria	Micrococcales	Intrasporangiaceae	Oryzihumus
ASV2920	-0.9537776	0.0021939	Actinobacteria	Actinobacteria	Frankiales	Nakamurellaceae	Nakamurella
ASV43518	-0.626037	0.0021939	Proteobacteria	Alphaproteobacteria	Micropepsales	Micropepsaceae	NA
ASV34369	-1.3505896	0.0022747	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	NA
ASV7737	1.0797683	0.0023362	Chloroflexi	Chloroflexia	Chloroflexales	Roseiflexaceae	Kouleothrix
ASV7260	1.4128166	0.0023587	Chloroflexi	Chloroflexia	Chloroflexales	Roseiflexaceae	NA
ASV18672	-0.7005071	0.0024834	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	TRA3-20	NA
ASV18146	-0.7828176	0.00275	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	MND1
ASV43936	-0.5977359	0.0030158	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Rhizobacter
ASV18611	-1.0362236	0.0030537	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	TRA3-20	NA
ASV30566	-0.7096901	0.0033906	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	NA
ASV5020	1.0902994	0.003501	Actinobacteria	Thermoleophilia	Gaiellales	NA	NA
ASV7056	0.9757383	0.003501	Chloroflexi	Chloroflexia	Chloroflexales	Roseiflexaceae	NA
ASV4154	-0.8982351	0.003601	Actinobacteria	Acidimicrobiia	Microtrichales	NA	NA
ASV7477	0.4088168	0.003601	Chloroflexi	Chloroflexia	Chloroflexales	Roseiflexaceae	NA
ASV7845	1.7189076	0.003601	Chloroflexi	Chloroflexia	Chloroflexales	Roseiflexaceae	NA
ASV15619	-0.614466	0.003601	Proteobacteria	Gammaproteobacteria	Steroidobacterales	Steroidobacteraceae	NA
ASV16025	-0.684704	0.003601	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Luteimonas

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ASV	$\log_2$FoldChange	padj	Phylum	Class	Order	Family	Genus
ASV18165	-1.4609913	0.003601	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	MND1
ASV42674	-0.524047	0.003601	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	NA
ASV26187	-0.5979628	0.0037119	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	Belnapia
ASV35593	-1.6348605	0.0037758	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	NA
ASV31637	2.35155	0.0037821	Gemmatimonadetes	Longimicrobia	Longimicrobiales	Longimicrobiaceae	NA
ASV6868	0.8431019	0.0037825	Chloroflexi	Chloroflexia	Chloroflexales	Roseiflexaceae	NA
ASV15677	-0.8362779	0.0038071	Proteobacteria	Gammaproteobacteria	R7C24	NA	NA
ASV20111	-0.9528636	0.0038071	Proteobacteria	Deltaproteobacteria	Myxococcales	Archangiaceae	Anaeromyxobacter
ASV16644	-0.6296734	0.0038508	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	A21b	NA
ASV16325	-3.533444	0.003901	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae	NA
ASV2084	0.674374	0.0040789	Actinobacteria	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium
ASV7125	0.687073	0.0040789	Chloroflexi	Chloroflexia	Chloroflexales	Roseiflexaceae	NA
ASV16027	-0.8206238	0.0040789	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	NA
ASV19318	-0.8880577	0.0040789	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	SC-I-84	NA
ASV18941	-0.9951912	0.004219	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	Ellin6067
ASV42958	-0.5570384	0.0042523	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Microvirga
ASV16435	-0.7658768	0.0044551	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	SC-I-84	NA
ASV31086	-0.4443656	0.0044551	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	Gemmatirosa
ASV16529	-2.1261679	0.004588	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	SC-I-84	NA
ASV42451	-0.9210085	0.004588	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiales_Incertae_Sedis	Nordella
ASV40992	-2.671908	0.0048093	Acidobacteria	Acidobacteriia	Acidobacteriales	NA	NA
ASV5971	-0.9614432	0.005033	Actinobacteria	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae	Solirubrobacter
ASV15454	-0.5930685	0.0052239	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria_Incertae_Sedis	Unknown_Family	Acidibacter
ASV18382	-0.5155498	0.0052239	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	Nitrosospira
ASV19041	-0.4660333	0.0052239	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Variovorax
ASV31254	-0.8545226	0.0052239	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	Gemmatimonas
ASV40081	-0.7771796	0.0052239	Acidobacteria	Acidobacteriia	Solibacterales	Solibacteraceae_(Subgroup_3)	Bryobacter
ASV2728	1.1248958	0.0060062	Actinobacteria	Actinobacteria	Micrococcales	Intrasporangiaceae	NA
ASV31406	-2.5643809	0.0060062	Gemmatimonadetes	Longimicrobia	Longimicrobiales	Longimicrobiaceae	NA
ASV19247	-0.7219092	0.0070382	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	A21b	NA
ASV30827	-1.6529282	0.0070382	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	Gemmatimonas
ASV18904	-0.4995517	0.0070505	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	Ellin6067
ASV31407	0.9917109	0.0070587	Gemmatimonadetes	Longimicrobia	Longimicrobiales	Longimicrobiaceae	NA
ASV1140	0.4411517	0.007445	Actinobacteria	Actinobacteria	Micromonosporales	Micromonosporaceae	NA
ASV31291	-0.6469905	0.0076597	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	Gemmatimonas
ASV19245	-1.069685	0.00769	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	A21b	NA
ASV31082	-0.7090507	0.0077189	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	Gemmatirosa
ASV16271	-0.6168231	0.007943	Proteobacteria	Gammaproteobacteria	Xanthomonadales	NA	NA
ASV7268	0.8716291	0.0080253	Chloroflexi	Chloroflexia	Chloroflexales	Roseiflexaceae	NA

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ASV	$\log_2$FoldChange	padj	Phylum	Class	Order	Family	Genus
ASV19243	-3.1655079	0.0080899	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	A21b	NA
ASV9891	0.8909807	0.0080978	Chloroflexi	NA	NA	NA	NA
ASV232	0.4396171	0.0085176	Actinobacteria	MB-A2-108	NA	NA	NA
ASV5751	-0.5520799	0.0088405	Actinobacteria	Thermoleophilia	Gaiellales	NA	NA
ASV39762	-0.7215286	0.0092084	Acidobacteria	Acidobacteriia	Solibacterales	Solibacteraceae_(Subgroup_3)	Candidatus_Solibacter
ASV461	0.8468281	0.0092223	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces
ASV15439	-0.591001	0.0092223	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria_Incertae_Sedis	Unknown_Family	Acidibacter
ASV17948	-0.5101252	0.0092223	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Ramlibacter
ASV30649	-0.6305458	0.0092223	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	Gemmatimonas
ASV41030	-1.7001549	0.0092223	Acidobacteria	Acidobacteriia	Acidobacteriales	Koribacteraceae	Candidatus_Koribacter
ASV42134	-0.9047029	0.0092223	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Hyphomicrobium
ASV44107	-0.4424004	0.0092223	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	TRA3-20	NA
ASV43940	-0.6199706	0.0096213	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Rhizobacter
ASV7495	-0.8881809	0.0096553	Chloroflexi	Chloroflexia	Chloroflexales	Roseiflexaceae	NA
ASV253	1.3129282	0.0101679	Actinobacteria	MB-A2-108	NA	NA	NA
ASV2147	-0.4824588	0.0101679	Actinobacteria	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Pseudonocardia
ASV3757	-1.1821069	0.0101679	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	NA
ASV8761	1.0010639	0.0101679	Chloroflexi	Dehalococcoidia	NA	NA	NA
ASV16375	-0.5523888	0.01025	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	SC-I-84	NA
ASV29330	-0.6655122	0.0113633	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Phenylobacterium
ASV16398	-1.5761918	0.0113703	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	SC-I-84	NA
ASV25934	-0.6260186	0.0113703	Proteobacteria	Alphaproteobacteria	Tistrellales	Geminicoccaceae	Candidatus_Alysiosphaera
ASV30807	-1.0530348	0.0113703	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	Gemmatimonas
ASV40236	-1.7970539	0.0115071	Acidobacteria	Acidobacteriia	Solibacterales	Solibacteraceae_(Subgroup_3)	Candidatus_Solibacter
ASV7376	1.8633056	0.0115274	Chloroflexi	Chloroflexia	Chloroflexales	Roseiflexaceae	NA
ASV42180	-0.332887	0.0124192	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methyloligellaceae	NA
ASV3500	0.568403	0.0124257	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Kribbella
ASV19317	-0.5326758	0.0124257	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	SC-I-84	NA
ASV7089	0.8954073	0.0127579	Chloroflexi	Chloroflexia	Chloroflexales	Roseiflexaceae	NA
ASV3200	1.3127207	0.0131218	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Arthrobacter
ASV3490	1.6990841	0.0131218	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Aeromicrobium
ASV16512	-0.3988206	0.0131218	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	SC-I-84	NA
ASV35122	-0.4176566	0.013202	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Terrimonas
ASV43877	-1.1842157	0.013202	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	NA
ASV43922	-0.5252877	0.0134096	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Rhizobacter
ASV18127	-0.7495279	0.0134103	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	MND1
ASV39757	-1.4759387	0.0134103	Acidobacteria	Acidobacteriia	Solibacterales	Solibacteraceae_(Subgroup_3)	Candidatus_Solibacter
ASV19581	-1.6515167	0.0135374	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	Geobacter
ASV22267	-1.4243639	0.0135983	Proteobacteria	Deltaproteobacteria	Myxococcales	Haliangiaceae	Haliangium

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ASV	$\log_2$FoldChange	padj	Phylum	Class	Order	Family	Genus
ASV31577	-1.0075762	0.0137864	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	NA
ASV6551	-1.5973998	0.0138339	Chloroflexi	Chloroflexia	Kallotenuales	AKIW781	NA
ASV6078	-0.4714848	0.0145636	Actinobacteria	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae	Solirubrobacter
ASV19321	-0.4917102	0.0145636	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	SC-I-84	NA
ASV2921	-1.122458	0.0146859	Actinobacteria	Actinobacteria	Frankiales	Nakamurellaceae	Nakamurella
ASV43966	-1.3009366	0.0151819	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	NA
ASV41031	-1.7098909	0.0153571	Acidobacteria	Acidobacteriia	Acidobacteriales	Koribacteraceae	Candidatus_Koribacter
ASV43210	-1.3210601	0.0153759	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiales_Incertae_Sedis	NA
ASV44379	-1.7315786	0.0160967	Actinobacteria	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae	JCM_18997
ASV7221	0.8096563	0.0171156	Chloroflexi	Chloroflexia	Chloroflexales	Roseiflexaceae	NA
ASV16381	-0.5691455	0.0171368	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	SC-I-84	NA
ASV40187	-0.5061316	0.0171368	Acidobacteria	Acidobacteriia	Solibacterales	Solibacteraceae_(Subgroup_3)	Candidatus_Solibacter
ASV16371	-0.7671839	0.0174392	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	SC-I-84	NA
ASV43809	-0.6156779	0.0174449	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Rhizobacter
ASV6094	-0.6066984	0.0180139	Actinobacteria	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae	Solirubrobacter
ASV44536	0.7773039	0.0180139	Actinobacteria	Thermoleophilia	Solirubrobacterales	67-14	NA
ASV20367	-0.3922474	0.0183112	Proteobacteria	Deltaproteobacteria	Myxococcales	Haliangiaceae	Haliangium
ASV42807	-1.3319222	0.0183112	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhodomicrobiaceae	Rhodomicrobium
ASV5260	0.6197685	0.0183573	Actinobacteria	Thermoleophilia	Gaiellales	NA	NA
ASV19794	-0.69347	0.0188322	Proteobacteria	Deltaproteobacteria	Myxococcales	Archangiaceae	NA
ASV35059	-0.5863952	0.0188575	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Terrimonas
ASV4351	0.4008307	0.0189641	Actinobacteria	Acidimicrobiia	IMCC26256	NA	NA
ASV7267	0.6202044	0.0189641	Chloroflexi	Chloroflexia	Chloroflexales	Roseiflexaceae	NA
ASV19334	-0.9973131	0.0189641	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	SC-I-84	NA
ASV6092	-1.3027852	0.0191647	Actinobacteria	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae	Solirubrobacter
ASV16439	-1.734343	0.0191647	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	SC-I-84	NA
ASV30828	-0.973356	0.0194374	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	Gemmatimonas
ASV34279	-0.508531	0.0205601	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	NA
ASV30292	-1.8482108	0.0211972	Proteobacteria	Deltaproteobacteria	NA	NA	NA
ASV21382	-1.425179	0.0212658	Proteobacteria	Deltaproteobacteria	Myxococcales	BIrii41	NA
ASV31190	-0.4769484	0.021274	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	NA
ASV6577	0.7552293	0.0216783	Chloroflexi	Chloroflexia	Chloroflexales	Roseiflexaceae	NA
ASV31264	-1.205769	0.0218154	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	Gemmatimonas
ASV7122	0.5825808	0.0220023	Chloroflexi	Chloroflexia	Chloroflexales	Roseiflexaceae	NA
ASV20496	-1.3819331	0.0223118	Proteobacteria	Deltaproteobacteria	Myxococcales	Sandaracinaceae	NA
ASV30793	-0.6480005	0.0223118	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	Gemmatimonas
ASV43944	-1.0066914	0.0223118	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Rhizobacter
ASV35472	-0.4466211	0.0225526	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Terrimonas
ASV8691	2.2455876	0.0226002	Chloroflexi	JG30-KF-CM66	NA	NA	NA

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ASV	$\log_2$FoldChange	padj	Phylum	Class	Order	Family	Genus
ASV4167	-1.1620396	0.0227638	Actinobacteria	Acidimicrobiia	Microtrichales	NA	NA
ASV5601	0.9771232	0.0227779	Actinobacteria	Thermoleophilia	Gaiellales	Gaiellaceae	Gaiella
ASV40460	-1.261591	0.0228197	Acidobacteria	Subgroup_5	NA	NA	NA
ASV30263	-1.6083061	0.023026	Proteobacteria	Deltaproteobacteria	Desulfarculales	Desulfarculaceae	NA
ASV43204	-1.0766532	0.023026	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiales_Incertae_Sedis	NA
ASV40263	-0.7410344	0.0234455	Acidobacteria	Acidobacteriia	Solibacterales	Solibacteraceae_(Subgroup_3)	Candidatus_Solibacter
ASV23759	-0.3699475	0.0253341	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus
ASV28189	-1.6326048	0.0253341	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Altererythrobacter
ASV194	0.5049167	0.0257881	Actinobacteria	MB-A2-108	NA	NA	NA
ASV6158	-0.5430434	0.0257881	Actinobacteria	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae	Conexibacter
ASV9967	0.5856591	0.0258361	Chloroflexi	NA	NA	NA	NA
ASV24220	-1.1675969	0.0262193	Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Ammoniphilus
ASV5978	-1.576733	0.0263413	Actinobacteria	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae	Conexibacter
ASV19492	-1.4339565	0.0266375	Proteobacteria	Deltaproteobacteria	Myxococcales	Archangiaceae	Anaeromyxobacter
ASV38899	-0.713278	0.0267252	Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	NA
ASV5701	0.6388871	0.027096	Actinobacteria	Thermoleophilia	Gaiellales	Gaiellaceae	Gaiella
ASV1	0.5161262	0.027111	Actinobacteria	Thermoleophilia	Solirubrobacterales	67-14	NA
ASV8572	1.5327471	0.0271573	Chloroflexi	Dehalococcoidia	S085	NA	NA
ASV30307	-1.0489793	0.0271573	Proteobacteria	Deltaproteobacteria	RCP2-54	NA	NA
ASV2587	1.0486994	0.0274535	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Marmoricola
ASV7493	-0.5796902	0.0274535	Chloroflexi	Chloroflexia	Chloroflexales	Roseiflexaceae	NA
ASV16048	-0.6661718	0.0276304	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Arenimonas
ASV16026	-1.0845037	0.0277761	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	NA
ASV15455	-0.4054137	0.0282508	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria_Incertae_Sedis	Unknown_Family	Acidibacter
ASV4463	-0.5324723	0.0285746	Actinobacteria	Acidimicrobiia	IMCC26256	NA	NA
ASV16278	-2.7280057	0.0287275	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae	Rhodanobacter
ASV40994	-1.5475115	0.0287275	Acidobacteria	Acidobacteriia	Acidobacteriales	NA	NA
ASV15526	-0.7366809	0.028891	Proteobacteria	Gammaproteobacteria	JG36-GS-52	NA	NA
ASV20108	-1.2920748	0.028891	Proteobacteria	Deltaproteobacteria	Myxococcales	Archangiaceae	Anaeromyxobacter
ASV43033	-0.8420775	0.028891	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Microvirga
ASV5580	0.5857632	0.0295572	Actinobacteria	Thermoleophilia	Gaiellales	Gaiellaceae	Gaiella
ASV42185	-0.7824108	0.0298693	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methyloligellaceae	NA
ASV18930	-1.0375639	0.030027	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	Ellin6067
ASV18003	-1.3940098	0.0300776	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	B1-7BS	NA
ASV19046	-0.6967218	0.0300776	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Variovorax
ASV38748	-0.6338758	0.0304433	Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	NA
ASV16028	-0.8718116	0.0306285	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Luteimonas
ASV27644	-0.4523866	0.0306285	Proteobacteria	Alphaproteobacteria	Azospirillales	Azospirillaceae	Skermanella
ASV31059	-0.4041148	0.0306285	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	NA

Table A.4 continued from previous page

ASV	$\log_2$FoldChange	padj	Phylum	Class	Order	Family	Genus
ASV31457	-0.574117	0.0309799	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	NA
ASV30600	-0.5781107	0.0310849	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	NA
ASV43520	-1.105336	0.0311566	Proteobacteria	Alphaproteobacteria	Micropepsales	Micropepsaceae	NA
ASV8208	0.8439832	0.0314819	Chloroflexi	Chloroflexia	Chloroflexales	NA	NA
ASV19420	-1.7945426	0.0314819	Proteobacteria	Deltaproteobacteria	NB1-j	NA	NA
ASV42743	-0.6514491	0.0322319	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Rhodoplanes
ASV345	0.5004677	0.032955	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces
ASV16436	-0.9968626	0.0332258	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	SC-I-84	NA
ASV43054	-0.9760196	0.0332258	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Bosea
ASV44014	-0.7377056	0.0332258	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Ramlibacter
ASV23231	-0.3642303	0.0332817	Nitrospirae	Nitrospira	Nitrospirales	Nitrospiraceae	Nitrospira
ASV18927	-1.8874642	0.0336362	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	Ellin6067
ASV19594	-2.0897251	0.0336362	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	Geobacter
ASV16142	-0.7320484	0.0340236	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Arenimonas
ASV38794	-0.4666638	0.0344197	Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	NA
ASV17927	-1.4779836	0.0352286	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Caenimonas
ASV17942	-1.053179	0.0352286	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	NA
ASV43340	-0.3793339	0.0355144	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	NA
ASV18884	-0.7303106	0.0369553	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	Ellin6067
ASV42327	-0.6069698	0.0369773	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Mesorhizobium
ASV6765	0.8495826	0.0370715	Chloroflexi	Chloroflexia	Chloroflexales	Roseiflexaceae	NA
ASV16377	-0.8930254	0.0370715	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	SC-I-84	NA
ASV18807	-0.480288	0.0370715	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	mle1-7
ASV18867	-0.9512489	0.0370715	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	Ellin6067
ASV7373	1.0288747	0.0373363	Chloroflexi	Chloroflexia	Chloroflexales	Roseiflexaceae	NA
ASV16050	-1.0861478	0.0373363	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Arenimonas
ASV42938	-0.5663315	0.0373363	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	NA
ASV19194	-2.0522743	0.0383212	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	IS-44
ASV162	0.6524847	0.0383332	Actinobacteria	MB-A2-108	NA	NA	NA
ASV12394	1.7285998	0.0390251	Patescibacteria	Saccharimonadia	Saccharimonadales	NA	NA
ASV2105	0.518267	0.0393369	Actinobacteria	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium
ASV5666	0.40661	0.0393369	Actinobacteria	Thermoleophilia	Gaiellales	Gaiellaceae	Gaiella
ASV6865	0.5474675	0.0393369	Chloroflexi	Chloroflexia	Chloroflexales	Roseiflexaceae	NA
ASV19244	-1.0320388	0.0393369	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	A21b	NA
ASV17781	-1.2035549	0.0395459	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium
ASV4720	-0.484697	0.0402839	Actinobacteria	Acidimicrobiia	NA	NA	NA
ASV15512	-0.5488675	0.0402922	Proteobacteria	Gammaproteobacteria	Steroidobacterales	Steroidobacteraceae	NA
ASV42102	-0.5707068	0.0409534	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Pedomicrobium
ASV18892	-0.9905316	0.0414049	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	Ellin6067

Table A.4 continued from previous page

ASV	$\log_2$FoldChange	padj	Phylum	Class	Order	Family	Genus
ASV42770	-0.4385148	0.0414049	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	NA
ASV31250	-1.1854088	0.0419866	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	Gemmatimonas
ASV33692	-0.6321345	0.0420214	Bacteroidetes	Bacteroidia	Cytophagales	Microscillaceae	Chryseolinea
ASV4749	-0.7269717	0.0420576	Actinobacteria	Acidimicrobiia	Microtrichales	Ilumatobacteraceae	Ilumatobacter
ASV2046	0.4022094	0.0426562	Actinobacteria	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium
ASV8040	0.7792856	0.0426562	Chloroflexi	Chloroflexia	Chloroflexales	Roseiflexaceae	NA
ASV19322	-0.9335891	0.0426562	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	SC-I-84	NA
ASV30838	-1.3406199	0.0426562	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	Gemmatimonas
ASV21198	-0.7161957	0.0426909	Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae	Sorangium
ASV3177	0.3486931	0.0432709	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Arthrobacter
ASV18980	-1.2941312	0.0432709	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	Ellin6067
ASV43356	-1.2695101	0.04357	Proteobacteria	Alphaproteobacteria	Rhizobiales	NA	NA
ASV15691	-0.8329453	0.0438783	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria_Incertae_Sedis	Unknown_Family	Acidibacter
ASV2247	0.7796086	0.0440844	Actinobacteria	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Pseudonocardia
ASV38222	-1.8799483	0.0449537	Spirochaetes	Leptospirae	Leptospirales	Leptospiraceae	Turneriella
ASV5958	-0.4484621	0.0461926	Actinobacteria	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae	Solirubrobacter
ASV6059	-1.1085553	0.0463494	Actinobacteria	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae	Solirubrobacter
ASV4464	-0.856699	0.04754	Actinobacteria	Acidimicrobiia	IMCC26256	NA	NA
ASV42140	-0.4855706	0.0481948	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Hyphomicrobium
ASV34784	-0.6239146	0.0484522	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Flavisolibacter
ASV18953	-0.9760763	0.0488871	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	Ellin6067
ASV5570	0.5484497	0.0489743	Actinobacteria	Thermoleophilia	Gaiellales	Gaiellaceae	Gaiella
ASV31193	-0.8038947	0.0489965	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	NA
ASV19287	-0.6469693	0.049109	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	SC-I-84	NA
ASV5454	0.5259828	0.0491468	Actinobacteria	Thermoleophilia	Gaiellales	NA	NA
ASV26186	-0.7851829	0.0491468	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	Belnapia
ASV30528	-0.8355488	0.0491468	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	NA
ASV35125	-0.6742211	0.0498122	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Terrimonas

APPENDIX B

SUPPLEMENTARY TABLES - CHAPTER II

Table B.1: Bacterial families and their respective phyla with the highest values of network node metrics in control and neonicotinoid-treated soil samples in a three-year soybean/corn rotation in l'Acadie, Quebec, Canada.

Metric	Treatment	Average value	Maximum value	Node with maximum value			
				Phylum	Family	Z-score	P-value
Betweenness centrality	Control	189.6	1974	Acidobacteria	Solibacteraceae	25.71	< 0.05
	Neonic	31.42	216.2	Proteobacteria	Sphingomonadaceae	5.45	< 0.05
Coreness	Control	3.39	8	Actinobacteria	Mycobacteriaceae	5.86	< 0.05
	Neonic	7.72	15	Actinobacteria	67-14	12.27	< 0.05
	Control	3.39	8			5.68	< 0.05
	Neonic	7.72	15	Actinobacteria	Gaiellaceae	11.55	< 0.05
	Control	3.39	8			5.94	< 0.05
	Neonic	7.72	15	Actinobacteria	Micromonosporaceae	11.98	< 0.05
	Control	3.39	8			5.9	< 0.05
	Neonic	7.72	15	Actinobacteria	Nocardioidaceae	11.94	< 0.05
	Control	3.39	8			6.35	< 0.05
	Neonic	7.72	15	Actinobacteria	Intrasporangiaceae	11.36	< 0.05
	Control	3.39	8			5.63	< 0.05
	Neonic	7.72	15	Actinobacteria	Pseudonocardiaceae	11.57	< 0.05
	Control	3.39	8			6.1	< 0.05
	Neonic	7.72	15	Actinobacteria	Geodermatophilaceae	10.64	< 0.05
	Control	3.39	8			5.73	< 0.05
	Neonic	7.72	15	Actinobacteria	Nakamurellaceae	10.7	< 0.05
	Control	3.39	8			5.75	< 0.05
	Neonic	7.72	15	Actinobacteria	Streptomycetaceae	11.29	< 0.05
	Control	3.39	8			6.01	< 0.05
	Neonic	7.72	15	Actinobacteria	Micrococcaceae	12.44	< 0.05
	Control	3.39	8			5.74	< 0.05
	Neonic	7.72	15	Actinobacteria	Thermomonosporaceae	10.44	< 0.05
	Neonic	7.72	15			11.84	< 0.05
	Neonic	7.72	15	Gemmatimonadetes	Longimicrobiaceae	10.8	< 0.05
	Neonic	7.72	15	Chloroflexi	Ktedonobacteraceae	11.97	< 0.05
	Neonic	7.72	15	Actinobacteria	Kineosporiaceae	10.65	< 0.05
	Neonic	7.72	15	Actinobacteria	Microbacteriacea	10.67	< 0.05
	Neonic	7.72	15	Bacteroidetes	Saprospiraceae	11.83	< 0.05
	Neonic	7.72	15	Actinobacteria	Nocardiaceae	10.48	< 0.05
	Neonic	7.72	15	Actinobacteria	Iamiaceae	10.59	< 0.05
	Neonic	7.72	15	Actinobacteria	Cellulomonadaceae	11.22	< 0.05

Table B.1 continued from previous page

Metric	Treatment	Average value	Maximum value	Node with maximum value			
				Phylum	Family	Z-score	P-value
	Neonic	7.72	15	Bacteroidetes	Hymenobacteraceae	10.33	< 0.05

APPENDIX C

SUPPLEMENTARY FIGURES - CHAPTER II

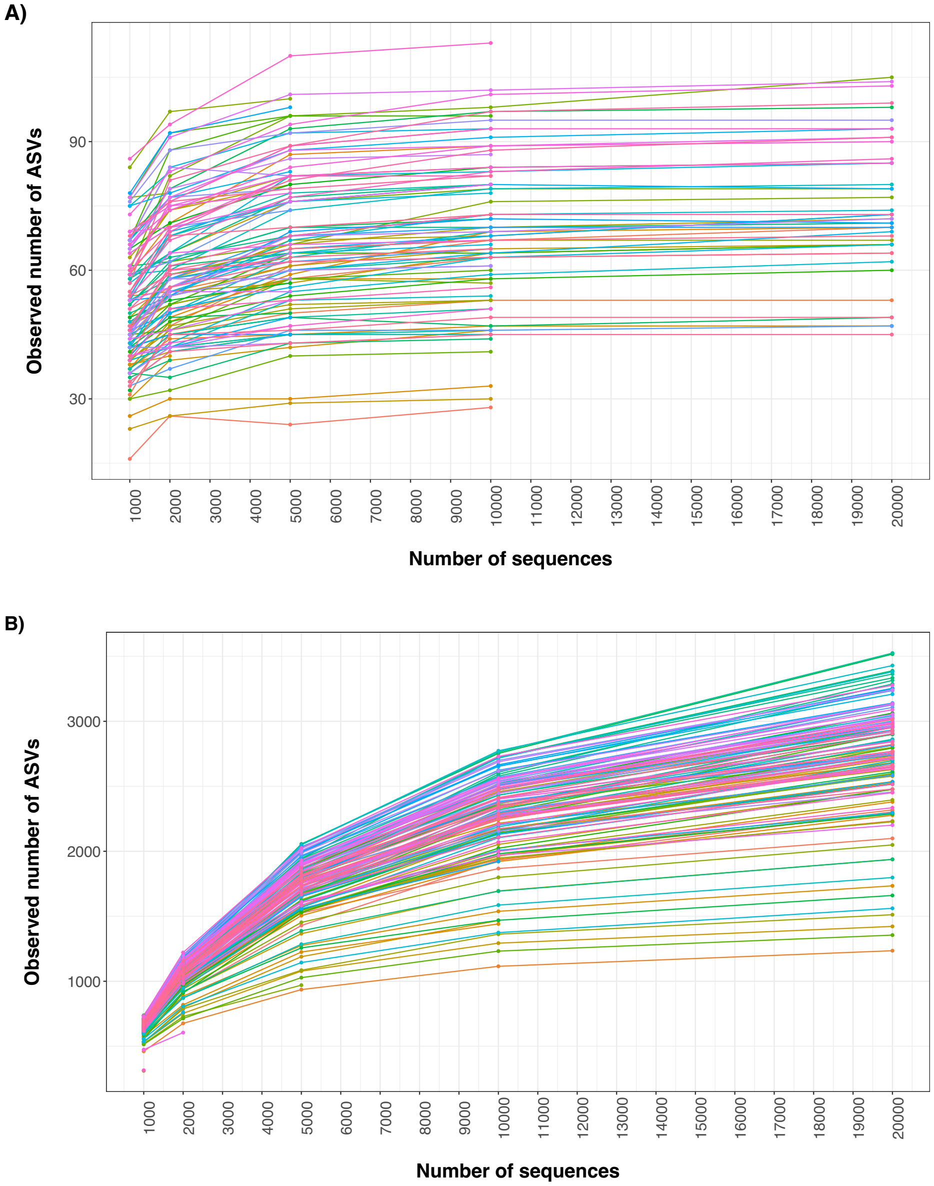

Figure C.1: Rarefaction curves of the soil microbial ASVs. Rarefaction curves for all microbial nematode (A) and bacterial (B) communities according to the observed ASVs richness in soil samples of a three-year soybean/corn rotation in l'Acadie, Quebec, Canada. Each line and color represent one soil sample. The maximum sequencing coverage (x-axis: number of sequences) is 2,000 reads with cutoffs at 100, 200, 500 and 1,000 reads for the nematode communities (A), and 20,000 reads with cutoffs at 1,000, 2,000, 5,000 and 10,000 reads for the nematode communities (B).

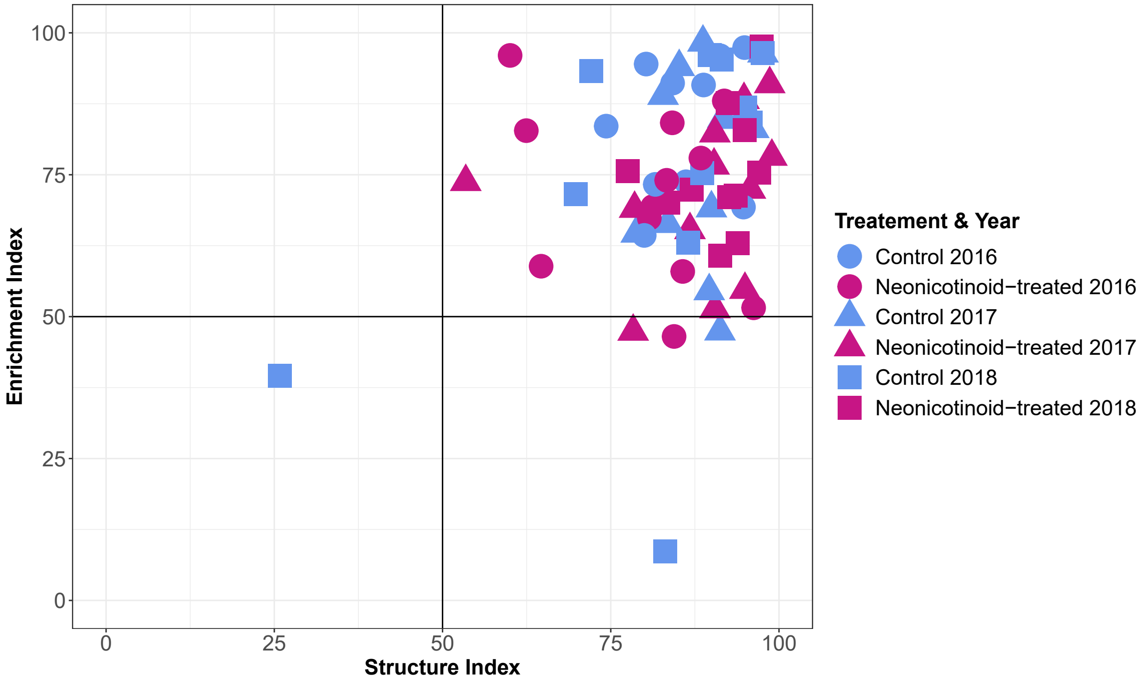

Figure C.2: Food web analysis of soil food web for nematodes. Plot represents the structure (x-axis) and enrichment (y-axis) conditions of the soil food web of a three-year soybean/corn rotation in l'Acadie, Quebec, Canada. Each point indicates a control (blue) or neonicotinoid-treated (pink) sample and the shape of it represents the year of the rotation (circle: 2016, triangle: 2017, square: 2018).

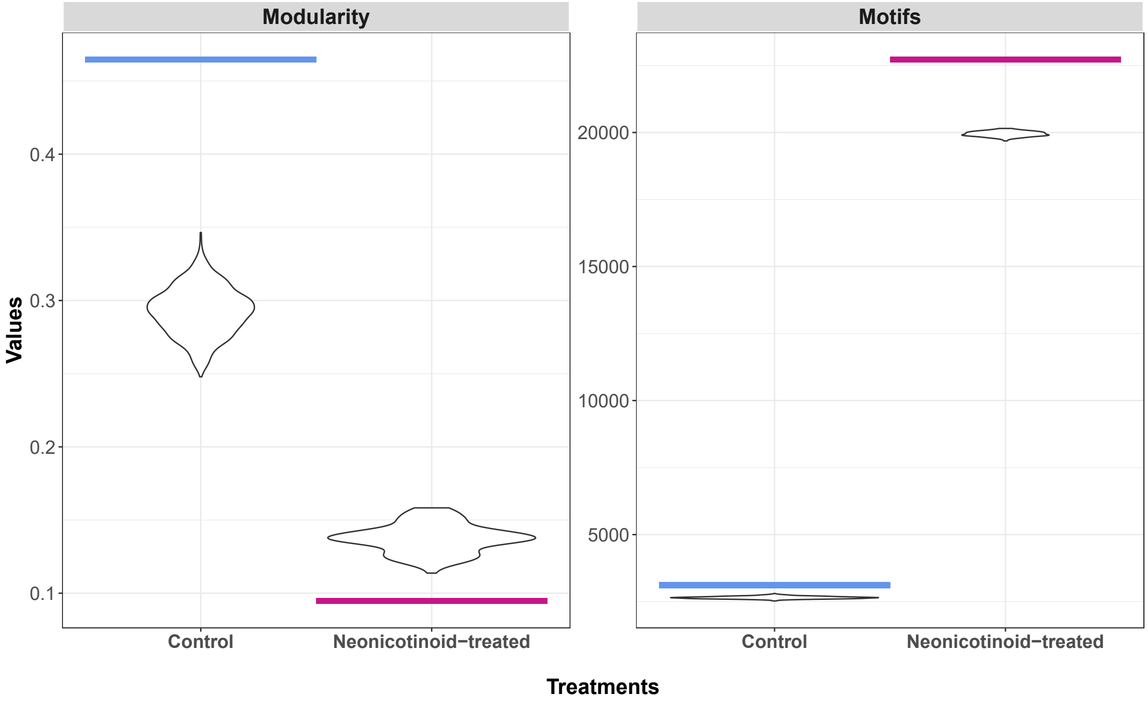

Figure C.3: Changes in topological properties of networks in response to neonicotinoid seed treatment. Violin plots use density curves to depict the distribution of the values of modularity and motifs present in the null models generated by randomizing control and neonicotinoid-treated matrices. The blue and pink lines show the calculated values of the mentioned metrics respectively for control and neonicotinoid-treated samples in a three-year soybean/corn rotation in l'Acadie, Quebec, Canada.

APPENDIX D

SUPPLEMENTARY TABLES - CHAPTER III

Table D.1: Variation in the expression of soil microbial genes between years (2017 vs. 2016), based on SEED hierarchical microbial functional annotation (level4) (DESeq2, adjusted $P < 0.05$).

year	gene	$\log_2$Fold Change	level1	level4
2017	gene3331	2.27	Electron transport and photophosphorylation	Photosystem II manganese-stabilizing protein (PsbO)
	gene2432	2.08	Plant Octadecanoids	Lipoxygenase, chloroplast precursor (EC 1.13.11.12)
	gene3327	2.01	Electron transport and photophosphorylation	photosystem I P700 chlorophyll a apoprotein subunit Ia (PsaA)
	gene3328	2.00	Electron transport and photophosphorylation	photosystem I P700 chlorophyll a apoprotein subunit Ib (PsaB)
	gene3333	1.99	Electron transport and photophosphorylation	photosystem II protein D2 (PsbD)
	gene1138	1.86	Respiration	Cytochrome b6-f complex subunit, cytochrome b6
	gene3334	1.86	Light-harvesting complexes	Phycobilisome core-membrane linker polypeptide
	gene1136	1.76	Respiration	Cytochrome b6-f complex subunit IV (PetD)
	gene2404	1.72	Tetrapyrroles	Light-independent protochlorophyllide reductase iron-sulfur ATP-binding protein ChlL (EC 1.18.-.-)
	gene3330	1.59	Electron transport and photophosphorylation	Photosystem II CP47 protein (PsbB)
	gene3332	1.56	Electron transport and photophosphorylation	photosystem II protein D1 (PsbA)
	gene3329	1.56	Electron transport and photophosphorylation	Photosystem II CP43 protein (PsbC)
	gene1541	1.54	Respiration	Ferredoxin-NADP(+) reductase (EC 1.18.1.2)
	gene2885	1.50	Electron donating reactions	NADH dehydrogenase I subunit 4, Involved in photosystem-1 cyclic electron flow
	gene2707	1.46	Tetrapyrroles	Mg protoporphyrin IX monomethyl ester oxidative cyclase (aerobic) (EC 1.14.13.81)
	gene1137	1.45	Respiration	Cytochrome b6-f complex subunit, apocytochrome f
	gene2959	1.42	Nitrogen Metabolism	Nitric oxide-dependent regulator DnrN or NorA
	gene2882	1.34	Electron donating reactions	NAD(P)H-quinone oxidoreductase chain K (EC 1.6.5.2)
	gene3404	1.28	CO2 fixation	Possible carbon dioxide concentrating mechanism protein CcmK
	gene2406	1.28	Tetrapyrroles	Light-independent protochlorophyllide reductase subunit N (EC 1.18.-.-)

Table D.1 continued from previous page

year	gene	$\log_2$Fold Change	level1	level4
	gene2405	1.21	Tetrapyrroles	Light-independent protochlorophyllide reductase subunit B (EC 1.18.-.-)
	gene1828	1.20	Tetrapyrroles	Geranylgeranyl hydrogenase BchP
	gene3903	1.19	Central carbohydrate metabolism	Pyruvate oxidase (EC 1.2.3.3)
	gene3319	1.16	CO2 fixation	Phosphoribulokinase (EC 2.7.1.19)
	gene4043	1.15	CO2 fixation	Ribulose bisphosphate carboxylase large chain (EC 4.1.1.39)
	gene3921	1.07	Electron donating reactions	Quinone-reactive Ni/Fe-hydrogenase small chain precursor (EC 1.12.5.1)
	gene3498	1.07	Clustering-based subsystems	Predicted oxidoreductase, Fe-S subunit
	gene2354	1.03	Central carbohydrate metabolism	L-malyl-CoA/beta-methylmalyl-CoA lyase (EC 4.1.3.-), actinobacterial type
	gene1176	1.02	Respiration	Cytochrome C553 (soluble cytochrome f)
	gene2960	1.01	Nitrogen Metabolism	Nitric-oxide reductase (EC 1.7.99.7), quinol-dependent
	gene4044	1.00	CO2 fixation	Ribulose bisphosphate carboxylase small chain (EC 4.1.1.39)
	gene1746	0.98	Monosaccharides	Fructose ABC transporter, permease component FrcC
	gene4393	0.98	Sulfur Metabolism	Sulfite reduction-associated complex DsrMKJOP protein DsrP (= HmeB)
	gene1747	0.92	Monosaccharides	Fructose ABC transporter, substrate-binding component FrcB
	gene4134	0.91	CO2 fixation	Sedoheptulose-1,7-bisphosphatase (EC 3.1.3.37)
	gene2867	0.91	Electron donating reactions	NAD-reducing hydrogenase subunit HoxE (EC 1.12.1.2)
	gene3736	0.91	Tetrapyrroles	Putative chaperon-like protein Ycf39 for quinone binding in Photosystem II
	gene3954	0.88	Nitrogen Metabolism	Respiratory nitrate reductase gamma chain (EC 1.7.99.4)
	gene4628	0.87	Biotin	Transmembrane component BioN of energizing module of biotin ECF transporter
	gene63	0.87	Lysine, threonine, methionine, and cysteine	2-hydroxy-3-keto-5-methylthiopentenyl-1-phosphate phosphatase related protein
	gene4907	0.85	Central carbohydrate metabolism	Valine dehydrogenase (EC 1.4.1.-)
	gene4843	0.83	Stress Response	Universal stress protein family COG0589
	gene1325	0.82	Tetrapyrroles	Divinyl protochlorophyllide a 8-vinyl-reductase (EC 1.3.1.75)
	gene4841	0.81	Stress Response	Universal stress protein family 4
	gene3544	0.80	Cell wall of Mycobacteria	Probable acyl-[acyl-carrier protein] desaturase DESA1 (Acyl-[ACP] desaturase) (Stearoyl-ACP desaturase) (Protein DES) (EC 1.14.19.2)
	gene3762	0.80	Electron donating reactions	putative Fe-S, FMN containing oxidoreductase
	gene928	0.79	Tetrapyrroles	Chlorophyll a synthase ChlG (EC 2.5.1.62)
	gene3043	0.78	Central carbohydrate metabolism	OpcA, an allosteric effector of glucose-6-phosphate dehydrogenase, cyanobacterial
	gene1557	0.78	Iron acquisition and metabolism	Ferrous iron transport permease EfeU, N-terminal extended
	gene1281	0.78	Peripheral pathways for catabolism of aromatic compounds	Dihydrodiol dehydrogenase (EC 1.3.1.56)
	gene3564	0.77	Clustering-based subsystems	Probable polyketide synthase, similar to many. e.g. gp|M63676|SERERYAA_1 S.erythraea first ORF of eryA gene, involved in complex polyketide formation in erythromycin biosynthesis.
	gene4598	0.77	Monosaccharides	Transcriptional repressor of the fructose operon, DeoR family
	gene1745	0.76	Monosaccharides	Fructose ABC transporter, ATP-binding component FrcA
	gene1135	0.76	Respiration	Cytochrome b6-f complex iron-sulfur subunit PetC1 (Rieske iron sulfur protein EC 1.10.99.1)
	gene2700	0.76	Sodium Ion-Coupled Energetics	Methylmalonyl-CoA:Pyruvate transcarboxylase 5S subunit (EC 2.1.3.1)
	gene1540	0.74	Inorganic sulfur assimilation	Ferredoxin-like protein involved in electron transfer
	gene2040	0.73	Iron acquisition and metabolism	Heme ABC transporter, cell surface heme and hemoprotein receptor HmuT
	gene3951	0.73	Nitrogen Metabolism	Respiratory nitrate reductase alpha chain (EC 1.7.99.4)
	gene547	0.72	Metabolism of central aromatic intermediates	AreB (Aryl-alcohol dehydrogenase) (EC 1.1.1.90)
	gene39	0.72	Plant Octadecanoids	12-oxophytodienoate reductase (OPR3)(DDE1)

Table D.1 continued from previous page

year	gene	$\log_2$Fold Change	level1	level4
	gene3713	0.71	Capsular and extracellular polysaccharides	PTS system, N-acetylglucosamine-specific IIA component (EC 2.7.1.69)
	gene2004	0.71	Cell Division and Cell Cycle	Group 2 RNA polymerase sigma factor
	gene1080	0.71	Central carbohydrate metabolism	Crotonyl-CoA carboxylase/reductase, ethylmalonyl-CoA producing
	gene127	0.71	RNA processing and modification	2H phosphoesterase superfamily protein Bsu1186 (yjcG)
	gene2353	0.70	Central carbohydrate metabolism	L-malyl-CoA/beta-methylmalyl-CoA lyase (EC 4.1.3.-)
	gene2889	0.70	CO2 fixation	NADH dehydrogenase subunit 5, Involved in CO2 fixation
	gene3920	0.70	Electron donating reactions	Quinone-reactive Ni/Fe-hydrogenase large chain (EC 1.12.5.1)
	gene2212	0.70	Bacterial cytostatics, differentiation factors and antibiotics	Inducers of aerial mycelium formation biosynthesis protein BldG
	gene4130	0.70	Cell wall of Mycobacteria	SECRETED ANTIGEN 85-A FBPA (MYCOLYL TRANSFERASE 85A) (FIBRONECTIN-BINDING PROTEIN A) (ANTIGEN 85 COMPLEX A)
	gene3953	0.70	Nitrogen Metabolism	Respiratory nitrate reductase delta chain (EC 1.7.99.4)
	gene122	0.69	Isoprenoids	2'-O-glycosyltransferase CruG
	gene2765	0.69	Resistance to antibiotics and toxic compounds	Multidrug efflux membrane fusion protein MexE
	gene1324	0.69	Electron accepting reactions	Dissimilatory sulfite reductase (desulfoviridin), alpha and beta subunits
	gene2083	0.69	Clustering-based subsystems	His repressor
	gene2678	0.68	Cell wall of Mycobacteria	METHOXY MYCOLIC ACID SYNTHASE 4 MMAA4 (METHYL MYCOLIC ACID SYNTHASE 4) (MMA4) (HYDROXY MYCOLIC ACID SYNTHASE)
	gene868	0.68	Cell Wall and Capsule	CBS domain protein sometimes clustered with YjeE
	gene3923	0.68	Tetrapyrroles	Radical SAM domain heme biosynthesis protein
	gene31	0.68	Monosaccharides	1-phosphofructokinase (EC 2.7.1.56)
	gene4372	0.68	Dessication stress	Sugar-1-epimerase YihR
	gene1487	0.67	Central carbohydrate metabolism	Ethylmalonyl-CoA mutase, methylsuccinyl-CoA-forming
	gene4840	0.67	Stress Response	Universal stress protein family
	gene20	0.67	Electron donating reactions	[NiFe] hydrogenase metallocenter assembly protein HypC
	gene792	0.67	Monosaccharides	Broad-specificity glycerol dehydrogenase (EC 1.1.99.22), subunit SldA
	gene2167	0.66	Clustering-based subsystems	Hypothetical protein of L-Asparaginase type 2-like superfamily
	gene4211	0.66	Membrane Transport	Signal transduction histidine kinase CitA regulating citrate metabolism
	gene1899	0.65	Lysine, threonine, methionine, and cysteine	Glutamine-dependent 2-keto-4-methylthiobutyrate transaminase
	gene2643	0.65	Quinone cofactors	Menaquinone via futalosine step 2
	gene3368	0.65	Dormancy and Sporulation	Polyketide chain length factor WhiE-CLF paralog
	gene2649	0.65	Quinone cofactors	Menaquinone-specific isochorismate synthase (EC 5.4.4.2)
	gene3787	0.65	One-carbon Metabolism	putative isobutyryl-CoA mutase, chain B
	gene1027	0.65	Tricarboxylate transporter	COG5591: Uncharacterized conserved protein
	gene3688	0.65	Tetrapyrroles	Protoporphyrinogen IX oxidase, oxygen-independent, HemG (EC 1.3.-.-)
	gene1830	0.64	Isoprenoids	Geranylgeranyl reductase (EC 1.3.1.83)
	gene3344	0.64	Clustering-based subsystems	Pirin-like protein YhaK
	gene4676	0.63	RNA processing and modification	tRNA nucleotidyltransferase, A-adding (EC 2.7.7.25)
	gene2701	0.63	Central carbohydrate metabolism	Methylsuccinyl-CoA dehydrogenase, predicted by (Erb et al, 2007)
	gene3974	0.63	Riboflavin, FMN, FAD	Riboflavin transporter PnuX
	gene4924	0.63	Pathogenicity islands	virulence cluster protein B VclB
	gene3718	0.63	Regulation and Cell signaling	Purine cyclase-related protein
	gene4176	0.62	Protein translocation across cytoplasmic membrane	Serine protease, DegP/HtrA, do-like (EC 3.4.21.-)
	gene4224	0.62	Plant-Prokaryote DOE project	Similar to non-heme chloroperoxidase, sll5080 homolog
	gene2565	0.62	Oxidative stress	Maleylpyruvate isomerase, mycothiol-dependent (EC 5.2.1.4)
	gene2332	0.62	Oxidative stress	L-cysteine:1D-myo-inositol 2-amino-2-deoxy-alpha-D-glucopyranoside ligase MshC

Table D.1 continued from previous page

year	gene	log$_2$Fold Change	level1	level4
	gene4245	0.62	Isoprenoids	Solanesyl diphosphate synthase (EC 2.5.1.11)
	gene4803	0.61	Cell Wall and Capsule	UDP-N-acetylmuramoylalanyl-D-glutamate–L,L-2,6-diaminopimelate ligase
	gene3460	0.61	Di- and oligosaccharides	Predicted galactoside ABC transporter type II, sugar-binding protein
	gene491	0.60	Proline and 4-hydroxyproline	Amino acid permease in 4-hydroxyproline catabolic gene cluster
	gene2129	0.60	Electron donating reactions	hydrogenase/sulfur reductase, alpha subunit
	gene4632	0.60	Cofactors, Vitamins, Prosthetic Groups, Pigments	Transmembrane component YkoC of energizing module of thiamin-regulated ECF transporter for HydroxyMethylPyrimidine
	gene3264	0.60	Central carbohydrate metabolism	Phosphoenolpyruvate-dihydroxyacetone phosphotransferase (EC 2.7.1.121), ADP-binding subunit DhaL
	gene867	0.60	Tetrapyrroles	CblZ, a non-orthologous displasment for Alpha-ribazole-5'-phosphate phosphatase
	gene998	0.60	Electron donating reactions	Coenzyme F420-reducing hydrogenase, gamma subunit
	gene4437	0.60	Fatty acids	TesB-like acyl-CoA thioesterase 3
	gene3791	0.60	Protein biosynthesis	Putative membrane protein found fused to lysyl-tRNA synthetase like protein
	gene227	0.60	One-carbon Metabolism	5-FCL-like protein, but predicted not to be 5-formyltetrahydrofolate cyclo-ligase (5-FCL)
	gene3707	0.59	Sugar alcohols	PTS system, mannitol-specific IIA component (EC 2.7.1.69)
	gene2130	0.59	Electron donating reactions	hydrogenase/sulfur reductase, delta subunit
	gene4409	0.59	Oxidative stress	superoxide dismutase [Fe-Zn] (EC 1.15.1.1)
	gene4865	0.59	DNA repair	Uracil-DNA glycosylase, putative family 6
	gene3689	0.59	Organic acids	PrpF protein involved in 2-methylcitrate cycle
	gene4325	0.59	Dormancy and Sporulation	Stage IV sporulation pro-sigma-K processing enzyme (SpoIVFB)
	gene130	0.58	Metabolism of central aromatic intermediates	3-carboxy-cis,cis-muconate cycloisomerase (EC 5.5.1.2)
	gene3362	0.58	Fatty Acids, Lipids, and Isoprenoids	polyhydroxyalkanoate granule-associated protein PhaI
	gene1258	0.58	Monosaccharides	Deoxyribonucleoside regulator DeoR (transcriptional repressor)
	gene3192	0.58	Phages, Prophages	Phage tape measure
	gene2128	0.58	Electron donating reactions	hydrogenase, subunit gamma related protein
	gene4685	0.57	Cell Wall and Capsule	tRNA-dependent lipid II–amino acid ligase
	gene2048	0.56	Iron acquisition and metabolism	Heme oxygenase (EC 1.14.99.3)
	gene156	0.56	Branched-chain amino acids	3-ketoacyl-CoA thiolase [isoleucine degradation] (EC 2.3.1.16)
	gene1000	0.56	Quinone cofactors	Coenzyme PQQ synthesis protein B
	gene4121	0.56	Urate degradation	salvage of nucleosides and nucleotides
	gene515	0.56	Sugar alcohols	Anaerobic glycerol-3-phosphate dehydrogenase subunit B (EC 1.1.5.3)
	gene2991	0.55	Folate and pterines	Non functional Dihydropteroate synthase 2
	gene3079	0.55	Resistance to antibiotics and toxic compounds	Outer membrane protein OprN
	gene2166	0.55	Probably GTP or GMP signaling related	hypothetical protein NAS141_09886
	gene4569	0.55	Central carbohydrate metabolism	Transcriptional regulator of succinyl CoA synthetase operon
	gene2721	0.55	Folate and pterines	Molybdate-binding domain of ModE
	gene1127	0.55	recX and regulatory cluster	Cysteinyl-tRNA synthetase related protein
	gene2894	0.55	Electron donating reactions	NADH-quinone oxidoreductase chain F 2 (EC 1.6.99.5)
	gene1529	0.55	Oxidative stress	Fe2+/Zn2+ uptake regulation proteins
	gene1534	0.54	Nitrogen Metabolism	Ferredoxin–nitrite reductase (EC 1.7.7.1)
	gene3832	0.54	Electron donating reactions	Putative succinate dehydrogenase cytochrome b subunit
	gene2870	0.54	Electron donating reactions	NAD-reducing hydrogenase subunit HoxU (EC 1.12.1.2)
	gene3558	0.54	Nitrogen Metabolism	probable iron binding protein from the HesB_IscA_SufA family in Nif operon
	gene2435	0.54	Clustering-based subsystems	Lon-like protease with PDZ domain

Table D.1 continued from previous page

year	gene	$\log_2$Fold Change	level1	level4
	gene2788	0.53	Oxidative stress	Mycothiol S-conjugate amidase Mca
	gene665	0.53	Biotin	ATPase component BioM of energizing module of biotin ECF transporter
	gene1536	0.53	Inorganic sulfur assimilation	Ferredoxin–sulfite reductase, actinobacterial type (EC 1.8.7.1)
	gene5006	0.53	Clustering-based subsystems	Zn-dependent hydrolase, RNA-metabolising
	gene1603	0.53	proteosome related	FIG019733: possible DNA-binding protein
	gene2652	0.53	Central carbohydrate metabolism	Mesaconyl-CoA hydratase
	gene1590	0.53	proteosome related	FIG005453: Putative DeoR-family transcriptional regulator
	gene2189	0.53	Cytochrome biogenesis	Hypothetical, related to broad specificity phosphatases COG0406
	gene1716	0.53	Respiration	Formate dehydrogenase N beta subunit (EC 1.2.1.2)
	gene3822	0.52	Monosaccharides	Putative regulator of the mannose operon, ManO
	gene4399	0.52	Cofactors, Vitamins, Prosthetic Groups, Pigments	Sulfur carrier protein ThiS
	gene4412	0.52	Oxidative stress	Superoxide dismutase [Mn/Fe] (EC 1.15.1.1)
	gene2386	0.52	Coenzyme F420	Lactyl (2) diphospho-(5')guanosine:7,8-didemethyl-8-hydroxy-5-deazariboflavin 2-phospho-L-lactate transferase
	gene874	0.52	Gram-Positive cell wall components	CDP-glycerol:poly(glycerophosphate) glycerophosphotransferase (EC 2.7.8.12)
	gene2348	0.52	Organic acids	L-lactate dehydrogenase (EC 1.1.2.3)
	gene4933	0.52	Regulation and Cell signaling	WhiB-type transcriptional regulator
	gene394	0.52	Arginine; urea cycle, polyamines	Agmatine/putrescine antiporter, associated with agmatine catabolism
	gene1533	0.51	Inorganic sulfur assimilation	Ferredoxin–NADP(+) reductase, actinobacterial (eukaryote-like) type (EC 1.18.1.2)
	gene3438	0.51	Monosaccharides	Predicted arabinose ABC transporter, permease protein 2
	gene3701	0.51	Monosaccharides	PTS system, fructose-specific IIC component (EC 2.7.1.69)
	gene2588	0.51	One-carbon Metabolism	Malyl-CoA lyase (EC 4.1.3.24)
	gene3767	0.51	Di- and oligosaccharides	Putative glucanase glgE (EC 3.2.1.-)
	gene21	0.50	Electron donating reactions	[NiFe] hydrogenase metallocenter assembly protein HypD
	gene25	0.50	Electron donating reactions	[NiFe] hydrogenase nickel incorporation-associated protein HypB
	gene4431	0.50	Gram-Positive cell wall components	Teichoic acid biosynthesis protein
	gene3345	0.50	Siderophores	Pirin-related protein, coexpressed with pyoverdine biosynthesis regulon
	gene1092	0.50	Transcription	Cyanobacteria-specific RpoD-like sigma factor, type-1
	gene3490	0.50	Aminosugars	Predicted N-acetyl-glucosamine kinase 2, ROK family (EC 2.7.1.59)
	gene1057	0.50	Resistance to antibiotics and toxic compounds	Copper resistance protein D
	gene2229	0.50	Phosphorus Metabolism	Inorganic pyrophospatase PpaX (EC 3.1.3.18)
	gene4344	0.50	RNA processing and modification	Substrate-specific component STY3230 of queuosine-regulated ECF transporter
	gene3394	0.50	Peripheral pathways for catabolism of aromatic compounds	Positive regulator of phenol hydroxylase
	gene1144	0.50	Electron accepting reactions	Cytochrome c oxidase (B(O/a)3-type) chain II (EC 1.9.3.1)
	gene217	0.49	Lysine, threonine, methionine, and cysteine	5-aminopentanamidase (EC 3.5.1.30)
	gene4175	0.49	Transcription	Serine protease precursor MucD/AlgY associated with sigma factor RpoE
	gene4345	0.49	Cofactors, Vitamins, Prosthetic Groups, Pigments	Substrate-specific component YkoE of thiamin-regulated ECF transporter for HydroxyMethylPyrimidine
	gene22	0.49	Electron donating reactions	[NiFe] hydrogenase metallocenter assembly protein HypE
	gene2651	0.48	Resistance to antibiotics and toxic compounds	Mercuric resistance operon regulatory protein
	gene1019	0.48	Gram-Positive cell wall components	COG1887: Putative glycosyl/glycerophosphate transferases involved in teichoic acid biosynthesis TagF/TagB/EpsJ/RodC
	gene23	0.48	Electron donating reactions	[NiFe] hydrogenase metallocenter assembly protein HypF
	gene2191	0.48	Cytochrome biogenesis	Hypothetical, similarity to phosphoglycerate mutase

Table D.1 continued from previous page

year	gene	$\log_2$Fold Change	level1	level4
	gene3905	0.48	Central carbohydrate metabolism	Pyruvate-flavodoxin oxidoreductase (EC 1.2.7.-)
	gene3879	0.48	Pyridoxine	Pyridoxine biosynthesis glutamine amidotransferase, synthase subunit (EC 2.4.2.-)
	gene898	0.48	Regulation and Cell signaling	Cell envelope-associated transcriptional attenuator LytR-CpsA-Psr, subfamily A1 (as in PMID19099556)
	gene4629	0.48	Membrane Transport	Transmembrane component CbrV of energizing module of predicted cobalamin ECF transporter
	gene1539	0.48	Fermentation	Ferredoxin-like protein
	gene3935	0.48	Oxidative stress	Redox-sensitive transcriptional regulator (AT-rich DNA-binding protein)
	gene3738	0.47	Electron accepting reactions	putative Cytochrome bd2, subunit I
	gene3950	0.47	Transposable elements	Resolvase/Integrase TinR protein
	gene4930	0.47	Regulation and Cell signaling	WhiB-like transcription regulator
	gene3278	0.47	Central carbohydrate metabolism	Phosphoglycolate phosphatase, archaeal type (EC 3.1.3.18)
	gene1942	0.47	Electron donating reactions	Glycerol-1-phosphate dehydrogenase [NAD(P)] (EC 1.1.1.261)
	gene1722	0.46	Respiration	Formate dehydrogenase putative subunit (EC 1.2.1.2)
	gene3679	0.46	Membrane Transport	Protein-L-isoaspartate O-methyltransferase (EC 2.1.1.77)
	gene3642	0.46	Central carbohydrate metabolism	Protein acetyltransferase
	gene327	0.46	Oxidative stress	Acetyl-CoA:Cys-GlcN-Ins acetyltransferase, mycothiol synthase MshD
	gene3087	0.46	Periplasmic Stress	Outer membrane stress sensor protease DegS
	gene1615	0.46	Clustering-based subsystems	FIG042796: Hypothetical protein
	gene2065	0.46	Isoprenoids	Heptaprenyl diphosphate synthase component I (EC 2.5.1.30)
	gene2316	0.46	Lysine, threonine, methionine, and cysteine	L-allo-threonine aldolase
	gene463	0.46	NA	Alpha-glucoside transport ATP-binding protein AglK
	gene1426	0.46	Membrane Transport	Duplicated ATPase component of energizing module of predicted ECF transporter in Mycobacteria
	gene1424	0.45	Membrane Transport	Duplicated ATPase component CbrU of energizing module of predicted cobalamin ECF transporter
	gene2966	0.45	Nitrogen Metabolism	Nitrite reductase [NAD(P)H] small subunit (EC 1.7.1.4)
	gene1582	0.45	Plant-Prokaryote DOE project	FIG003620: Proteophosphoglycan precursor (Fragment)
	gene4099	0.45	CO2 fixation	RuBisCO operon transcriptional regulator
	gene1955	0.45	Osmotic stress	Glycine betaine ABC transport system permease protein
	gene1973	0.45	Selenoproteins	Glycine/sarcosine/betaine reductase component C chain 1
	gene4340	0.45	Membrane Transport	Substrate-specific component CbrT of predicted cobalamin ECF transporter
	gene19	0.45	Electron donating reactions	[Ni/Fe] hydrogenase, group 1, small subunit
	gene410	0.45	Catabolism of an unknown compound	Aldehyde dehydrogenase in hypothetical Actinobacterial gene cluster
	gene312	0.45	Fermentation	Acetoin catabolism protein X
	gene4456	0.45	Folate and pterines	Thiamin-phosphate synthase ThiN (EC 2.5.1.3)
	gene4580	0.45	Oxidative stress	Transcriptional regulator, FUR family
	gene4549	0.44	NA	Transcriptional regulator AglR, LacI family
	gene3094	0.44	Protein biosynthesis	Oxytetracycline resistance protein OtrA
	gene777	0.44	Peripheral pathways for catabolism of aromatic compounds	Biphenyl-2,3-diol 1,2-dioxygenase (EC 1.13.11.39)
	gene3042	0.44	Central carbohydrate metabolism	OpcA, an allosteric effector of glucose-6-phosphate dehydrogenase, actinobacterial
	gene4441	0.44	Respiration	Tetrachloroethene reductive dehalogenase PceA (EC 1.97.1.8)
	gene3904	0.44	Central carbohydrate metabolism	Pyruvate oxidase [ubiquinone, cytochrome] (EC 1.2.2.2)
	gene4931	0.44	Regulation and Cell signaling	WhiB-like transcriptional regulator
	gene4637	0.44	Clustering-based subsystems	Transposase for insertion sequence element IS1557
	gene2285	0.44	Aromatic amino acids and derivatives	Isochorismate synthase (EC 5.4.4.2) of siderophore biosynthesis

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year	gene	$\log_2$Fold Change	level1	level4
	gene2869	0.44	Electron donating reactions	NAD-reducing hydrogenase subunit HoxH (EC 1.12.1.2)
	gene3420	0.44	Potassium metabolism	Potassium-transporting ATPase A chain (EC 3.6.3.12) (TC 3.A.3.7.1)
	gene4365	0.44	Di- and oligosaccharides	Sucrose-6-phosphate hydrolase (EC 3.2.1.26)
	gene2813	0.44	Capsular and extracellular polysaccharides	N-acetylmannosamine kinase (EC 2.7.1.60)
	gene2389	0.44	Peripheral pathways for catabolism of aromatic compounds	Large subunit naph/bph dioxygenase
	gene4347	0.44	Electron donating reactions	Succinate dehydrogenase cytochrome b subunit
	gene869	0.44	Respiration	Ccs1/ResB-related putative cytochrome C-type biogenesis protein
	gene2932	0.44	Membrane Transport	Nickel ABC transporter, periplasmic nickel-binding protein nikA2 (TC 3.A.1.5.3)
	gene2862	0.44	Respiration	NAD-dependent formate dehydrogenase gamma subunit
	gene1555	0.43	Iron acquisition and metabolism	Ferrous iron transport periplasmic protein EfeO, contains peptidase-M75 domain and (frequently) cupredoxin-like domain
	gene4035	0.43	Protein biosynthesis	Ribosome protection-type tetracycline resistance related proteins
	gene3878	0.43	Pyridoxine	Pyridoxine biosynthesis glutamine amidotransferase, glutaminase subunit (EC 2.4.2.-)
	gene1803	0.43	Isoprenoid/cell wall biosynthesis: PREDICTED UNDECAPRENYL DIPHOSPHATE PHOSPHATASE	GCN5-related N-acetyltransferase, FIGfam019367
	gene4394	0.43	Organic sulfur assimilation	sulfonate monooxygenase
	gene2858	0.43	Respiration	NAD-dependent formate dehydrogenase (EC 1.2.1.2)
	gene2111	0.43	Membrane Transport	HoxN/HupN/NixA family nickel/cobalt transporter
	gene3206	0.43	Metabolism of Aromatic Compounds	Phenylacetaldehyde dehydrogenase (EC 1.2.1.39)
	gene3939	0.43	Clustering-based subsystems	Regulator of polyketide synthase expression
	gene402	0.42	Fermentation	Alcohol dehydrogenase (EC 1.1.1.1)
	gene936	0.42	Aromatic amino acids and derivatives	Chorismate mutase II (EC 5.4.99.5)
	gene1001	0.42	Quinone cofactors	Coenzyme PQQ synthesis protein C
	gene24	0.42	Electron donating reactions	[NiFe] hydrogenase nickel incorporation protein HypA
	gene2916	0.42	Quinone cofactors	Naphthoate synthase (EC 4.1.3.36)
	gene3428	0.42	Tetrapyrroles	Precorrin-2 oxidase (EC 1.3.1.76)
	gene257	0.42	Coenzyme F420	7,8-didemethyl-8-hydroxy-5-deazariboflavin synthase subunit 2
	gene2265	0.42	Iron acquisition and metabolism	Iron-dependent repressor IdeR/DtxR
	gene1939	0.42	Electron donating reactions	Glycerol dehydrogenase (EC 1.1.1.6)
	gene3759	0.42	Protein translocation across cytoplasmic membrane	Putative ESX-1 secretion system component Rv3877
	gene3521	0.42	Transcription	Predicted transcriptional regulator of 4-carboxymuconolactone decarboxylase, Rrf2 family
	gene3739	0.42	Electron accepting reactions	putative Cytochrome bd2, subunit II
	gene1721	0.42	Respiration	Formate dehydrogenase O putative subunit
	gene4827	0.41	Detoxification	Uncharacterized protein YaiN in in formaldehyde detoxification operon
	gene951	0.41	Cell Division and Cell Cycle	Circadian phase modifier
	gene893	0.41	Cell Division	Cell division protein YlmG/Ycf19 (putative), YggT family
	gene331	0.41	Central carbohydrate metabolism	Acetyl-coenzyme A synthetase (EC 6.2.1.1)
	gene2866	0.41	Central carbohydrate metabolism	NAD-independent protein deacetylase AcuC
	gene3735	0.41	Gram-Positive cell wall components	Putative CDP-glycosylpolyol phosphate:glycosylpolyol glycosylpolyolphosphotransferase
	gene3063	0.41	Osmotic stress	Osmotically activated L-carnitine/choline ABC transporter, permease protein OpuCB
	gene494	0.41	Aromatic amino acids and derivatives	Amino acid-binding ACT
	gene1051	0.41	Fatty Acids, Lipids, and Isoprenoids	Conserved protein IgrD

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year	gene	$\log_2$Fold Change	level1	level4
	gene489	0.41	NAD and NADP	Amidases related to nicotinamidase
	gene1537	0.41	Inorganic sulfur assimilation	Ferredoxin–sulfite reductase, bacillial type (EC 1.8.7.1)
	gene1938	0.41	Sugar alcohols	Glycerol dehydratase large subunit (EC 4.2.1.30)
	gene2871	0.41	Electron donating reactions	NAD-reducing hydrogenase subunit HoxY (EC 1.12.1.2)
	gene3934	0.41	Oxidative stress	Redox-sensitive transcriptional activator SoxR
	gene2113	0.41	Heat shock	HspR, transcriptional repressor of DnaK operon
	gene3831	0.41	Electron donating reactions	putative succinate dehydrogenase [membrane anchor subunit] (succinic dehydrogenase)
	gene4663	0.41	Potassium metabolism	Trk system potassium uptake protein TrkA
	gene157	0.40	Coenzyme A	3-methyl-2-oxobutanoate hydroxymethyltransferase (EC 2.1.2.11)
	gene3265	0.40	Central carbohydrate metabolism	Phosphoenolpyruvate-dihydroxyacetone phosphotransferase (EC 2.7.1.121), dihydroxyacetone binding subunit DhaK
	gene3784	0.40	Oxidative stress	Putative hydrolase in cluster with formaldehyde/S-nitrosomycothiol reductase MscR
	gene4114	0.40	Oxidative stress	S-nitrosomycothiol reductase MscR
	gene2290	0.40	Central carbohydrate metabolism	Isocitrate lyase (EC 4.1.3.1), group III, Mycobacterial type ICL2
	gene834	0.40	Electron donating reactions	carbon monoxide dehydrogenase G protein
	gene4324	0.40	Dormancy and Sporulation	Stage II sporulation protein D (SpoIID)
	gene1460	0.40	Central carbohydrate metabolism	Enoyl-CoA hydratase [branched-chain amino acid degradation] (EC 4.2.1.17)
	gene1531	0.40	Electron accepting reactions	Ferredoxin reductase
	gene589	0.40	Coenzyme A	Aspartate 1-decarboxylase (EC 4.1.1.11)
	gene2115	0.40	Periplasmic Stress	HtrA protease/chaperone protein
	gene4652	0.40	Di- and oligosaccharides	Trehalose synthase, nucleoside diphosphate glucose dependent
	gene324	0.40	Central carbohydrate metabolism	Acetyl-CoA synthetase (ADP-forming) beta chain (EC 6.2.1.13)
	gene2914	0.40	Oxidative stress	NADPH-dependent mycothiol reductase Mtr
	gene3421	0.40	Potassium metabolism	Potassium-transporting ATPase B chain (EC 3.6.3.12) (TC 3.A.3.7.1)
	gene4342	0.40	Membrane Transport	Substrate-specific component NikM of nickel ECF transporter
	gene1094	0.39	Transcription	Cyanobacteria-specific RpoD-like sigma factor, type-13
	gene1556	0.39	Iron acquisition and metabolism	Ferrous iron transport permease EfeU
	gene262	0.39	NA	ABC alpha-glucoside transporter, inner membrane subunit AglF
	gene3633	0.39	Protein degradation	Proteasome subunit beta (EC 3.4.25.1), bacterial
	gene2315	0.39	CO2 fixation	L-alanine:glyoxylate aminotransferase (EC 2.6.1.44)
	gene2770	0.39	Resistance to antibiotics and toxic compounds	Multidrug efflux transporter MexF
	gene831	0.39	Electron donating reactions	carbon monoxide dehydrogenase E protein
	gene3467	0.39	Cofactors, Vitamins, Prosthetic Groups, Pigments	Predicted hydroxymethylpyrimidine transporter CytX
	gene929	0.39	Light-harvesting complexes	Chlorosome protein I, 2Fe-2S ferredoxin
	gene778	0.39	Peripheral pathways for catabolism of aromatic compounds	biphenyl-2,3-diol 1,2-dioxygenase III-related protein
	gene3579	0.39	Protein degradation	Prokaryotic ubiquitin-like protein Pup
	gene840	0.39	Electron donating reactions	Carbon monoxide dehydrogenase small chain (EC 1.2.99.2)
	gene4270	0.39	Dormancy and Sporulation	SpoVS-related protein, type 5
	gene4985	0.39	Folate and pterines	YjbQ (alternate ThiE)
	gene1427	0.39	Membrane Transport	Duplicated ATPase component YkoD of energizing module of thiamin-regulated ECF transporter for HydroxyMethylPyrimidine
	gene4238	0.39	Tetrapyrroles	Sirohydrochlorin ferrochelatase (EC 4.99.1.4)
	gene4075	0.39	Transcription	RNA polymerase sporulation specific sigma factor SigH
	gene488	0.39	Nitrogen Metabolism	Amidase clustered with urea ABC transporter and nitrile hydratase functions
	gene290	0.39	Inorganic sulfur assimilation	ABC-type probable sulfate transporter, periplasmic binding protein
	gene838	0.39	Electron donating reactions	Carbon monoxide dehydrogenase medium chain (EC 1.2.99.2)

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year	gene	$\log_2$Fold Change	level1	level4
	gene291	0.39	Inorganic sulfur assimilation	ABC-type probable sulfate transporter, permease protein
	gene1465	0.39	DNA repair	Error-prone repair protein UmuD
	gene1143	0.39	Electron accepting reactions	Cytochrome c oxidase (B(O/a)3-type) chain I (EC 1.9.3.1)
	gene4897	0.38	Tetrapyrroles	Uroporphyrinogen-III methyltransferase (EC 2.1.1.107)
	gene4054	0.38	Transcription	RNA polymerase principal sigma factor HrdC
	gene901	0.38	Regulation and Cell signaling	Cell envelope-associated transcriptional attenuator LytR-CpsA-Psr, subfamily M (as in PMID19099556)
	gene3422	0.38	Potassium metabolism	Potassium-transporting ATPase C chain (EC 3.6.3.12) (TC 3.A.3.7.1)
	gene802	0.38	Resistance to antibiotics and toxic compounds	Cadmium-transporting ATPase (EC 3.6.3.3)
	gene2261	0.38	RNA processing and modification	Iron binding protein IscA for iron-sulfur cluster assembly
	gene3167	0.38	Oxidative stress	Peroxide stress regulator PerR, FUR family
	gene1020	0.38	Catabolism of an unknown compound	COG2071: predicted glutamine amidotransferases in hypothetical Actinobacterial gene cluster
	gene602	0.38	Nitrogen Metabolism	Assimilatory nitrate reductase large subunit (EC:1.7.99.4)
	gene2868	0.38	Electron donating reactions	NAD-reducing hydrogenase subunit HoxF (EC 1.12.1.2)
	gene2032	0.38	Heat shock	Heat-inducible transcription repressor HrcA
	gene2177	0.38	One-carbon Metabolism	Hypothetical protein with distant similarity to Ribonuclease E inhibitor RraA (former MenG)
	gene4977	0.38	Miscellaneous	YbbM seven transmembrane helix protein
	gene3148	0.38	Cofactors, Vitamins, Prosthetic Groups, Pigments	Periplasmic aromatic aldehyde oxidoreductase, iron-sulfur subunit YagT
	gene3326	0.37	Electron transport and photophosphorylation	photosystem I biogenesis protein BtpA
	gene1053	0.37	Resistance to antibiotics and toxic compounds	CopG protein
	gene1736	0.37	Folate and pterines	Formiminotetrahydrofolate cyclodeaminase (EC 4.3.1.4)
	gene1392	0.37	Transcription	DNA-directed RNA polymerase delta (= beta") subunit (EC 2.7.7.6)
	gene835	0.37	Electron donating reactions	Carbon monoxide dehydrogenase large chain (EC 1.2.99.2)
	gene1704	0.37	Folate and pterines	Folate transporter 3
	gene3648	0.37	Clustering-based subsystems	protein from nitrogen regulatory protein P-II (GLNB) family, ortholog YAAQ B. subtilis
	gene2063	0.37	Tetrapyrroles	Hemoprotein HemQ, essential component of heme biosynthetic pathway in Gram-positive bacteria
	gene2060	0.37	Iron acquisition and metabolism	Hemoglobin, heme-dependent two component system sensory histidine kinase ChrS
	gene1956	0.37	Osmotic stress	Glycine betaine ABC transport system, ATP-binding protein OpuAA (EC 3.6.3.32)
	gene830	0.37	Electron donating reactions	carbon monoxide dehydrogenase D protein
	gene2987	0.37	Protein export	NLP/P60 family protein
	gene459	0.37	Monosaccharides	Alpha-glucosidase (EC 3.2.1.20)
	gene3324	0.37	Monosaccharides	Phosphotransferase system, phosphocarrier protein HPr
	gene1935	0.37	Oxidative stress	Glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) (GAPDH)
	gene3624	0.36	Protein degradation	Proteasome subunit alpha (EC 3.4.25.1), bacterial
	gene2043	0.36	Tetrapyrroles	Heme biosynthesis protein related to NirL and NirH
	gene3952	0.36	Nitrogen Metabolism	Respiratory nitrate reductase beta chain (EC 1.7.99.4)
	gene1937	0.36	Monosaccharides	Glycerate kinase (EC 2.7.1.31)
	gene3255	0.36	Lysine, threonine, methionine, and cysteine	Phosphoadenylyl-sulfate reductase [thioredoxin] (EC 1.8.4.8)
	gene995	0.36	Coenzyme F420	Coenzyme F420-1:L-glutamate ligase
	gene4411	0.36	Oxidative stress	Superoxide dismutase [Mn] (EC 1.15.1.1)
	gene1718	0.36	Respiration	Formate dehydrogenase O alpha subunit (EC 1.2.1.2)
	gene1558	0.36	Iron acquisition and metabolism	Ferrous iron transport peroxidase EfeB

Table D.1 continued from previous page

year	gene	$\log_2$Fold Change	level1	level4
	gene4376	0.36	Lysine, threonine, methionine, and cysteine	Sulfate adenylyltransferase subunit 1 (EC 2.7.7.4)
	gene578	0.36	Resistance to antibiotics and toxic compounds	Arsenical pump-driving ATPase (EC 3.6.3.16)
	gene1011	0.36	RNA processing and modification	COG1180: Radical SAM, Pyruvate-formate lyase-activating enzyme like
	gene1437	0.36	Electron donating reactions	Electron transport complex protein RnfA
	gene3664	0.36	RNA processing and modification	Protein often found in Actinomycetes clustered with signal peptidase and/or RNaseHII
	gene1885	0.36	Oxidative stress	Glutamate–cysteine ligase archaeal (EC 6.3.2.2)
	gene3402	0.36	Plant-Prokaryote DOE project	Possible alpha/beta hydrolase superfamily, slr1916 homolog
	gene4898	0.36	Tetrapyrroles	Uroporphyrinogen-III synthase (EC 4.2.1.75)
	gene1291	0.35	Central carbohydrate metabolism	Dihydrolipoamide dehydrogenase of acetoin dehydrogenase (EC 1.8.1.4)
	gene3352	0.35	Bacterial cytostatics, differentiation factors and antibiotics	Pleiotropic negative regulator for morphological and physiological development in Streptomyces, BldD
	gene3371	0.35	Dormancy and Sporulation	Polyketide hydroxylase WhiE VIII
	gene4845	0.35	Two related proteases	Unspecified monosaccharide ABC transport system, ATP-binding protein
	gene255	0.35	Folate and pterines	7,8 dihydropteroate synthase (methanopterin)
	gene3444	0.35	Di- and oligosaccharides	Predicted beta-glucoside-regulated ABC transport system, sugar binding component, COG1653
	gene3123	0.35	Resistance to antibiotics and toxic compounds	Peptidase M48, Ste24p precursor
	gene340	0.35	Aromatic amino acids and derivatives	Acting phosphoribosylanthranilate isomerase (EC 5.3.1.24)
	gene3687	0.35	Tetrapyrroles	Protoporphyrinogen IX oxidase, aerobic (EC 1.3.3.4)
	gene1179	0.35	Electron accepting reactions	Cytochrome d ubiquinol oxidase subunit I (EC 1.10.3.-)
	gene2010	0.35	Probably GTP or GMP signaling related	GTP pyrophosphokinase (EC 2.7.6.5), (p)ppGpp synthetase I
	gene2333	0.35	Organic sulfur assimilation	L-Cystine ABC transporter, ATP-binding protein TcyC
	gene514	0.35	Sugar alcohols	Anaerobic glycerol-3-phosphate dehydrogenase subunit A (EC 1.1.5.3)
	gene1477	0.35	Sugar alcohols	Ethanolamine two-component response regulator
	gene4363	0.34	Di- and oligosaccharides	Sucrose phosphorylase (EC 2.4.1.7)
	gene3114	0.34	Monosaccharides	Pectin degradation protein KdgF
	gene3816	0.34	Peripheral pathways for catabolism of aromatic compounds	Putative phthalate 4,5-dioxygenase oxygenase subunit (OhpA2)
	gene4053	0.34	Transcription	RNA polymerase principal sigma factor HrdA
	gene1941	0.34	Osmotic stress	Glycerol uptake facilitator protein
	gene2793	0.34	Oxidative stress	N-acetyl-1-D-myo-inosityl-2-amino-2-deoxy-alpha-D-glucopyranoside deacetylase MshB
	gene1306	0.34	Phospholipids	Dihydroxyacetone kinase family protein
	gene434	0.34	Sulfur Metabolism	Alkylhydroperoxidase protein D
	gene836	0.34	Electron donating reactions	Carbon monoxide dehydrogenase large chain (EC 1.2.99.2) parolog without usual motifs
	gene2828	0.34	Amino Acids and Derivatives	N-carbamoylsarcosine amidase (EC 3.5.1.59)
	gene3716	0.34	Protein degradation	Pup ligase PafA, possible component of postulated heterodimer PafA-PafA'
	gene2727	0.34	Folate and pterines	Molybdenum cofactor biosynthesis protein MoaE
	gene507	0.34	Nitrogen Metabolism	ammonium/methylammonium permease
	gene1841	0.34	Di- and oligosaccharides	Glucoamylase (EC 3.2.1.3)
	gene4809	0.34	Miscellaneous	Uncharacterized ATP-dependent helicase MJ0294
	gene3801	0.34	Detoxification	Putative Nudix hydrolase YfcD (EC 3.6.-.-)
	gene832	0.34	Electron donating reactions	Carbon monoxide dehydrogenase F protein
	gene823	0.34	Arginine; urea cycle, polyamines	Carbamate kinase (EC 2.7.2.2)

Table D.1 continued from previous page

year	gene	$\log_2$Fold Change	level1	level4
	gene4138	0.34	Selenoproteins	selenocysteine-containing
	gene3424	0.33	Folate and pterines	PqqC-like protein
	gene724	0.33	Osmotic stress	Beta-(1–>2)glucan export ATP-binding/permease protein NdvA (EC 3.6.3.42)
	gene523	0.33	Stress Response	anti sigma b factor antagonist RsbV
	gene4859	0.33	Pyrimidines	Uracil permease
	gene2657	0.33	Resistance to antibiotics and toxic compounds	Metal-dependent hydrolases of the beta-lactamase superfamily III
	gene1024	0.33	Clustering-based subsystems	COG2740: Predicted nucleic-acid-binding protein implicated in transcription termination
	gene4982	0.33	Programmed Cell Death and Toxin-antitoxin Systems	YefM protein (antitoxin to YoeB)
	gene3222	0.33	Folate and pterines	Phenylalanine-4-hydroxylase (EC 1.14.16.1)
	gene567	0.33	Aromatic amino acids and derivatives	Arogenate dehydrogenase (EC 1.3.1.43)
	gene4581	0.33	Catabolism of an unknown compound	Transcriptional regulator, GntR family, in hypothetical Actinobacterial gene cluster
	gene4876	0.33	Arginine; urea cycle, polyamines	Urea carboxylase-related amino acid permease
	gene2371	0.33	Selenoproteins	L-seryl-tRNA(Sec) selenium transferase (EC 2.9.1.1)
	gene110	0.33	Peripheral pathways for catabolism of aromatic compounds	2,3-dihydroxybiphenyl 1,2-dioxygenase
	gene1971	0.32	Alanine, serine, and glycine	Glycine riboswitch
	gene464	0.32	NA	Alpha-glucoside transport system permease protein AglG
	gene2069	0.32	Electron accepting reactions	heterodisulfide reductase, subunit A/methylviologen reducing hydrogenase, subunit delta
	gene4223	0.32	Plant-Prokaryote DOE project	Similar to non-heme chloroperoxidase
	gene4963	0.32	Monosaccharides	Xylose ABC transporter, permease protein XylH
	gene3926	0.32	Protein translocation across cytoplasmic membrane	RD1 region associated protein Rv3876
	gene2306	0.32	DNA repair	Ku domain protein
	gene1891	0.32	Glutamine, glutamate, aspartate, asparagine; ammonia assimilation	Glutamine amidotransferase protein GlxB (EC 2.4.2.-)
	gene2982	0.32	Nitrogen Metabolism	Nitrous oxide reductase maturation protein NosF (ATPase)
	gene2448	0.32	Programmed Cell Death and Toxin-antitoxin Systems	LrgA-associated membrane protein LrgB
	gene3168	0.32	Resistance to antibiotics and toxic compounds	PF00070 family, FAD-dependent NAD(P)-disulphide oxidoreductase
	gene956	0.32	Bacterial cytostatics, differentiation factors and antibiotics	clavaldehyde dehydrogenase
	gene3700	0.32	Monosaccharides	PTS system, fructose-specific IIA component (EC 2.7.1.69)
	gene1846	0.32	Monosaccharides	Gluconate 2-dehydrogenase (EC 1.1.99.3), membrane-bound, gamma subunit
	gene3260	0.32	Central carbohydrate metabolism	Phosphoenolpyruvate carboxykinase [GTP] (EC 4.1.1.32)
	gene1376	0.32	Resistance to antibiotics and toxic compounds	DNA-binding heavy metal response regulator
	gene4530	0.32	Plant-Prokaryote DOE project	Transcription regulator [contains diacylglycerol kinase catalytic domain]
	gene2795	0.31	Aminosugars	N-Acetyl-D-glucosamine ABC transport system, permease protein 1
	gene1829	0.31	Isoprenoids	Geranylgeranyl pyrophosphate synthetase (EC 2.5.1.29)
	gene4912	0.31	Resistance to antibiotics and toxic compounds	Vancomycin response regulator VanR
	gene3270	0.31	Central carbohydrate metabolism	Phosphogluconate dehydratase (EC 4.2.1.12)
	gene1601	0.31	Three hypotheticals linked to lipoprotein biosynthesis	FIG018229: hypothetical protein
	gene806	0.31	Isoprenoids	Candidate gene for the hypothesized phosphomevalonate decarboxylase
	gene2996	0.31	Oxidative stress	Non-specific DNA-binding protein Dps

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year	gene	$\log_2$Fold Change	level1	level4
	gene938	0.31	Resistance to antibiotics and toxic compounds	Chromate resistance protein ChrB
	gene1895	0.31	Catabolism of an unknown compound	Glutamine synthetase family protein in hypothetical Actinobacterial gene cluster
	gene554	0.31	Arginine; urea cycle, polyamines	Arginine deiminase (EC 3.5.3.6)
	gene289	0.31	Inorganic sulfur assimilation	ABC-type probable sulfate transporter, ATPase component
	gene4082	0.31	Resistance to antibiotics and toxic compounds	RND efflux system, membrane fusion protein CmeA
	gene862	0.31	Metabolism of central aromatic intermediates	Catechol 1,2-dioxygenase 1 (EC 1.13.11.1)
	gene1379	0.31	DNA Metabolism	DNA-binding protein HBsu
	gene1294	0.31	Central carbohydrate metabolism	Dihydrolipoamide succinyltransferase component (E2) of 2-oxoglutarate dehydrogenase complex (EC 2.3.1.61)
	gene1263	0.31	Central carbohydrate metabolism	DHA-specific EI component
	gene3958	0.31	Phosphorus Metabolism	response regulator in two-component regulatory system with PhoQ
	gene1299	0.31	Pyrimidines	Dihydroorotate dehydrogenase electron transfer subunit (EC 1.3.3.1)
	gene2743	0.31	Carbohydrates	Monooxygenase component A
	gene2737	0.31	Folate and pterines	Molybdopterin-guanine dinucleotide biosynthesis protein MobA
	gene3795	0.30	Miscellaneous	Putative membrane-bound ClpP-class protease associated with aq_911
	gene863	0.30	Metabolism of central aromatic intermediates	Catechol 2,3-dioxygenase (EC 1.13.11.2)
	gene3028	0.30	Lipoic acid	Octanoate-[acyl-carrier-protein]-protein-N-octanoyltransferase
	gene4392	0.30	Lysine, threonine, methionine, and cysteine	Sulfite reductase [NADPH] hemoprotein beta-component (EC 1.8.1.2)
	gene1751	0.30	Central carbohydrate metabolism	Fructose-1,6-bisphosphatase, GlpX type (EC 3.1.3.11)
	gene495	0.30	Catabolism of an unknown compound	Amino acid/metabolite permease in hypothetical Actinobacterial gene cluster
	gene1414	0.30	DNA repair	Domain often clustered or fused with uracil-DNA glycosylase
	gene2672	0.30	Lysine, threonine, methionine, and cysteine	Methionine gamma-lyase (EC 4.4.1.11)
	gene1013	0.30	RNA processing and modification	COG1355, Predicted dioxygenase
	gene4973	0.30	Fermentation	Xylulose-5-phosphate phosphoketolase (EC 4.1.2.9)
	gene1729	0.30	Respiration	Formate hydrogenlyase subunit 4
	gene3522	0.30	Transcription	Predicted transcriptional regulator of cysteine synthase, Rrf2 family
	gene4940	0.30	Purines	Xanthine and CO dehydrogenases maturation factor, XdhC/CoxF family
	gene38	0.30	Quinone cofactors	1,4-dihydroxy-2-naphthoate octaprenyltransferase (EC 2.5.1.74)
	gene1719	0.30	Respiration	Formate dehydrogenase O beta subunit (EC 1.2.1.2)
	gene1592	0.30	proteosome related	FIG005666: putative helicase
	gene4775	0.30	Electron accepting reactions	Ubiquinol–cytochrome c reductase, cytochrome B subunit (EC 1.10.2.2)
	gene3164	0.30	Oxidative stress	Peroxidase (EC 1.11.1.7)
	gene4942	0.30	Purines	Xanthine dehydrogenase, FAD binding subunit (EC 1.17.1.4)
	gene1312	0.30	Sulfur Metabolism	Dimethylsulfoniopropionate (DMSP) acyl CoA transferase DddD
	gene2275	0.30	Cytochrome biogenesis	Iron-sulfur cluster regulator SufR
	gene247	0.30	Di- and oligosaccharides	6-phospho-beta-glucosidase (EC 3.2.1.86)
	gene3960	0.30	Nitrogen Metabolism	Response regulator NasT
	gene4617	0.29	Protein biosynthesis	Translation elongation factor G-related protein
	gene4461	0.29	Cofactors, Vitamins, Prosthetic Groups, Pigments	Thiazole biosynthesis protein ThiG
	gene3023	0.29	Lysine, threonine, methionine, and cysteine	O-acetylhomoserine sulfhydrylase (EC 2.5.1.49)
	gene2594	0.29	Oxidative stress	Manganese superoxide dismutase (EC 1.15.1.1)
	gene504	0.29	Protein degradation	Aminopeptidase YpdF (MP-, MA-, MS-, AP-, NP- specific)
	gene1436	0.29	Fermentation	Electron transfer flavoprotein, beta subunit

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year	gene	$\log_2$Fold Change	level1	level4
	gene517	0.29	Nitrogen Metabolism	Anaerobic nitric oxide reductase flavorubredoxin
	gene2282	0.29	Aromatic amino acids and derivatives	Isochorismatase (EC 3.3.2.1)
	gene4061	0.29	Transcription	RNA polymerase sigma factor RpoH-related protein
	gene1903	0.29	Tetrapyrroles	Glutamyl-tRNA reductase (EC 1.2.1.70)
	gene633	0.29	DNA repair	ATP-dependent DNA ligase (EC 6.5.1.1)
	gene2384	0.29	Organic acids	Lactate-responsive regulator LldR in Firmicutes, GntR family
	gene4708	0.29	Protein translocation across cytoplasmic membrane	Twin-arginine translocation protein TatA
	gene3449	0.29	Membrane Transport	Predicted cobalt transporter CbtA
	gene1167	0.29	Respiration	Cytochrome c2
	gene3600	0.29	One-carbon Metabolism	Propionyl-CoA carboxylase beta chain (EC 6.4.1.3)
	gene115	0.29	Arginine; urea cycle, polyamines	2,4-diaminopentanoate dehydrogenase (EC 1.4.1.12)
	gene3717	0.29	Protein degradation	Pup ligase PafA' paralog, possible component of postulated heterodimer PafA-PafA'
	gene3917	0.29	NAD and NADP	Quinolinate synthetase (EC 2.5.1.72)
	gene2804	0.28	Aminosugars	N-acetylglucosamine kinase of eukaryotic type (EC 2.7.1.59)
	gene4164	0.28	Folate and pterines	Sepiapterin reductase (EC 1.1.1.153)
	gene3379	0.28	Central carbohydrate metabolism	Polyphosphate glucokinase (EC 2.7.1.63)
	gene4480	0.28	Lysine, threonine, methionine, and cysteine	Threonine dehydrogenase and related Zn-dependent dehydrogenases
	gene1968	0.28	Cofactors, Vitamins, Prosthetic Groups, Pigments	Glycine oxidase ThiO (EC 1.4.3.19)
	gene4489	0.28	Folate and pterines	Thymidylate synthase thyX (EC 2.1.1.-)
	gene1107	0.28	Clustering-based subsystems	Cyclopropane-fatty-acyl-phospholipid synthase (EC 2.1.1.79)
	gene4860	0.28	Pyrimidines	Uracil phosphoribosyltransferase (EC 2.4.2.9)
	gene1259	0.28	Monosaccharides	Deoxyribose-phosphate aldolase (EC 4.1.2.4)
	gene3557	0.28	Clustering-based subsystems	probable iron binding protein from the HesB_IscA_SufA family
	gene516	0.28	Electron donating reactions	Anaerobic glycerol-3-phosphate dehydrogenase subunit C (EC 1.1.5.3)
	gene1123	0.28	Lysine, threonine, methionine, and cysteine	Cysteine synthase A (EC 2.5.1.47)
	gene1209	0.28	Proline and 4-hydroxyproline	D-amino-acid oxidase (EC 1.4.3.3)
	gene3527	0.28	Transcription	Predicted transcriptional regulator of sulfate adenylyltransferase, Rrf2 family
	gene1896	0.28	Cell Wall and Capsule	Glutamine synthetase type I (EC 6.3.1.2)
	gene252	0.28	Central carbohydrate metabolism	6-phosphogluconolactonase (EC 3.1.1.31), eukaryotic type
	gene3523	0.27	Aminosugars	Predicted transcriptional regulator of N-Acetylglucosamine utilization, GntR family
	gene634	0.27	DNA repair	ATP-dependent DNA ligase (EC 6.5.1.1) clustered with Ku protein, LigD
	gene1214	0.27	Resistance to antibiotics and toxic compounds	D-cysteine desulfhydrase (EC 4.4.1.15)
	gene2286	0.27	Central carbohydrate metabolism	Isocitrate dehydrogenase [NAD] (EC 1.1.1.41)
	gene3764	0.27	Respiration	Putative formate dehydrogenase oxidoreductase protein
	gene3175	0.27	Phage Host Interactions	Phage endolysin
	gene2134	0.27	Oxidative stress	Hydroxyacylglutathione hydrolase (EC 3.1.2.6)
	gene2005	0.27	Folate and pterines	GTP cyclohydrolase I (EC 3.5.4.16) type 1
	gene2837	0.27	Lysine, threonine, methionine, and cysteine	N-succinyl-L,L-diaminopimelate aminotransferase alternative (EC 2.6.1.17)
	gene4557	0.27	Monosaccharides	Transcriptional regulator in cluster with unspecified monosaccharide ABC transport system
	gene2550	0.27	Putative asociate of RNA polymerase sigma-54 factor rpoN	macromolecule metabolism
	gene73	0.27	Aromatic amino acids and derivatives	2-keto-3-deoxy-D-arabino-heptulosonate-7-phosphate synthase II (EC 2.5.1.54)

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year	gene	$\log_2$Fold Change	level1	level4
	gene1435	0.27	Fermentation	Electron transfer flavoprotein, alpha subunit
	gene3696	0.27	Folate and pterines	Pterin-4-alpha-carbinolamine dehydratase (EC 4.2.1.96)
	gene1452	0.27	DNA repair	Endonuclease V (EC 3.1.21.7)
	gene1535	0.27	Inorganic sulfur assimilation	Ferredoxin–sulfite reductase (EC 1.8.7.1)
	gene2590	0.27	Membrane Transport	Manganese ABC transporter, ATP-binding protein SitB
	gene313	0.27	Central carbohydrate metabolism	Acetoin dehydrogenase E1 component alpha-subunit (EC 1.2.4.-)
	gene3013	0.27	Clustering-based subsystems	Nucleoside-diphosphate-sugar epimerases
	gene1289	0.27	Branched-chain amino acids	Dihydrolipoamide dehydrogenase (EC 1.8.1.4)
	gene4408	0.27	Oxidative stress	Superoxide dismutase [Cu-Zn] precursor (EC 1.15.1.1)
	gene1250	0.27	Proline and 4-hydroxyproline	Delta-1-pyrroline-5-carboxylate dehydrogenase (EC 1.5.1.12)
	gene1710	0.27	DNA repair	Formamidopyrimidine-DNA glycosylase (EC 3.2.2.23)
	gene2204	0.27	Pigment biosynthesis	Indigoidine synthase A-like protein, uncharacterized enzyme involved in pigment biosynthesis
	gene465	0.27	NA	Alpha-glucosides-binding periplasmic protein AglE precursor
	gene1156	0.27	Protein folding	Cytochrome c-type biogenesis protein CcdA (DsbD analog)
	gene3015	0.27	Protein degradation	Nucleotide excision repair protein, with UvrB/UvrC motif
	gene2834	0.27	Nucleosides and Nucleotides	N-methylhydantoinase B (EC 3.5.2.14)
	gene1070	0.27	Amino Acids and Derivatives	Creatinine amidohydrolase (EC 3.5.2.10)
	gene4090	0.27	Flagellar motility in Prokaryota	Rrf2 family transcriptional regulator
	gene980	0.26	Tetrapyrroles	Cobalt-precorrin-6 synthase, anaerobic
	gene3149	0.26	Cofactors, Vitamins, Prosthetic Groups, Pigments	Periplasmic aromatic aldehyde oxidoreductase, molybdenum binding subunit YagR
	gene621	0.26	Protein degradation	ATP-dependent Clp protease, ATP-binding subunit ClpC
	gene1064	0.26	Resistance to antibiotics and toxic compounds	Copper-translocating P-type ATPase (EC 3.6.3.4)
	gene2192	0.26	Purines	Hypoxanthine-guanine phosphoribosyltransferase (EC 2.4.2.8)
	gene4577	0.26	ABC transporters	Transcriptional regulator YbiH, TetR family
	gene2779	0.26	Sugar alcohols	Multiple polyol-specific dehydrogenase (EC 1.1.1.-)
	gene4926	0.26	Tetrapyrroles	Vitamin B12 ABC transporter, B12-binding component BtuF
	gene2418	0.26	Lipoic acid	Lipoate synthase
	gene1764	0.26	Central carbohydrate metabolism	Fumarate hydratase class II (EC 4.2.1.2)
	gene4636	0.26	Electron accepting reactions	Transport ATP-binding protein CydD
	gene579	0.26	Resistance to antibiotics and toxic compounds	Arsenical resistance operon repressor
	gene841	0.26	Stress Response	Carbon starvation protein A
	gene1857	0.26	Aminosugars	Glucosamine-6-phosphate deaminase [isomerizing], alternative (EC 3.5.99.6)
	gene2231	0.26	Purines	Inosine-5'-monophosphate dehydrogenase (EC 1.1.1.205)
	gene3351	0.26	Methylamine utilization	PlcB, ORFX, ORFP, ORFB, ORFA, ldh gene
	gene2953	0.26	Nitrogen Metabolism	Nitrate/nitrite transporter
	gene4920	0.26	Detoxification	Various polyols ABC transporter, permease component 2
	gene3128	0.26	Clustering-based subsystems	Peptide deformylase (EC 3.5.1.88)
	gene4350	0.26	One-carbon Metabolism	Succinate dehydrogenase flavoprotein subunit (EC 1.3.99.1)
	gene3895	0.26	Central carbohydrate metabolism	Pyruvate carboxyl transferase subunit B (EC 6.4.1.1)
	gene400	0.26	Protein biosynthesis	Alanyl-tRNA synthetase domain protein
	gene2833	0.26	Nucleosides and Nucleotides	N-methylhydantoinase A (EC 3.5.2.14)
	gene4777	0.26	Electron accepting reactions	Ubiquinol-cytochrome C reductase iron-sulfur subunit (EC 1.10.2.2)
	gene4856	0.26	Miscellaneous	UPF0434 protein YcaR
	gene2601	0.26	Monosaccharides	Mannose-1-phosphate guanylyltransferase (EC 2.7.7.13)
	gene3464	0.26	Polysaccharides	Predicted glycogen synthase, ADP-glucose transglucosylase (EC 2.4.1.21), Actinobacterial type

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year	gene	$\log_2$Fold Change	level1	level4
	gene4377	0.26	Lysine, threonine, methionine, and cysteine	Sulfate adenylyltransferase subunit 2 (EC 2.7.7.4)
	gene2125	0.26	Respiration	Hydrogenase-4 component F (EC 1.-.-.-)
	gene2104	0.26	Folate and pterines	Homogentisate 1,2-dioxygenase (EC 1.13.11.5)
	gene4516	0.26	Monosaccharides	Transaldolase (EC 2.2.1.2)
	gene4908	0.26	Alanine, serine, and glycine	Valine–pyruvate aminotransferase (EC 2.6.1.66)
	gene4139	0.25	Selenoproteins	Selenocysteine-specific translation elongation factor
	gene1288	0.25	Central carbohydrate metabolism	Dihydrolipoamide acyltransferase component of branched-chain alpha-keto acid dehydrogenase complex (EC 2.3.1.168)
	gene106	0.25	Fermentation	2,3-butanediol dehydrogenase, R-alcohol forming, (R)- and (S)-acetoin-specific (EC 1.1.1.4)
	gene498	0.25	Aromatic amino acids and derivatives	Aminodeoxychorismate lyase (EC 4.1.3.38)
	gene3391	0.25	Tetrapyrroles	Porphobilinogen synthase (EC 4.2.1.24)
	gene1865	0.25	Central carbohydrate metabolism	Glucose-6-phosphate 1-dehydrogenase (EC 1.1.1.49)
	gene1224	0.25	Capsular and extracellular polysacchrides	D-glycero-D-manno-heptose 1-phosphate guanosyltransferase
	gene2284	0.25	Aromatic amino acids and derivatives	Isochorismate synthase (EC 5.4.4.2)
	gene2864	0.25	Central carbohydrate metabolism	NAD-dependent malic enzyme (EC 1.1.1.38)
	gene2860	0.25	Respiration	NAD-dependent formate dehydrogenase beta subunit
	gene1530	0.25	Inorganic sulfur assimilation	Ferredoxin
	gene1554	0.25	Tetrapyrroles	Ferrochelatase, protoheme ferro-lyase (EC 4.99.1.1)
	gene216	0.25	Tetrapyrroles	5-aminolevulinate synthase (EC 2.3.1.37)
	gene4449	0.25	Cofactors, Vitamins, Prosthetic Groups, Pigments	Thiamin ABC transporter, ATPase component
	gene4815	0.25	Oxidative stress	Uncharacterized monothiol glutaredoxin ycf64-like
	gene2381	0.25	Central carbohydrate metabolism	Lactate 2-monooxygenase (EC 1.13.12.4)
	gene2589	0.25	Metabolism of central aromatic intermediates	mandelate racemase/muconate lactonizing enzyme family protein
	gene1501	0.24	DNA repair	Excinuclease ABC subunit C
	gene4141	0.24	Selenoproteins	Selenoprotein O and cysteine-containing homologs
	gene1994	0.24	Oxidative stress	Glycosyltransferase MshA involved in mycothiol biosynthesis (EC 2.4.1.-)
	gene2295	0.24	Isoprenoids	Isopentenyl-diphosphate delta-isomerase (EC 5.3.3.2)
	gene3339	0.24	Isoprenoids	Phytoene dehydrogenase and related proteins
	gene4339	0.24	Biotin	Substrate-specific component BioY of biotin ECF transporter
	gene3770	0.24	Organic sulfur assimilation	Putative glutathione transporter,solute-binding component
	gene2780	0.24	Di- and oligosaccharides	Multiple sugar ABC transporter, ATP-binding protein
	gene2690	0.24	Folate and pterines	Methylenetetrahydrofolate dehydrogenase (NADP+) (EC 1.5.1.5) / Methenyltetrahydrofolate cyclohydrolase (EC 3.5.4.9)
	gene4026	0.24	Heat shock	Ribosomal RNA small subunit methyltransferase E (EC 2.1.1.-)
	gene2895	0.24	Electron donating reactions	NADH-ubiquinone oxidoreductase chain B (EC 1.6.5.3)
	gene4991	0.24	Miscellaneous	YrbA protein
	gene1345	0.24	Recombination related cluster	DNA polymerase X family
	gene1923	0.24	Oxidative stress	Glutathione S-transferase, omega (EC 2.5.1.18)
	gene102	0.24	Quinone cofactors	2-succinyl-5-enolpyruvyl-6-hydroxy-3-cyclohexene-1-carboxylic-acid synthase (EC 2.2.1.9)
	gene283	0.24	Cytochrome biogenesis	ABC-type multidrug transport system, ATPase component
	gene4464	0.24	Plant-Prokaryote DOE project	ThiJ/PfpI family protein
	gene1120	0.24	Alanine, serine, and glycine	Cysteine desulfurase (EC 2.8.1.7), SufS subfamily
	gene145	0.24	Fermentation	3-hydroxybutyryl-CoA dehydrogenase (EC 1.1.1.157)
	gene2205	0.24	Aromatic amino acids and derivatives	Indole-3-glycerol phosphate synthase (EC 4.1.1.48)

Table D.1 continued from previous page

year	gene	$\log_2$Fold Change	level1	level4
	gene987	0.24	Resistance to antibiotics and toxic compounds	Cobalt-zinc-cadmium resistance protein CzcD
	gene3016	0.24	Purines	Nucleotide pyrophosphatase (EC 3.6.1.9)
	gene478	0.24	Di- and oligosaccharides	Alpha,alpha-trehalose-phosphate synthase [UDP-forming] (EC 2.4.1.15)
	gene1117	0.24	Lysine, threonine, methionine, and cysteine	Cysteine desulfurase (EC 2.8.1.7)
	gene3398	0.24	Monosaccharides	Possible alpha-xyloside ABC transporter, permease component
	gene1810	0.24	Quinone cofactors	Gene SCO4494, often clustered with other genes in menaquinone via futalosine pathway
	gene1300	0.24	Pyrimidines	Dihydroorotate dehydrogenase, catalytic subunit (EC 1.3.3.1)
	gene2884	0.24	Electron donating reactions	NADH dehydrogenase (EC 1.6.99.3)
	gene3969	0.24	RNA processing and modification	Rhodanese-like domain protein
	gene309	0.24	Branched-chain amino acids	Acetoacetyl-CoA synthetase (EC 6.2.1.16)
	gene688	0.24	Protein degradation	Bacterial proteasome-activating AAA-ATPase (PAN)
	gene1876	0.24	Cell Wall and Capsule	Glutamate racemase (EC 5.1.1.3)
	gene752	0.24	Osmotic stress	Betaine aldehyde dehydrogenase (EC 1.2.1.8)
	gene1451	0.24	DNA repair	Endonuclease IV (EC 3.1.21.2)
	gene57	0.24	Monosaccharides	2-dehydro-3-deoxyphosphogluconate aldolase (EC 4.1.2.14)
	gene953	0.23	One-carbon Metabolism	Citrate synthase (si) (EC 2.3.3.1)
	gene520	0.23	Aromatic amino acids and derivatives	Anthranilate phosphoribosyltransferase (EC 2.4.2.18)
	gene3488	0.23	Membrane Transport	Predicted molybdate-responsive regulator YvgK in bacilli
	gene600	0.23	Protein biosynthesis	Aspartyl-tRNA(Asn) amidotransferase subunit C (EC 6.3.5.6)
	gene1238	0.23	Monosaccharides	D-xylose transport ATP-binding protein XylG
	gene2329	0.23	NAD and NADP	L-aspartate oxidase (EC 1.4.3.16)
	gene4941	0.23	Purines	Xanthine dehydrogenase iron-sulfur subunit (EC 1.17.1.4)
	gene2094	0.23	Histidine Metabolism	Histidinol-phosphatase [alternative form] (EC 3.1.3.15)
	gene1086	0.23	Resistance to antibiotics and toxic compounds	Cu(I)-responsive transcriptional regulator
	gene1450	0.23	DNA repair	Endonuclease III (EC 4.2.99.18)
	gene1998	0.23	Protein biosynthesis	Glycyl-tRNA synthetase (EC 6.1.1.14)
	gene4542	0.23	Di- and oligosaccharides	Transcriptional activator of maltose regulon, MalT
	gene4472	0.23	Selenoproteins	Thioredoxin
	gene1063	0.23	Resistance to antibiotics and toxic compounds	Copper-sensing two-component system response regulator CusR
	gene4454	0.23	Folate and pterines	Thiamin-phosphate pyrophosphorylase (EC 2.5.1.3)
	gene1118	0.23	Alanine, serine, and glycine	Cysteine desulfurase (EC 2.8.1.7), IscS subfamily
	gene3783	0.23	Clustering-based subsystems	Putative Holliday junction resolvase (EC 3.1.-.-)
	gene2270	0.23	Phages, Prophages	Iron-sulfur cluster assembly protein SufB
	gene2007	0.23	Folate and pterines	GTP cyclohydrolase II (EC 3.5.4.25)
	gene635	0.23	DNA repair	ATP-dependent DNA ligase (EC 6.5.1.1) LigC
	gene1115	0.23	Lysine, threonine, methionine, and cysteine	Cystathionine gamma-lyase (EC 4.4.1.1)
	gene2796	0.22	Aminosugars	N-Acetyl-D-glucosamine ABC transport system, permease protein 2
	gene1981	0.22	Central carbohydrate metabolism	Glycolate dehydrogenase (EC 1.1.99.14), subunit GlcD
	gene12	0.22	Branched-chain amino acids	(R)-citramalate synthase (EC 2.3.1.182)
	gene3970	0.22	Clustering-based subsystems	Rhodanese-related sulfurtransferase
	gene1283	0.22	Lysine, threonine, methionine, and cysteine	Dihydrodipicolinate synthase (EC 4.2.1.52)
	gene682	0.22	Organic acids	B12 binding domain of Methylmalonyl-CoA mutase (EC 5.4.99.2)
	gene1295	0.22	Folate and pterines	Dihydroneopterin aldolase (EC 4.1.2.25)
	gene1845	0.22	Monosaccharides	Gluconate 2-dehydrogenase (EC 1.1.99.3), membrane-bound, flavoprotein

Table D.1 continued from previous page

year	gene	$\log_2$Fold Change	level1	level4
	gene1949	0.22	Electron donating reactions	Glycerol-3-phosphate dehydrogenase (EC 1.1.5.3)
	gene193	0.22	Isoprenoids	4-hydroxy-3-methylbut-2-enyl diphosphate reductase (EC 1.17.1.2)
	gene203	0.22	Folate and pterines	4-hydroxyphenylpyruvate dioxygenase (EC 1.13.11.27)
	gene3749	0.22	Monosaccharides	Putative deoxyribose-specific ABC transporter, permease protein
	gene1457	0.22	Fatty acids	Enoyl-[acyl-carrier-protein] reductase [NADH] (EC 1.3.1.9)
	gene2066	0.21	Isoprenoids	Heptaprenyl diphosphate synthase component II (EC 2.5.1.30)
	gene878	0.21	Cell Division and Cell Cycle	Cell division initiation protein DivIVA
	gene1873	0.21	Acid stress	Glutamate decarboxylase (EC 4.1.1.15)
	gene4006	0.21	Monosaccharides	Ribose operon repressor
	gene1713	0.21	Respiration	Formate dehydrogenase chain D (EC 1.2.1.2)
	gene3269	0.21	Chromosome Replication	Phosphoglucomutase (EC 5.4.2.2)
	gene3342	0.21	Isoprenoids	Phytoene desaturase, pro-zeta-carotene producing (EC 1.-.-.-)
	gene27	0.21	Phosphorus Metabolism	1-acyl-sn-glycerol-3-phosphate acyltransferase (EC 2.3.1.51)
	gene4000	0.21	Monosaccharides	Ribose 5-phosphate isomerase B (EC 5.3.1.6)
	gene557	0.21	Arginine; urea cycle, polyamines	Arginine pathway regulatory protein ArgR, repressor of arg regulon
	gene3099	0.21	Coenzyme A	Pantoate–beta-alanine ligase (EC 6.3.2.1)
	gene4029	0.21	Protein biosynthesis	Ribosomal subunit interface protein
	gene1285	0.21	Folate and pterines	Dihydrofolate reductase (EC 1.5.1.3)
	gene978	0.21	Tetrapyrroles	Cobalt-precorrin-3b C17-methyltransferase
	gene603	0.21	Histidine Metabolism	ATP phosphoribosyltransferase (EC 2.4.2.17)
	gene2232	0.21	Purines	Inosine-uridine preferring nucleoside hydrolase (EC 3.2.2.1)
	gene4650	0.21	Di- and oligosaccharides	Trehalose phosphorylase (EC 2.4.1.64)
	gene877	0.21	Dormancy and Sporulation	Cell division inhibitor
	gene2056	0.21	Iron acquisition and metabolism	Hemoglobin-dependent two component system response regulator HrrA
	gene2685	0.21	DNA repair	Methylated-DNA–protein-cysteine methyltransferase (EC 2.1.1.63)
	gene1544	0.21	Respiration	Ferredoxin, 2Fe-2S
	gene396	0.21	Alanine, serine, and glycine	Alanine racemase (EC 5.1.1.1)
	gene3741	0.21	Nitrogen Metabolism	putative cytochrome P450 hydroxylase
	gene3245	0.21	Phosphorus Metabolism	Phosphate transport system regulatory protein PhoU
	gene617	0.21	Protein degradation	ATP-dependent Clp protease adaptor protein ClpS
	gene4221	0.21	Oxidative stress	Similar to Hydroxyacylglutathione hydrolase, but in an organism lacking glutathione biosynthesis
	gene4885	0.21	Arginine; urea cycle, polyamines	Urease gamma subunit (EC 3.5.1.5)
	gene2272	0.20	RNA processing and modification	Iron-sulfur cluster assembly scaffold protein IscU
	gene2943	0.20	Oxidative stress	Nicotinate phosphoribosyltransferase (EC 2.4.2.11)
	gene3897	0.20	Central carbohydrate metabolism	Pyruvate dehydrogenase E1 component (EC 1.2.4.1)
	gene3839	0.20	Inorganic sulfur assimilation	Putative sulfate permease
	gene1908	0.20	Protein biosynthesis	Glutamyl-tRNA(Gln) synthetase (EC 6.1.1.24)
	gene3514	0.20	DNA polymerase III epsilon cluster	Predicted signal-transduction protein containing cAMP-binding and CBS domains
	gene3298	0.20	Coenzyme A	Phosphopantetheine adenylyltransferase (EC 2.7.7.3)
	gene4068	0.20	Transcription	RNA polymerase sigma-70 factor
	gene3892	0.20	Proline and 4-hydroxyproline	Pyrroline-5-carboxylate reductase (EC 1.5.1.2)
	gene4605	0.20	Central carbohydrate metabolism	Transketolase (EC 2.2.1.1)
	gene3546	0.20	Clustering-based subsystems	Probable carboxyvinyl-carboxyphosphonate phosphorylmutase (EC 2.7.8.23)
	gene1567	0.20	Heat shock	FIG001341: Probable Fe(2+)-trafficking protein YggX
	gene103	0.20	Quinone cofactors	2-succinyl-6-hydroxy-2,4-cyclohexadiene-1-carboxylate synthase (EC 4.2.99.20)
	gene4687	0.20	Protein biosynthesis	tRNA-Gly
	gene1960	0.20	Alanine, serine, and glycine	Glycine cleavage system H protein

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year	gene	$\log_2$Fold Change	level1	level4
	gene4490	0.20	Protein degradation	TldD family protein, Actinobacterial subgroup
	gene322	0.20	Central carbohydrate metabolism	Acetyl-CoA synthetase (ADP-forming) alpha and beta chains, putative
	gene3414	0.20	Potassium metabolism	Potassium channel protein
	gene4410	0.20	Oxidative stress	Superoxide dismutase [Fe] (EC 1.15.1.1)
	gene2244	0.20	Bacteriophage integration/excision/lysogeny	Integrase
	gene4417	0.19	Di- and oligosaccharides	Tagatose 1,6-bisphosphate aldolase (EC 4.1.2.40)
	gene2586	0.19	Polysaccharides	Maltose/maltodextrin ABC transporter, substrate binding periplasmic protein MalE
	gene3086	0.19	Periplasmic Stress	Outer membrane stress sensor protease DegQ, serine protease
	gene1766	0.19	Electron donating reactions	Fumarate reductase flavoprotein subunit (EC 1.3.99.1)
	gene4031	0.19	Protein biosynthesis	Ribosomal-protein-S18p-alanine acetyltransferase (EC 2.3.1.-)
	gene2058	0.19	Stress Response	Hemoglobin-like protein HbO
	gene3806	0.19	Carbohydrates	Putative oxidoreductase YeaE, aldo/keto reductase family
	gene3390	0.19	Tetrapyrroles	Porphobilinogen deaminase (EC 2.5.1.61)
	gene3916	0.19	NAD and NADP	Quinolinate phosphoribosyltransferase [decarboxylating] (EC 2.4.2.19)
	gene1292	0.19	Central carbohydrate metabolism	Dihydrolipoamide dehydrogenase of branched-chain alpha-keto acid dehydrogenase (EC 1.8.1.4)
	gene166	0.19	Metabolism of central aromatic intermediates	3-oxoadipate CoA-transferase subunit A (EC 2.8.3.6)
	gene4487	0.19	Pyrimidines	Thymidylate kinase (EC 2.7.4.9)
	gene3321	0.19	Alanine, serine, and glycine	Phosphoserine phosphatase (EC 3.1.3.3)
	gene4944	0.19	Purines	Xanthine dehydrogenase, molybdenum binding subunit (EC 1.17.1.4)
	gene2178	0.19	Protein biosynthesis	Hypothetical protein YaeJ with similarity to translation release factor
	gene4534	0.19	Resistance to antibiotics and toxic compounds	Transcription repressor of multidrug efflux pump acrAB operon, TetR (AcrR) family
	gene856	0.19	Regulation and Cell signaling	Catabolite control protein A
	gene642	0.19	Protein degradation	ATP-dependent protease HslV (EC 3.4.25.-)
	gene4352	0.19	One-carbon Metabolism	Succinate dehydrogenase iron-sulfur protein (EC 1.3.99.1)
	gene4179	0.19	Stress Response	Serine-protein kinase rsbW (EC 2.7.11.1)
	gene1754	0.19	Central carbohydrate metabolism	Fructose-bisphosphate aldolase class I (EC 4.1.2.13)
	gene2202	0.19	Cell Wall and Capsule	Inactive homolog of metal-dependent proteases, putative molecular chaperone
	gene356	0.19	Central carbohydrate metabolism	Acylphosphate phosphohydrolase (EC 3.6.1.7), putative
	gene437	0.19	Nitrogen Metabolism	Allantoinase (EC 3.5.2.5)
	gene3047	0.19	Oxidative stress	Organic hydroperoxide resistance protein
	gene785	0.19	Central carbohydrate metabolism	Branched-chain alpha-keto acid dehydrogenase, E1 component, alpha subunit (EC 1.2.4.4)
	gene490	0.19	Purines	Amidophosphoribosyltransferase (EC 2.4.2.14)
	gene1114	0.18	Lysine, threonine, methionine, and cysteine	Cystathionine beta-synthase (EC 4.2.1.22)
	gene2945	0.18	Tetrapyrroles	Nicotinate-nucleotide–dimethylbenzimidazole phosphoribosyltransferase (EC 2.4.2.21)
	gene2357	0.18	Osmotic stress	L-proline glycine betaine ABC transport system permease protein ProV (TC 3.A.1.12.1)
	gene1246	0.18	Detoxification	DedA protein
	gene319	0.18	Fermentation	Acetyl-CoA acetyltransferase (EC 2.3.1.9)
	gene1018	0.18	RNA processing and modification	COG1720: Uncharacterized conserved protein
	gene1566	0.18	DNA recombination	FIG000859: hypothetical protein
	gene1272	0.18	Riboflavin, FMN, FAD	Diaminohydroxyphosphoribosylaminopyrimidine deaminase (EC 3.5.4.26)
	gene1318	0.18	ABC transporters	Dipeptide transport system permease protein DppC (TC 3.A.1.5.2)
	gene187	0.18	Metabolism of central aromatic intermediates	4-carboxymuconolactone decarboxylase (EC 4.1.1.44)

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year	gene	$\log_2$Fold Change	level1	level4
	gene2100	0.18	Fatty acids	Holo-[acyl-carrier protein] synthase (EC 2.7.8.7)
	gene2938	0.18	Oxidative stress	Nicotinamidase (EC 3.5.1.19)
	gene2385	0.18	Oxidative stress	Lactoylglutathione lyase (EC 4.4.1.5)
	gene2686	0.18	Branched-chain amino acids	Methylcrotonyl-CoA carboxylase biotin-containing subunit (EC 6.4.1.4)
	gene2816	0.18	Cell Wall and Capsule	N-acetylmuramic acid 6-phosphate etherase (EC 4.2.-.-)
	gene4177	0.18	One-carbon Metabolism	Serine–glyoxylate aminotransferase (EC 2.6.1.45)
	gene436	0.18	Nitrogen Metabolism	Allantoicase (EC 3.5.3.4)
	gene1886	0.18	Tetrapyrroles	Glutamate-1-semialdehyde aminotransferase (EC 5.4.3.8)
	gene214	0.18	Inorganic sulfur assimilation	4Fe-4S ferredoxin, iron-sulfur binding
	gene222	0.18	Aromatic amino acids and derivatives	5-carboxymethyl-2-oxo-hex-3- ene-1,7-dioate decarboxylase (EC 4.1.1.68)
	gene30	0.18	Isoprenoid/cell wall biosynthesis: PREDICTED UNDECAPRENYL DIPHOSPHATE PHOSPHATASE	1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate synthase (EC 1.17.7.1)
	gene935	0.18	Aromatic amino acids and derivatives	Chorismate mutase I (EC 5.4.99.5)
	gene3973	0.18	Riboflavin, FMN, FAD	Riboflavin synthase alpha chain (EC 2.5.1.9)
	gene985	0.18	Resistance to antibiotics and toxic compounds	Cobalt-zinc-cadmium resistance protein
	gene337	0.17	One-carbon Metabolism	Aconitate hydratase (EC 4.2.1.3)
	gene1890	0.17	NAD and NADP	Glutamine amidotransferase chain of NAD synthetase
	gene3024	0.17	Quinone cofactors	O-succinylbenzoate-CoA synthase (EC 4.2.1.-)
	gene3227	0.17	Peripheral pathways for catabolism of aromatic compounds	Phenylpropionate dioxygenase and related ring-hydroxylating dioxygenases, large terminal subunit
	gene1920	0.17	Oxidative stress	Glutathione S-transferase (EC 2.5.1.18)
	gene788	0.17	Branched-chain amino acids	Branched-chain amino acid aminotransferase (EC 2.6.1.42)
	gene1940	0.17	Sugar alcohols	Glycerol kinase (EC 2.7.1.30)
	gene2724	0.17	Folate and pterines	Molybdenum cofactor biosynthesis protein MoaB
	gene871	0.17	Phospholipids	CDP-diacylglycerol–glycerol-3-phosphate 3-phosphatidyltransferase (EC 2.7.8.5)
	gene1794	0.17	Plant-Prokaryote DOE project	Gamma-glutamyl phosphate reductase (EC 1.2.1.41)
	gene598	0.17	Protein biosynthesis	Aspartyl-tRNA(Asn) amidotransferase subunit A (EC 6.3.5.6)
	gene3898	0.17	Central carbohydrate metabolism	Pyruvate dehydrogenase E1 component alpha subunit (EC 1.2.4.1)
	gene2092	0.17	Histidine Metabolism	Histidinol dehydrogenase (EC 1.1.1.23)
	gene3242	0.17	Phosphorus Metabolism	Phosphate transport regulator (distant homolog of PhoU)
	gene1193	0.17	Purines	Cytosine/purine/uracil/thiamine/allantoin permease family protein
	gene3101	0.17	Coenzyme A	Pantothenate kinase type III, CoaX-like (EC 2.7.1.33)
	gene3299	0.17	Coenzyme A	Phosphopantothenoylcysteine decarboxylase (EC 4.1.1.36)
	gene432	0.17	Oxidative stress	Alkyl hydroperoxide reductase subunit C-like protein
	gene4863	0.17	DNA repair	Uracil-DNA glycosylase, family 4
	gene500	0.17	Alanine, serine, and glycine	Aminomethyltransferase (glycine cleavage system T protein) (EC 2.1.2.10)
	gene1274	0.17	Lysine, threonine, methionine, and cysteine	Diaminopimelate epimerase (EC 5.1.1.7)
	gene2398	0.17	Branched-chain amino acids	Leucine dehydrogenase (EC 1.4.1.9)
	gene346	0.17	Fatty acid metabolic cluster	Acyl dehydratase
	gene2892	0.17	Electron donating reactions	NADH ubiquinone oxidoreductase chain A (EC 1.6.5.3)
	gene2698	0.17	One-carbon Metabolism	Methylmalonyl-CoA mutase (EC 5.4.99.2)
	gene2732	0.17	Folate and pterines	Molybdopterin biosynthesis protein MoeA
	gene4586	0.17	Resistance to antibiotics and toxic compounds	Transcriptional regulator, MerR family
	gene573	0.17	Aromatic amino acids and derivatives	Aromatic-L-amino-acid decarboxylase (EC 4.1.1.28)

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year	gene	$\log_2$Fold Change	level1	level4
	gene3541	0.17	Programmed Cell Death and Toxin-antitoxin Systems	Prevent host death protein, Phd antitoxin
	gene3301	0.16	Monosaccharides	Phosphopentomutase (EC 5.4.2.7)
	gene3316	0.16	Purines	Phosphoribosylformylglycinamidine synthase, synthetase subunit (EC 6.3.5.3)
	gene167	0.16	Metabolism of central aromatic intermediates	3-oxoadipate CoA-transferase subunit B (EC 2.8.3.6)
	gene424	0.16	Phosphorus Metabolism	Alkaline phosphatase synthesis transcriptional regulatory protein PhoP
	gene738	0.16	Metabolism of central aromatic intermediates	Beta-ketoadipate enol-lactone hydrolase (EC 3.1.1.24)
	gene1866	0.16	Central carbohydrate metabolism	Glucose-6-phosphate isomerase (EC 5.3.1.9)
	gene3029	0.16	Isoprenoids	Octaprenyl-diphosphate synthase (EC 2.5.1.-)
	gene3036	0.16	ABC transporters	Oligopeptide transport ATP-binding protein OppF (TC 3.A.1.5.1)
	gene1234	0.16	D-tyrosyl-tRNA(Tyr) deacylase (EC 3.1.-.-) cluster	D-tyrosyl-tRNA(Tyr) deacylase
	gene2896	0.16	Electron donating reactions	NADH-ubiquinone oxidoreductase chain C (EC 1.6.5.3)
	gene2642	0.16	Quinone cofactors	Menaquinone via futalosine step 1
	gene374	0.16	Purines	Adenylosuccinate lyase (EC 4.3.2.2)
	gene2109	0.16	Lysine, threonine, methionine, and cysteine	Homoserine O-acetyltransferase (EC 2.3.1.31)
	gene3130	0.16	Protein processing and modification	Peptide methionine sulfoxide reductase MsrB (EC 1.8.4.12)
	gene3129	0.16	Protein processing and modification	Peptide methionine sulfoxide reductase MsrA (EC 1.8.4.11)
	gene3581	0.16	Proline and 4-hydroxyproline	Proline iminopeptidase (EC 3.4.11.5)
	gene161	0.16	Fatty acids	3-oxoacyl-[acyl-carrier protein] reductase (EC 1.1.1.100)
	gene3364	0.16	Fatty Acids, Lipids, and Isoprenoids	Polyhydroxyalkanoic acid synthase
	gene358	0.15	DNA repair	ADA regulatory protein
	gene4851	0.15	Miscellaneous	UPF0234 protein YajQ
	gene314	0.15	Central carbohydrate metabolism	Acetoin dehydrogenase E1 component beta-subunit (EC 1.2.4.-)
	gene2584	0.15	Di- and oligosaccharides	Maltose/maltodextrin ABC transporter, permease protein MalF
	gene1374	0.15	DNA repair	DNA-3-methyladenine glycosylase II (EC 3.2.2.21)
	gene2865	0.15	Oxidative stress	NAD-dependent protein deacetylase of SIR2 family
	gene2006	0.15	Folate and pterines	GTP cyclohydrolase I (EC 3.5.4.16) type 2
	gene2850	0.15	Uni- Sym- and Antiporters	Na+/H+ antiporter NhaA type
	gene2874	0.15	Phosphorus Metabolism	NAD(P) transhydrogenase alpha subunit (EC 1.6.1.2)
	gene1781	0.15	Di- and oligosaccharides	Galactokinase (EC 2.7.1.6)
	gene3261	0.15	Fermentation	Phosphoenolpyruvate carboxylase (EC 4.1.1.31)
	gene3756	0.15	Polysaccharides	putative esterase
	gene1500	0.15	DNA repair	Excinuclease ABC subunit B
	gene121	0.15	RNA processing and modification	2'-5' RNA ligase
	gene2095	0.15	Histidine Metabolism	Histidinol-phosphate aminotransferase (EC 2.6.1.9)
	gene349	0.15	Branched-chain amino acids	Acyl-CoA dehydrogenase, short-chain specific (EC 1.3.99.2)
	gene4045	0.15	Clustering-based subsystems	Ribulose-phosphate 3-epimerase (EC 5.1.3.1)
	gene3320	0.15	Pyridoxine	Phosphoserine aminotransferase (EC 2.6.1.52)
	gene795	0.15	Electron accepting reactions	Butyryl-CoA dehydrogenase (EC 1.3.99.2)
	gene2444	0.15	Alanine, serine, and glycine	Low-specificity L-threonine aldolase (EC 4.1.2.5)
	gene592	0.15	Pyrimidines	Aspartate carbamoyltransferase (EC 2.1.3.2)
	gene2439	0.15	Clustering-based subsystems	Low molecular weight protein tyrosine phosphatase (EC 3.1.3.48)
	gene937	0.15	Aromatic amino acids and derivatives	Chorismate synthase (EC 4.2.3.5)
	gene3972	0.15	Riboflavin, FMN, FAD	Riboflavin kinase (EC 2.7.1.26)
	gene2859	0.14	Respiration	NAD-dependent formate dehydrogenase alpha subunit

Table D.1 continued from previous page

year	gene	$\log_2$Fold Change	level1	level4
	gene2863	0.14	Oxidative stress	NAD-dependent glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12)
	gene4262	0.14	Cell Division and Cell Cycle	Sporulation initiation inhibitor protein Soj
	gene1212	0.14	Fatty Acids, Lipids, and Isoprenoids	D-beta-hydroxybutyrate dehydrogenase (EC 1.1.1.30)
	gene3056	0.14	Pyrimidines	Orotidine 5'-phosphate decarboxylase (EC 4.1.1.23)
	gene4482	0.14	Lysine, threonine, methionine, and cysteine	Threonine synthase (EC 4.2.3.1)
	gene4701	0.14	NAD and NADP	Tryptophan 2,3-dioxygenase (EC 1.13.11.11)
	gene409	0.14	Central carbohydrate metabolism	Aldehyde dehydrogenase B (EC 1.2.1.22)
	gene407	0.14	Metabolism of Aromatic Compounds	Aldehyde dehydrogenase (EC 1.2.1.3), PaaZ
	gene2694	0.14	Branched-chain amino acids	Methylmalonate-semialdehyde dehydrogenase (EC 1.2.1.27)
	gene3272	0.14	Central carbohydrate metabolism	Phosphoglycerate kinase (EC 2.7.2.3)
	gene248	0.14	Monosaccharides	6-phosphofructokinase (EC 2.7.1.11)
	gene2644	0.14	Quinone cofactors	Menaquinone via futalosine step 3
	gene2725	0.14	Folate and pterines	Molybdenum cofactor biosynthesis protein MoaC
	gene596	0.14	Lysine, threonine, methionine, and cysteine	Aspartokinase (EC 2.7.2.4)
	gene2373	0.14	Alanine, serine, and glycine	L-threonine 3-dehydrogenase (EC 1.1.1.103)
	gene1965	0.14	Alanine, serine, and glycine	Glycine dehydrogenase [decarboxylating] (glycine cleavage system P1 protein) (EC 1.4.4.2)
	gene1564	0.14	DNA uptake, competence	FIG000557: hypothetical protein co-occurring with RecR
	gene3580	0.14	Proline and 4-hydroxyproline	Proline dehydrogenase (EC 1.5.99.8) (Proline oxidase)
	gene2703	0.14	Lysine, threonine, methionine, and cysteine	Methylthioribose-1-phosphate isomerase (EC 5.3.1.23)
	gene2689	0.14	One-carbon Metabolism	Methylenetetrahydrofolate dehydrogenase (NADP+) (EC 1.5.1.5)
	gene329	0.14	Fatty acids	Acetyl-coenzyme A carboxyl transferase beta chain (EC 6.4.1.2)
	gene1458	0.13	Fatty acids	Enoyl-[acyl-carrier-protein] reductase [NADPH] (EC 1.3.1.10)
	gene4494	0.13	Protein degradation	TldE/PmbA family protein, Actinobacterial subgroup
	gene1122	0.13	Lysine, threonine, methionine, and cysteine	Cysteine synthase (EC 2.5.1.47)
	gene4673	0.13	RNA processing and modification	tRNA dihydrouridine synthase B (EC 1.-.-.-)
	gene3055	0.13	Plant-Prokaryote DOE project	Orotate phosphoribosyltransferase (EC 2.4.2.10)
	gene362	0.13	Purines	Adenine phosphoribosyltransferase (EC 2.4.2.7)
	gene3238	0.13	Phosphorus Metabolism	Phosphate regulon transcriptional regulatory protein PhoB (SphR)
	gene234	0.13	Lysine, threonine, methionine, and cysteine	5-methyltetrahydropteroyltriglutamate–homocysteine methyltransferase (EC 2.1.1.14)
	gene1744	0.13	Di- and oligosaccharides	Fructokinase (EC 2.7.1.4)
	gene1251	0.13	Pyrimidines	Deoxycytidine triphosphate deaminase (EC 3.5.4.13)
	gene4987	0.13	Cell Wall and Capsule	YjeF protein, function unknown
	gene1335	0.13	DNA replication	DNA polymerase I (EC 2.7.7.7)
	gene1196	0.13	Pyridoxine	D-3-phosphoglycerate dehydrogenase (EC 1.1.1.95)
	gene3559	0.13	Phosphorus Metabolism	Probable low-affinity inorganic phosphate transporter
	gene4773	0.13	Protein biosynthesis	Tyrosyl-tRNA synthetase (EC 6.1.1.1)
	gene2557	0.13	One-carbon Metabolism	Malate dehydrogenase (EC 1.1.1.37)
	gene1297	0.13	Pyrimidines	Dihydroorotase (EC 3.5.2.3)
	gene2047	0.12	Respiration	Heme O synthase, protoheme IX farnesyltransferase (EC 2.5.1.-) COX10-CtaB
	gene1842	0.12	Central carbohydrate metabolism	Glucokinase (EC 2.7.1.2)
	gene335	0.12	Arginine; urea cycle, polyamines	Acetylornithine deacetylase (EC 3.5.1.16)
	gene1455	0.12	Fatty acids	Enoyl-[acyl-carrier-protein] reductase [FMN] (EC 1.3.1.9)
	gene2139	0.12	Branched-chain amino acids	Hydroxymethylglutaryl-CoA lyase (EC 4.1.3.4)

Table D.1 continued from previous page

year	gene	$\log_2$Fold Change	level1	level4
	gene1343	0.12	DNA uptake, competence	DNA polymerase III subunits gamma and tau (EC 2.7.7.7)
	gene1344	0.12	DNA repair	DNA polymerase IV (EC 2.7.7.7)
	gene4125	0.12	Sarcosine oxidase	Sarcosine oxidase beta subunit (EC 1.5.3.1)
	gene3062	0.12	Potassium metabolism	Osmosensitive K+ channel histidine kinase KdpD (EC 2.7.3.-)
	gene1243	0.12	Programmed Cell Death and Toxin-antitoxin Systems	Death on curing protein, Doc toxin
	gene3307	0.12	Purines	Phosphoribosylaminoimidazole carboxylase catalytic subunit (EC 4.1.1.21)
	gene4479	0.12	Branched-chain amino acids	Threonine dehydratase, catabolic (EC 4.3.1.19)
	gene3380	0.12	Purines	Polyphosphate kinase (EC 2.7.4.1)
	gene70	0.12	Branched-chain amino acids	2-isopropylmalate synthase (EC 2.3.3.13)
	gene4696	0.12	Clustering-based subsystems	tRNA(Ile)-lysidine synthetase
	gene3026	0.12	Lysine, threonine, methionine, and cysteine	O-succinylhomoserine sulfhydrylase (EC 2.5.1.48)
	gene595	0.12	Lysine, threonine, methionine, and cysteine	Aspartate-semialdehyde dehydrogenase (EC 1.2.1.11)
	gene1511	0.11	Phosphorus Metabolism	Exopolyphosphatase (EC 3.6.1.11)
	gene4690	0.11	RNA processing and modification	tRNA-i(6)A37 methylthiotransferase
	gene763	0.11	Aromatic amino acids and derivatives	Biosynthetic Aromatic amino acid aminotransferase beta (EC 2.6.1.57)
	gene1507	0.11	DNA repair	Exodeoxyribonuclease VII large subunit (EC 3.1.11.6)
	gene4675	0.11	RNA processing and modification	tRNA nucleotidyltransferase (EC 2.7.7.21) (EC 2.7.7.25)
	gene1763	0.11	One-carbon Metabolism	Fumarate hydratase class I, aerobic (EC 4.2.1.2)
	gene3975	0.11	Monosaccharides	Ribokinase (EC 2.7.1.15)
	gene1800	0.11	Oxidative stress	Gamma-glutamyltranspeptidase (EC 2.3.2.2)
	gene4169	0.11	Lysine, threonine, methionine, and cysteine	Serine acetyltransferase (EC 2.3.1.30)
	gene3313	0.11	Purines	Phosphoribosylformylglycinamidine cyclo-ligase (EC 6.3.3.1)
	gene4793	0.11	Cell Wall and Capsule	UDP-N-acetylglucosamine 1-carboxyvinyltransferase (EC 2.5.1.7)
	gene1157	0.11	Respiration	Cytochrome c-type biogenesis protein CcmC, putative heme lyase for CcmE
	gene779	0.11	Pyrimidines	Bis(5'-nucleosyl)-tetraphosphatase (asymmetrical) (EC 3.6.1.17)
	gene1583	0.11	TldD cluster	FIG003879: Predicted amidohydrolase
	gene3858	0.11	Arginine; urea cycle, polyamines	Putrescine transport ATP-binding protein PotA (TC 3.A.1.11.1)
	gene3875	0.10	Pyridoxine	Pyridoxamine 5'-phosphate oxidase (EC 1.4.3.5)
	gene4983	0.10	Clustering-based subsystems	YgjD/Kae1/Qri7 family, required for threonylcarbamoyladenosine (t(6)A) formation in tRNA
	gene4854	0.10	Clustering-based subsystems	UPF0301 protein YqgE
	gene2807	0.10	Cell Wall and Capsule	N-acetylglucosamine-1-phosphate uridyltransferase (EC 2.7.7.23)
	gene845	0.10	Regulation and Cell signaling	Carbonic anhydrase (EC 4.2.1.1)
	gene2003	0.10	Purines	GMP synthase [glutamine-hydrolyzing] (EC 6.3.5.2)
	gene3285	0.10	Monosaccharides	Phosphomannomutase (EC 5.4.2.8)
	gene786	0.09	Central carbohydrate metabolism	Branched-chain alpha-keto acid dehydrogenase, E1 component, beta subunit (EC 1.2.4.4)
	gene3585	0.09	Protein processing and modification	Prolipoprotein diacylglyceryl transferase (EC 2.4.99.-)
	gene4935	0.09	Protein degradation	Xaa-Pro aminopeptidase (EC 3.4.11.9)
	gene58	0.09	Coenzyme A	2-dehydropantoate 2-reductase (EC 1.1.1.169)
	gene566	0.09	Protein biosynthesis	Arginyl-tRNA synthetase (EC 6.1.1.19)
	gene1964	0.09	Alanine, serine, and glycine	Glycine dehydrogenase [decarboxylating] (glycine cleavage system P protein) (EC 1.4.4.2)
	gene28	0.08	Isoprenoid/cell wall biosynthesis: PREDICTED UNDECAPRENYL DIPHOSPHATE PHOSPHATASE	1-deoxy-D-xylulose 5-phosphate reductoisomerase (EC 1.1.1.267)
	gene3932	0.08	DNA uptake, competence	Recombination protein RecR

Table D.1 continued from previous page

year	gene	$\log_2$Fold Change	level1	level4
	gene3305	0.08	Purines	Phosphoribosylamine–glycine ligase (EC 6.3.4.13)
	gene4896	0.08	Tetrapyrroles	Uroporphyrinogen III decarboxylase (EC 4.1.1.37)
	gene2897	0.08	Electron donating reactions	NADH-ubiquinone oxidoreductase chain D (EC 1.6.5.3)
	gene742	0.07	Resistance to antibiotics and toxic compounds	Beta-lactamase (EC 3.5.2.6)
	gene590	0.06	Glutamine, glutamate, aspartate, asparagine; ammonia assimilation	Aspartate aminotransferase (EC 2.6.1.1)
2016	gene2011	-0.10	Protein biosynthesis	GTP-binding and nucleic acid-binding protein YchF
	gene2602	-0.10	Monosaccharides	Mannose-1-phosphate guanylyltransferase (GDP) (EC 2.7.7.22)
	gene2900	-0.10	Electron donating reactions	NADH-ubiquinone oxidoreductase chain H (EC 1.6.5.3)
	gene3947	-0.10	DNA replication	Replicative DNA helicase (EC 3.6.1.-)
	gene4788	-0.11	Capsular and extracellular polysacchrides	UDP-glucose dehydrogenase (EC 1.1.1.22)
	gene4018	-0.12	RNA processing and modification	Ribosomal protein S12p Asp88 (E. coli) methylthiotransferase
	gene4483	-0.12	Protein biosynthesis	Threonyl-tRNA synthetase (EC 6.1.1.3)
	gene2551	-0.12	Putative asociate of RNA polymerase sigma-54 factor rpoN	macromolecule synthesis, modification
	gene791	-0.12	ABC transporters	Branched-chain amino acid transport system permease protein LivM (TC 3.A.1.4.1)
	gene2513	-0.12	Protein biosynthesis	LSU ribosomal protein L9p
	gene3536	-0.13	Aromatic amino acids and derivatives	Prephenate dehydratase (EC 4.2.1.51)
	gene2482	-0.13	Protein biosynthesis	LSU ribosomal protein L25p
	gene2414	-0.13	Gram-Negative cell wall components	Lipid A export ATP-binding/permease protein MsbA (EC 3.6.3.25)
	gene729	-0.13	Sulfur Metabolism	Beta-galactosidase (EC 3.2.1.23)
	gene4739	-0.13	DNA Metabolism	Type I restriction-modification system, DNA-methyltransferase subunit M (EC 2.1.1.72)
	gene4086	-0.13	Cell Division and Cell Cycle	Rod shape-determining protein MreC
	gene946	-0.14	Cell Division and Cell Cycle	Chromosome partition protein smc
	gene3093	-0.14	Clustering-based subsystems	Oxidoreductase, short-chain dehydrogenase/reductase family (EC 1.1.1.-)
	gene4136	-0.14	Cell Division and Cell Cycle	Segregation and condensation protein B
	gene3009	-0.14	Purines	Nucleoside diphosphate kinase (EC 2.7.4.6)
	gene597	-0.14	Protein biosynthesis	Aspartyl-tRNA synthetase (EC 6.1.1.12)
	gene4618	-0.14	Heat shock	Translation elongation factor LepA
	gene4252	-0.14	DNA repair	SOS-response repressor and protease LexA (EC 3.4.21.88)
	gene4005	-0.14	Motility and Chemotaxis	Ribose ABC transporter, periplasmic ribose-binding protein RbsB (TC 3.A.1.2.1)
	gene4512	-0.14	Resistance to antibiotics and toxic compounds	Topoisomerase IV subunit B (EC 5.99.1.-)
	gene4003	-0.15	Monosaccharides	Ribose ABC transport system, periplasmic ribose-binding protein RbsB (TC 3.A.1.2.1)
	gene2658	-0.15	Clustering-based subsystems	Metallo-beta-lactamase family protein, RNA-specific
	gene1932	-0.15	Potassium metabolism	Glutathione-regulated potassium-efflux system ATP-binding protein
	gene3520	-0.15	Branched-chain amino acids	Predicted transcriptional regulator LiuR of leucine degradation pathway, MerR family
	gene1415	-0.15	Capsular and extracellular polysacchrides	dTDP-4-dehydrorhamnose 3,5-epimerase (EC 5.1.3.13)
	gene4246	-0.15	Cell Wall and Capsule	Soluble lytic murein transglycosylase precursor (EC 3.2.1.-)
	gene4794	-0.15	Capsular and extracellular polysacchrides	UDP-N-acetylglucosamine 2-epimerase (EC 5.1.3.14)
	gene1864	-0.15	Capsular and extracellular polysacchrides	Glucose-1-phosphate thymidylyltransferase (EC 2.7.7.24)
	gene4285	-0.15	Clustering-based subsystems	SSU ribosomal protein S16p

Table D.1 continued from previous page

year	gene	$\log_2$Fold Change	level1	level4
	gene2855	-0.15	NAD and NADP	NAD synthetase (EC 6.3.1.5)
	gene685	-0.15	Capsular and extracellular polysacchrides	Bacillosamine/Legionaminic acid biosynthesis aminotransferase PglE
	gene198	-0.15	Membrane Transport	4-hydroxybenzoyl-CoA thioesterase family active site
	gene750	-0.15	Nucleosides and Nucleotides	Beta-ureidopropionase (EC 3.5.1.6)
	gene2025	-0.15	Probably GTP or GMP signaling related	Guanosine-3',5'-bis(diphosphate) 3'-pyrophosphohydrolase (EC 3.1.7.2)
	gene630	-0.15	DNA repair	ATP-dependent DNA helicase UvrD/PcrA, proteobacterial paralog
	gene2098	-0.16	DNA replication	Holliday junction DNA helicase RuvA
	gene1900	-0.16	Protein biosynthesis	Glutaminyl-tRNA synthetase (EC 6.1.1.18)
	gene1164	-0.16	Respiration	Cytochrome c-type biogenesis protein ResA
	gene1266	-0.16	Uni- Sym- and Antiporters	Di-/tripeptide transporter
	gene784	-0.16	Branched-chain amino acids	Branched-chain acyl-CoA dehydrogenase (EC 1.3.99.12)
	gene1742	-0.16	Folate and pterines	Formyltetrahydrofolate deformylase (EC 3.5.1.10)
	gene59	-0.16	Monosaccharides	2-deoxy-D-gluconate 3-dehydrogenase (EC 1.1.1.125)
	gene2247	-0.16	DNA Metabolism	Integration host factor beta subunit
	gene354	-0.16	Phospholipids	Acyl-phosphate:glycerol-3-phosphate O-acyltransferase PlsY
	gene1334	-0.16	DNA repair	DNA mismatch repair protein MutS
	gene4234	-0.17	DNA repair	Single-stranded-DNA-specific exonuclease RecJ (EC 3.1.-.-)
	gene3162	-0.17	RNA processing and modification	Permease of the drug/metabolite transporter (DMT) superfamily
	gene3545	-0.17	Branched-chain amino acids	Probable acyl-CoA dehydrogenase (EC 1.3.99.3)
	gene1352	-0.17	DNA repair	DNA recombination protein RmuC
	gene4626	-0.17	Protein biosynthesis	Translation initiation factor 3
	gene4518	-0.17	Clustering-based subsystems	Transcription accessory protein (S1 RNA-binding domain)
	gene4276	-0.18	Protein biosynthesis	SSU ribosomal protein S12p (S23e)
	gene2485	-0.18	Clustering-based subsystems	LSU ribosomal protein L27p
	gene2763	-0.18	Resistance to antibiotics and toxic compounds	Multi antimicrobial extrusion protein (Na(+)/drug antiporter), MATE family of MDR efflux pumps
	gene918	-0.18	Flagellar motility in Prokaryota	Chemotaxis regulator - transmits chemoreceptor signals to flagelllar motor components CheY
	gene4732	-0.18	Gram-Negative cell wall components	Two-component system response regulator OmpR
	gene940	-0.18	DNA replication	Chromosomal replication initiator protein DnaA
	gene232	-0.18	Detoxification	5-methyl-dCTP pyrophosphohydrolase (EC 3.6.1.-)
	gene1641	-0.18	Potassium metabolism	FKBP-type peptidyl-prolyl cis-trans isomerase FkpA precursor (EC 5.2.1.8)
	gene2630	-0.18	Clustering-based subsystems	Membrane protein containing HD superfamily hydrolase domain, YQFF ortholog
	gene1315	-0.18	ABC transporters	Dipeptide transport ATP-binding protein DppD (TC 3.A.1.5.2)
	gene2423	-0.18	Gram-Negative cell wall components	Lipopolysaccharide ABC transporter, ATP-binding protein LptB
	gene2994	-0.19	Siderophores	Non-ribosomal peptide synthetase modules, pyoverdine
	gene3127	-0.19	Protein biosynthesis	Peptide chain release factor 3
	gene1172	-0.19	Respiration	Cytochrome c551/c552
	gene1944	-0.19	Sugar alcohols	Glycerol-3-phosphate ABC transporter, periplasmic glycerol-3-phosphate-binding protein (TC 3.A.1.1.3)
	gene4578	-0.19	Oxidative stress	transcriptional regulator, Crp/Fnr family
	gene3068	-0.19	Membrane Transport	Outer membrane lipoprotein omp16 precursor
	gene1904	-0.19	Protein biosynthesis	Glutamyl-tRNA synthetase (EC 6.1.1.17)
	gene920	-0.19	Motility and Chemotaxis	Chemotaxis response regulator protein-glutamate methylesterase CheB (EC 3.1.1.61)
	gene804	-0.19	Regulation and Cell signaling	cAMP-binding proteins - catabolite gene activator and regulatory subunit of cAMP-dependent protein kinases

Table D.1 continued from previous page

year	gene	$\log_2$Fold Change	level1	level4
	gene4740	-0.19	DNA Metabolism	Type I restriction-modification system, restriction subunit R (EC 3.1.21.3)
	gene2474	-0.19	Clustering-based subsystems	LSU ribosomal protein L21p
	gene2459	-0.19	Protein biosynthesis	LSU ribosomal protein L13p (L13Ae)
	gene4770	-0.19	Capsular and extracellular polysacchrides	Tyrosine-protein kinase EpsD (EC 2.7.10.2)
	gene4414	-0.19	Periplasmic Stress	Survival protein SurA precursor (Peptidyl-prolyl cis-trans isomerase SurA) (EC 5.2.1.8)
	gene4958	-0.19	Monosaccharides	Xylanase
	gene4511	-0.19	Resistance to antibiotics and toxic compounds	Topoisomerase IV subunit A (EC 5.99.1.-)
	gene2420	-0.20	Lipoic acid	Lipoate-protein ligase A
	gene3384	-0.20	Glycoside hydrolases	Polysaccharide deacetylase
	gene1805	-0.20	Capsular and extracellular polysacchrides	GDP-mannose 4,6-dehydratase (EC 4.2.1.47)
	gene422	-0.20	Phosphorus Metabolism	Alkaline phosphatase (EC 3.1.3.1)
	gene4465	-0.20	Protein degradation	Thimet oligopeptidase (EC 3.4.24.15)
	gene3340	-0.20	Isoprenoids	Phytoene desaturase (EC 1.14.99.-)
	gene4966	-0.20	Monosaccharides	Xylose isomerase (EC 5.3.1.5)

Table D.2: Variation in the expression of soil microbial genes between months (September vs. June), based on SEED hierarchical microbial functional annotation (level4) (DESeq2, adjusted $P < 0.05$).

month	gene	$\log_2$Fold Change	level1	level4
September	gene1931	3.09	Potassium metabolism	Glutathione-regulated potassium-efflux system ancillary protein KefG
	gene3231	2.46	Phosphorus Metabolism	PhnI protein
	gene3230	2.41	Phosphorus Metabolism	PhnG protein
	gene1361	2.40	DNA replication	DNA replication protein DnaC
	gene3349	2.22	DNA replication	Plasmid replication protein RepC
	gene3292	2.11	Phosphorus Metabolism	Phosphonates transport ATP-binding protein PhnK
	gene3232	1.91	Phosphorus Metabolism	PhnJ protein
	gene3293	1.76	Phosphorus Metabolism	Phosphonates transport ATP-binding protein PhnL
	gene2245	1.71	Transposable elements	Integrase/recombinase clustered with segregation and condensation protein B
	gene2300	1.71	Transposable elements	ISPsy4, transposase
	gene3348	1.66	DNA replication	Plasmid replication protein RepB
	gene3347	1.43	DNA replication	Plasmid replication protein RepA
	gene201	1.41	Metabolism of Aromatic Compounds	4-hydroxyphenylacetate 3-monooxygenase, reductase component (EC 1.6.8.-)
	gene2572	1.27	Organic acids	Malonate transporter, MadM subunit
	gene4814	1.21	Electron donating reactions	Uncharacterized MobA-related protein
	gene1693	1.13	Respiration	Flavocytochrome c flavin subunit
	gene3874	1.13	Pyridoxine	Pyridoxal kinase (EC 2.7.1.35)
	gene41	1.08	Adhesion	16 kDa heat shock protein B
	gene2150	1.02	Electron donating reactions	Hypothetical lactonase in carbon monoxide dehydrogenase cluster
	gene2188	1.00	Iron acquisition and metabolism	Hypothetical, distant similarity with heme-degrading oxygenase IsdG
	gene4997	1.00	Protein degradation	Zinc D-Ala-D-Ala carboxypeptidase (EC 3.4.17.14)

Table D.2 continued from previous page

month	gene	$\log_2$Fold Change	level1	level4
	gene4438	0.95	Fatty acids	TesB-like acyl-CoA thioesterase 4
	gene3544	0.94	Cell wall of Mycobacteria	Probable acyl-[acyl-carrier protein] desaturase DESA1 (Acyl-[ACP] desaturase) (Stearoyl-ACP desaturase) (Protein DES) (EC 1.14.19.2)
	gene4857	0.93	Electron donating reactions	Uptake hydrogenase large subunit (EC 1.12.99.6)
	gene4504	0.92	Metabolism of Aromatic Compounds	Toluene-4-monooxygenase, subunit TmoE
	gene4858	0.91	Electron donating reactions	Uptake hydrogenase small subunit precursor (EC 1.12.99.6)
	gene3498	0.89	Clustering-based subsystems	Predicted oxidoreductase, Fe-S subunit
	gene1045	0.89	Type III, Type IV, Type VI, ESAT secretion systems	Conjugative transfer protein TrbI
	gene2661	0.89	Central carbohydrate metabolism	Methane monooxygenase component A alpha chain (EC 1.14.13.25)
	gene1088	0.88	Nitrogen Metabolism	Cyanate ABC transporter, permease protein
	gene2663	0.87	Central carbohydrate metabolism	Methane monooxygenase component C (EC 1.14.13.25)
	gene3929	0.87	DNA repair	RecD-like DNA helicase Atu2026
	gene2654	0.87	Phosphorus Metabolism	Metal-dependent hydrolase involved in phosphonate metabolism
	gene960	0.86	Respiration	CO dehydrogenase accessory protein CooC (nickel insertion)
	gene2030	0.86	Protein folding	Heat shock protein 60 family co-chaperone GroES
	gene505	0.85	Nitrogen Metabolism	Ammonium transporter
	gene4638	0.85	Transposable elements	Transposase OrfAB, subunit B
	gene2662	0.85	Central carbohydrate metabolism	Methane monooxygenase component A beta chain (EC 1.14.13.25)
	gene4502	0.84	Metabolism of Aromatic Compounds	Toluene-4-monooxygenase, subunit TmoA
	gene2029	0.83	Protein folding	Heat shock protein 60 family chaperone GroEL
	gene3564	0.82	Clustering-based subsystems	Probable polyketide synthase, similar to many. e.g. gp|M63676|SERERYAA_1 S.erythraea first ORF of eryA gene, involved in complex polyketide formation in erythromycin biosynthesis.
	gene1173	0.81	Respiration	Cytochrome c552
	gene4224	0.80	Plant-Prokaryote DOE project	Similar to non-heme chloroperoxidase, sll5080 homolog
	gene1513	0.79	Biosynthesis of galactoglycans and related lipopolysacharides	Exopolysaccharide production protein ExoF precursor
	gene781	0.79	Pyruvate kinase associated cluster	bll7545
	gene2965	0.78	Nitrogen Metabolism	Nitrite reductase [NAD(P)H] large subunit (EC 1.7.1.4)
	gene40	0.78	Adhesion	16 kDa heat shock protein A
	gene4338	0.78	Protein biosynthesis	Structure-specific tRNA-binding protein
	gene1780	0.78	Sulfur Metabolism	Galactocerebrosidase precursor (EC 3.2.1.46)
	gene1897	0.76	Cell Wall and Capsule	Glutamine synthetase type II, eukaryotic (EC 6.3.1.2)
	gene1974	0.75	Selenoproteins	Glycine/sarcosine/betaine reductase protein A
	gene429	0.75	Organic sulfur assimilation	Alkanesulfonates-binding protein
	gene2677	0.75	Protein biosynthesis	Methionyl-tRNA synthetase-related protein 2
	gene2054	0.74	Iron acquisition and metabolism	Hemin uptake protein
	gene171	0.73	Stress Response	3-phenylpropionate dioxygenase, alpha subunit (EC 1.14.12.19)
	gene2664	0.72	Central carbohydrate metabolism	Methane monooxygenase regulatory protein B
	gene3064	0.72	Osmotic stress	Osmotically inducible protein OsmY
	gene2083	0.72	Clustering-based subsystems	His repressor
	gene3278	0.72	Central carbohydrate metabolism	Phosphoglycolate phosphatase, archaeal type (EC 3.1.3.18)
	gene990	0.71	Tetrapyrroles	CobW GTPase involved in cobalt insertion for B12 biosynthesis
	gene4268	0.68	Dormancy and Sporulation	SpoVS-related protein, type 2
	gene2948	0.68	Nitrogen Metabolism	Nitrate ABC transporter, nitrate-binding protein
	gene4121	0.68	Urate degradation	salvage of nucleosides and nucleotides
	gene1898	0.67	Cell Wall and Capsule	Glutamine synthetase type III, GlnN (EC 6.3.1.2)
	gene2976	0.67	Nitrogen Metabolism	Nitrogen regulatory protein P-II
	gene2361	0.67	Monosaccharides	L-rhamno-gamma-lactonase (EC 3.1.1.65)

Table D.2 continued from previous page

month	gene	$\log_2$Fold Change	level1	level4
	gene4442	0.67	Respiration	Tetrachloroethene reductive dehalogenase TceA
	gene488	0.65	Nitrogen Metabolism	Amidase clustered with urea ABC transporter and nitrile hydratase functions
	gene3703	0.65	Monosaccharides	PTS system, glucitol/sorbitol-specific IIC component (EC 2.7.1.69)
	gene3442	0.65	Di- and oligosaccharides	Predicted beta-glucoside-regulated ABC transport system, permease component 1, COG1175
	gene907	0.65	Heat shock	Chaperone protein DnaK
	gene3702	0.64	Monosaccharides	PTS system, glucitol/sorbitol-specific IIB component and second of two IIC components (EC 2.7.1.69)
	gene3362	0.64	Fatty Acids, Lipids, and Isoprenoids	polyhydroxyalkanoate granule-associated protein PhaI
	gene3113	0.63	Monosaccharides	Pectate lyase precursor (EC 4.2.2.2)
	gene4865	0.63	DNA repair	Uracil-DNA glycosylase, putative family 6
	gene502	0.62	Protein degradation	Aminopeptidase S (Leu, Val, Phe, Tyr preference) (EC 3.4.11.24)
	gene3429	0.62	Tetrapyrroles	Precorrin-6A synthase (EC 2.1.1.152)
	gene3044	0.61	Osmotic stress	OpgC protein
	gene79	0.60	Metabolism of Aromatic Compounds	2-keto-4-pentenoate hydratase/2-oxohepta-3-ene-1,7-dioic acid hydratase (catechol pathway)
	gene2706	0.59	Miscellaneous	MFS superfamily export protein YceL
	gene2949	0.59	Nitrogen Metabolism	Nitrate ABC transporter, permease protein
	gene2113	0.58	Heat shock	HspR, transcriptional repressor of DnaK operon
	gene493	0.58	Clustering-based subsystems	Amino acid regulated cytosolic protein
	gene3816	0.58	Peripheral pathways for catabolism of aromatic compounds	Putative phthalate 4,5-dioxygenase oxygenase subunit (OhpA2)
	gene833	0.58	Electron donating reactions	Carbon monoxide dehydrogenase form I, large chain(EC:1.2.99.2)
	gene4967	0.57	Monosaccharides	Xylose oligosaccharides ABC transporter, ATP-binding protein 1
	gene4601	0.57	Carbohydrates	Transcriptional repressor UidR
	gene703	0.57	Peripheral pathways for catabolism of aromatic compounds	Benzoate 1,2-dioxygenase beta subunit (EC 1.14.12.10)
	gene964	0.56	Proteolytic pathway	Coagulation factor VIII
	gene386	0.56	Tetrapyrroles	Aerobic cobaltochelatase CobS subunit (EC 6.6.1.2)
	gene146	0.56	Fermentation	3-hydroxybutyryl-CoA epimerase (EC 5.1.2.3)
	gene2031	0.56	Heat shock	Heat shock protein GrpE
	gene3134	0.55	Protein export	peptidoglycan lytic protein P45
	gene1447	0.55	Di- and oligosaccharides	Endo-beta-1,3-1,4 glucanase (Licheninase) (EC 3.2.1.73)
	gene1985	0.55	Carbohydrates	Glycosyl transferase, family 2
	gene1534	0.55	Nitrogen Metabolism	Ferredoxin–nitrite reductase (EC 1.7.7.1)
	gene724	0.54	Osmotic stress	Beta-(1–>2)glucan export ATP-binding/permease protein NdvA (EC 3.6.3.42)
	gene4869	0.54	Arginine; urea cycle, polyamines	Urea ABC transporter, permease protein UrtB
	gene3729	0.54	Clustering-based subsystems	Putative antibiotic transport-associated protein
	gene3718	0.54	Regulation and Cell signaling	Purine cyclase-related protein
	gene2389	0.53	Peripheral pathways for catabolism of aromatic compounds	Large subunit naph/bph dioxygenase
	gene20	0.53	Electron donating reactions	[NiFe] hydrogenase metallocenter assembly protein HypC
	gene961	0.53	Electron donating reactions	CO dehydrogenases maturation factor, CoxF family
	gene3672	0.52	RNA processing and modification	Protein with similarity to RtcB
	gene963	0.52	Proteolytic pathway	Coagulation factor V
	gene4635	0.52	Electron accepting reactions	Transport ATP-binding protein CydCD
	gene4249	0.52	Monosaccharides	Sorbitol-6-phosphate 2-dehydrogenase (EC 1.1.1.140)
	gene4441	0.52	Respiration	Tetrachloroethene reductive dehalogenase PceA (EC 1.97.1.8)
	gene4223	0.52	Plant-Prokaryote DOE project	Similar to non-heme chloroperoxidase
	gene929	0.51	Light-harvesting complexes	Chlorosome protein I, 2Fe-2S ferredoxin

Table D.2 continued from previous page

month	gene	$\log_2$Fold Change	level1	level4
	gene2185	0.51	Carbohydrates	Hypothetical sugar ABC transporter, solute-binding protein UgtE
	gene545	0.51	Miscellaneous	Archease
	gene4258	0.50	Clustering-based subsystems	Spore coat protein A
	gene4652	0.50	Di- and oligosaccharides	Trehalose synthase, nucleoside diphosphate glucose dependent
	gene2656	0.50	Resistance to antibiotics and toxic compounds	Metal-dependent hydrolases of the beta-lactamase superfamily II
	gene4500	0.50	Membrane Transport	TolA protein
	gene1052	0.49	Bacteriocins, ribosomally synthesized antibacterial peptides	Conserved uncharacterized protein CreA
	gene2255	0.49	Pyruvate kinase associated cluster	InterPro IPR001440 COGs COG0457
	gene3045	0.49	Tetrapyrroles	Optional hypothetical component of the B12 transporter BtuN
	gene1970	0.48	Selenoproteins	Glycine reductase component B gamma subunit (EC 1.21.4.2)
	gene4248	0.48	Monosaccharides	Sorbitol operon transcription regulator
	gene2136	0.48	Plant-Prokaryote DOE project	Hydroxyethylthiazole kinase (EC 2.7.1.50)
	gene4250	0.47	Adhesion	Sortase
	gene2306	0.47	DNA repair	Ku domain protein
	gene910	0.47	Protein folding	Chaperone protein HtpG
	gene2416	0.47	Fatty Acids, Lipids, and Isoprenoids	Lipid carrier protein IgrF
	gene22	0.47	Electron donating reactions	[NiFe] hydrogenase metallocenter assembly protein HypE
	gene1093	0.47	Transcription	Cyanobacteria-specific RpoD-like sigma factor, type-12
	gene4054	0.47	Transcription	RNA polymerase principal sigma factor HrdC
	gene4394	0.47	Organic sulfur assimilation	sulfonate monooxygenase
	gene1460	0.47	Central carbohydrate metabolism	Enoyl-CoA hydratase [branched-chain amino acid degradation] (EC 4.2.1.17)
	gene4990	0.47	Miscellaneous	YpfJ protein, zinc metalloprotease superfamily
	gene1000	0.46	Quinone cofactors	Coenzyme PQQ synthesis protein B
	gene4409	0.46	Oxidative stress	superoxide dismutase [Fe-Zn] (EC 1.15.1.1)
	gene4330	0.46	Dormancy and Sporulation	Stage V sporulation protein required for dehydratation of the spore core and assembly of the coat (SpoVS)
	gene4779	0.46	Quinone cofactors	Ubiquinone/menaquinone biosynthesis methyltransferase UbiE (EC 2.1.1.-)
	gene2718	0.46	Probably GTP or GMP signaling related	Mll7752 protein
	gene4427	0.45	Organic acids	TcuA: flavoprotein used to oxidize tricarballylate to cis-aconitate
	gene906	0.45	Heat shock	Chaperone protein DnaJ
	gene1514	0.45	Biosynthesis of galactoglycans and related lipopolysacharides	Exopolysaccharide production protein ExoQ
	gene1573	0.45	Cell Division	FIG001960: FtsZ-interacting protein related to cell division
	gene1616	0.45	Gram-Negative cell wall components	FIG043197: Inositol monophosphatase family protein
	gene996	0.45	Electron accepting reactions	Coenzyme F420-dependent oxidoreductase
	gene3925	0.44	Cell Wall and Capsule	Rare lipoprotein A precursor
	gene3420	0.44	Potassium metabolism	Potassium-transporting ATPase A chain (EC 3.6.3.12) (TC 3.A.3.7.1)
	gene1021	0.44	RNA processing and modification	COG2078: Uncharacterized ACR
	gene2966	0.44	Nitrogen Metabolism	Nitrite reductase [NAD(P)H] small subunit (EC 1.7.1.4)
	gene4653	0.44	Di- and oligosaccharides	Trehalose-6-phosphate hydrolase (EC 3.2.1.93)
	gene1929	0.43	Detoxification	Glutathione-dependent formaldehyde-activating enzyme (EC 4.4.1.22)
	gene2403	0.43	Tetrapyrroles	Light-dependent protochlorophyllide reductase (EC 1.3.1.33)
	gene507	0.43	Nitrogen Metabolism	ammonium/methylammonium permease
	gene1973	0.43	Selenoproteins	Glycine/sarcosine/betaine reductase component C chain 1
	gene634	0.43	DNA repair	ATP-dependent DNA ligase (EC 6.5.1.1) clustered with Ku protein, LigD

Table D.2 continued from previous page

month	gene	$\log_2$Fold Change	level1	level4
	gene3147	0.43	Purines	Periplasmic aromatic aldehyde oxidoreductase, FAD binding subunit YagS
	gene494	0.43	Aromatic amino acids and derivatives	Amino acid-binding ACT
	gene425	0.42	Organic sulfur assimilation	Alkanesulfonate monooxygenase (EC 1.14.14.5)
	gene199	0.42	Peripheral pathways for catabolism of aromatic compounds	4-hydroxycinnamoyl CoA hydratase/lyase (Enoyl-CoA hydratase/lyase) (EC 4.2.1.17)
	gene2947	0.42	Nitrogen Metabolism	Nitrate ABC transporter, ATP-binding protein
	gene1835	0.42	Regulation and Cell signaling	GltC, transcription activator of glutamate synthase operon
	gene1635	0.42	Putative GGDEF domain protein related to agglutinin secretion	FIGfam020323
	gene2743	0.42	Carbohydrates	Monooxygenase component A
	gene1366	0.41	DNA replication	DNA topoisomerase IB (poxvirus type) (EC 5.99.1.2)
	gene4428	0.41	Organic acids	TcuB: works with TcuA to oxidize tricarballylate to cis-aconitate
	gene3788	0.41	Putative Isoquinoline 1-oxidoreductase subunit	Putative Isoquinoline 1-oxidoreductase subunit, Mll3835 protein
	gene1050	0.41	Branched-chain amino acids	conserved protein associated with acetyl-CoA C-acyltransferase
	gene2659	0.41	Clustering-based subsystems	Metallo-beta-lactamase superfamily protein PA0057
	gene1383	0.41	Regulation and Cell signaling	DNA-binding response regulator ChvI
	gene1051	0.41	Fatty Acids, Lipids, and Isoprenoids	Conserved protein IgrD
	gene154	0.41	Lysine, threonine, methionine, and cysteine	3-keto-5-aminohexanoate cleavage enzyme
	gene19	0.41	Electron donating reactions	[Ni/Fe] hydrogenase, group 1, small subunit
	gene156	0.40	Branched-chain amino acids	3-ketoacyl-CoA thiolase [isoleucine degradation] (EC 2.3.1.16)
	gene4685	0.40	Cell Wall and Capsule	tRNA-dependent lipid II–amino acid ligase
	gene3148	0.40	Cofactors, Vitamins, Prosthetic Groups, Pigments	Periplasmic aromatic aldehyde oxidoreductase, iron-sulfur subunit YagT
	gene3149	0.40	Cofactors, Vitamins, Prosthetic Groups, Pigments	Periplasmic aromatic aldehyde oxidoreductase, molybdenum binding subunit YagR
	gene290	0.40	Inorganic sulfur assimilation	ABC-type probable sulfate transporter, periplasmic binding protein
	gene4259	0.40	Dormancy and Sporulation	Spore cortex-lytic enzyme, lytic transglycosylase SleB
	gene898	0.40	Regulation and Cell signaling	Cell envelope-associated transcriptional attenuator LytR-CpsA-Psr, subfamily A1 (as in PMID19099556)
	gene4677	0.40	RNA processing and modification	tRNA nucleotidyltransferase, CC-adding (EC 2.7.7.21)
	gene2001	0.40	Nitrogen Metabolism	Glyoxylate carboligase (EC 4.1.1.47)
	gene506	0.40	Nitrogen Metabolism	Ammonium transporter family
	gene1896	0.40	Cell Wall and Capsule	Glutamine synthetase type I (EC 6.3.1.2)
	gene4412	0.40	Oxidative stress	Superoxide dismutase [Mn/Fe] (EC 1.15.1.1)
	gene1007	0.39	Membrane Transport	COG0523: Putative GTPases (G3E family)
	gene691	0.39	Tetrapyrroles	Bacteriochlorophyllide c C8 methyltransefase BchQ
	gene4270	0.39	Dormancy and Sporulation	SpoVS-related protein, type 5
	gene3489	0.38	Pyrimidines	Predicted monooxygenase RutA in novel pyrimidine catabolism pathway
	gene2267	0.38	Oxidative stress	Iron-responsive regulator Irr
	gene4408	0.38	Oxidative stress	Superoxide dismutase [Cu-Zn] precursor (EC 1.15.1.1)
	gene2172	0.38	Central carbohydrate metabolism	hypothetical protein that often co-occurs with aconitase
	gene4659	0.38	Electron accepting reactions	Trimethylamine-N-oxide reductase (EC 1.6.6.9)
	gene24	0.38	Electron donating reactions	[NiFe] hydrogenase nickel incorporation protein HypA
	gene3389	0.38	Electron accepting reactions	Polysulfide reductase, subunit C, putative
	gene1068	0.38	Tetrapyrroles	Coproporphyrinogen III oxidase, oxygen-independent (EC 1.3.99.22), divergent, putative 2
	gene3378	0.38	Resistance to antibiotics and toxic compounds	Polymyxin transporter PmxC

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month	gene	$\log_2$Fold Change	level1	level4
	gene2577	0.38	Di- and oligosaccharides	Malto-oligosyltrehalose trehalohydrolase (EC 3.2.1.141)
	gene3444	0.37	Di- and oligosaccharides	Predicted beta-glucoside-regulated ABC transport system, sugar binding component, COG1653
	gene860	0.37	Folate and pterines	Catalyzes the cleavage of p-aminobenzoyl-glutamate to p-aminobenzoate and glutamate, subunit B
	gene2788	0.37	Oxidative stress	Mycothiol S-conjugate amidase Mca
	gene3421	0.37	Potassium metabolism	Potassium-transporting ATPase B chain (EC 3.6.3.12) (TC 3.A.3.7.1)
	gene2065	0.37	Isoprenoids	Heptaprenyl diphosphate synthase component I (EC 2.5.1.30)
	gene1090	0.37	Nitrogen Metabolism	Cyanate hydratase (EC 4.2.1.104)
	gene861	0.37	Metabolism of central aromatic intermediates	Catechol 1,2-dioxygenase (EC 1.13.11.1)
	gene3344	0.37	Clustering-based subsystems	Pirin-like protein YhaK
	gene4354	0.37	Central carbohydrate metabolism	Succinyl-CoA ligase [ADP-forming] beta chain (EC 6.2.1.5)
	gene463	0.37	NA	Alpha-glucoside transport ATP-binding protein AglK
	gene632	0.36	DNA repair	ATP-dependent DNA helicase, RecQ family
	gene958	0.36	Protein folding	ClpB protein
	gene2967	0.36	Nitrogen Metabolism	Nitrite reductase accessory protein NirV
	gene1533	0.36	Inorganic sulfur assimilation	Ferredoxin–NADP(+) reductase, actinobacterial (eukaryote-like) type (EC 1.18.1.2)
	gene3151	0.36	Di- and oligosaccharides	Periplasmic beta-glucosidase (EC 3.2.1.21)
	gene1291	0.36	Central carbohydrate metabolism	Dihydrolipoamide dehydrogenase of acetoin dehydrogenase (EC 1.8.1.4)
	gene21	0.36	Electron donating reactions	[NiFe] hydrogenase metallocenter assembly protein HypD
	gene3896	0.36	Central carbohydrate metabolism	Pyruvate decarboxylase (EC 4.1.1.1)
	gene2144	0.36	Cofactors, Vitamins, Prosthetic Groups, Pigments	Hydroxymethylpyrimidine ABC transporter, transmembrane component
	gene1263	0.36	Central carbohydrate metabolism	DHA-specific EI component
	gene2111	0.35	Membrane Transport	HoxN/HupN/NixA family nickel/cobalt transporter
	gene3274	0.35	Membrane Transport	Phosphoglycerate transport system transcriptional regulatory protein PgtA
	gene3565	0.35	Resistance to antibiotics and toxic compounds	Probable RND efflux membrane fusion protein
	gene1905	0.35	Protein biosynthesis	Glutamyl-tRNA(Gln) amidotransferase subunit A (EC 6.3.5.7)
	gene4251	0.35	Iron acquisition and metabolism	Sortase A, LPXTG specific
	gene1327	0.35	Regulation and Cell signaling	Dna binding response regulator PrrA (RegA)
	gene4834	0.35	Gram-Negative cell wall components	Uncharacterized protein, Bsl7517 homolog
	gene1364	0.35	Clustering-based subsystems	DNA topoisomerase I (EC 5.99.1.2)
	gene3679	0.35	Membrane Transport	Protein-L-isoaspartate O-methyltransferase (EC 2.1.1.77)
	gene1412	0.35	Protein folding	DnaJ-class molecular chaperone CbpA
	gene489	0.35	NAD and NADP	Amidases related to nicotinamidase
	gene936	0.35	Aromatic amino acids and derivatives	Chorismate mutase II (EC 5.4.99.5)
	gene3118	0.35	Dormancy and Sporulation	Penicillin-binding protein DacC
	gene582	0.35	Organic sulfur assimilation	Arylsulfatase (EC 3.1.6.1)
	gene4970	0.34	Monosaccharides	Xylose oligosaccharides ABC transporter, sugar-binding protein
	gene1718	0.34	Respiration	Formate dehydrogenase O alpha subunit (EC 1.2.1.2)
	gene3443	0.34	Di- and oligosaccharides	Predicted beta-glucoside-regulated ABC transport system, permease component 2, COG0395
	gene777	0.34	Peripheral pathways for catabolism of aromatic compounds	Biphenyl-2,3-diol 1,2-dioxygenase (EC 1.13.11.39)
	gene4913	0.34	Electron accepting reactions	Vanillate O-demethylase oxidoreductase (EC 1.14.13.-)
	gene1619	0.34	Sulfatases and sulfatase modifying factor 1 (and a hypothetical)	FIG068086: hypothetical protein
	gene2987	0.34	Protein export	NLP/P60 family protein

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month	gene	$\log_2$Fold Change	level1	level4
	gene3116	0.34	Cell Division and Cell Cycle	Penicillin-binding protein 1A/1B (PBP1)
	gene3359	0.34	Gram-Positive cell wall components	Poly(glycerol-phosphate) alpha-glucosyltransferase (EC 2.4.1.52)
	gene3957	0.33	Membrane Transport	Response regulator CitB of citrate metabolism
	gene4407	0.33	Oxidative stress	Superoxide dismutase [Cu-Zn] (EC 1.15.1.1)
	gene4959	0.33	Monosaccharides	Xylonate dehydratase (EC 4.2.1.82)
	gene4721	0.33	Resistance to antibiotics and toxic compounds	Two-component response regulator VncR
	gene3656	0.33	Gram-Negative cell wall components	Protein of unknown function DUF374
	gene4993	0.32	Isoprenoids	Zeaxanthin glucosyl transferase
	gene2207	0.32	Aromatic amino acids and derivatives	Indoleacetamide hydrolase (EC 3.5.1.-)
	gene2364	0.32	Monosaccharides	L-rhamnose operon transcriptional activator RhaR
	gene989	0.31	Tetrapyrroles	CobN component of cobalt chelatase involved in B12 biosynthesis
	gene4082	0.31	Resistance to antibiotics and toxic compounds	RND efflux system, membrane fusion protein CmeA
	gene948	0.31	Cell Division and Cell Cycle	Circadian clock protein KaiC
	gene1306	0.31	Phospholipids	Dihydroxyacetone kinase family protein
	gene472	0.31	Monosaccharides	Alpha-mannosidase (EC 3.2.1.24)
	gene3251	0.31	Phospholipids	Phosphatidylglycerophosphatase B (EC 3.1.3.27)
	gene4395	0.31	Coenzyme M	Sulfopyruvate decarboxylase - alpha subunit (EC 4.1.1.79)
	gene722	0.31	Peripheral pathways for catabolism of aromatic compounds	Benzoylformate decarboxylase (EC 4.1.1.7)
	gene3422	0.30	Potassium metabolism	Potassium-transporting ATPase C chain (EC 3.6.3.12) (TC 3.A.3.7.1)
	gene3015	0.30	Protein degradation	Nucleotide excision repair protein, with UvrB/UvrC motif
	gene2795	0.30	Aminosugars	N-Acetyl-D-glucosamine ABC transport system, permease protein 1
	gene4396	0.30	Coenzyme M	Sulfopyruvate decarboxylase - beta subunit (EC 4.1.1.79)
	gene4912	0.30	Resistance to antibiotics and toxic compounds	Vancomycin response regulator VanR
	gene2762	0.30	Metabolism of central aromatic intermediates	Muconolactone isomerase (EC 5.3.3.4),putative
	gene603	0.29	Histidine Metabolism	ATP phosphoribosyltransferase (EC 2.4.2.17)
	gene3265	0.29	Central carbohydrate metabolism	Phosphoenolpyruvate-dihydroxyacetone phosphotransferase (EC 2.7.1.121), dihydroxyacetone binding subunit DhaK
	gene832	0.29	Electron donating reactions	Carbon monoxide dehydrogenase F protein
	gene621	0.29	Protein degradation	ATP-dependent Clp protease, ATP-binding subunit ClpC
	gene467	0.29	Organic sulfur assimilation	Alpha-ketoglutarate-dependent taurine dioxygenase (EC 1.14.11.17)
	gene2032	0.29	Heat shock	Heat-inducible transcription repressor HrcA
	gene110	0.29	Peripheral pathways for catabolism of aromatic compounds	2,3-dihydroxybiphenyl 1,2-dioxygenase
	gene262	0.29	NA	ABC alpha-glucoside transporter, inner membrane subunit AglF
	gene4651	0.29	Di- and oligosaccharides	Trehalose synthase (EC 5.4.99.16)
	gene4400	0.28	Sulfur Metabolism	Sulfur oxidation molybdopterin C protein
	gene931	0.28	Osmotic stress	Choline binding protein A
	gene516	0.28	Electron donating reactions	Anaerobic glycerol-3-phosphate dehydrogenase subunit C (EC 1.1.5.3)
	gene4113	0.28	Detoxification	S-formylglutathione hydrolase (EC 3.1.2.12)
	gene4192	0.28	Capsular and extracellular polysacchrides	Sialic acid transporter (permease) NanT
	gene633	0.28	DNA repair	ATP-dependent DNA ligase (EC 6.5.1.1)
	gene3398	0.28	Monosaccharides	Possible alpha-xyloside ABC transporter, permease component
	gene3031	0.28	Di- and oligosaccharides	Oligo-1,6-glucosidase (EC 3.2.1.10)
	gene450	0.28	Di- and oligosaccharides	Alpha-amylase (EC 3.2.1.1)
	gene1766	0.27	Electron donating reactions	Fumarate reductase flavoprotein subunit (EC 1.3.99.1)

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month	gene	$\log_2$Fold Change	level1	level4
	gene1312	0.27	Sulfur Metabolism	Dimethylsulfoniopropionate (DMSP) acyl CoA transferase DddD
	gene1841	0.27	Di- and oligosaccharides	Glucoamylase (EC 3.2.1.3)
	gene2234	0.27	Sugar alcohols	Inositol oxygenase (EC 1.13.99.1)
	gene4815	0.27	Oxidative stress	Uncharacterized monothiol glutaredoxin ycf64-like
	gene4926	0.27	Tetrapyrroles	Vitamin B12 ABC transporter, B12-binding component BtuF
	gene4122	0.27	Oxidative stress	SAM-dependent methyltransferase 2, in cluster with Hydroxyacylglutathione hydrolase (EC 3.1.2.6)
	gene1244	0.27	Protein degradation	Deblocking aminopeptidase (EC 3.4.11.-)
	gene2433	0.26	Lysine, threonine, methionine, and cysteine	LL-diaminopimelate aminotransferase, predicted alternative
	gene4720	0.26	Iron acquisition and metabolism	Two-component response regulator SA14-24
	gene251	0.26	Central carbohydrate metabolism	6-phosphogluconolactonase (EC 3.1.1.31)
	gene950	0.26	Cell Division and Cell Cycle	Circadian oscillation regulator KaiB
	gene4908	0.26	Alanine, serine, and glycine	Valine–pyruvate aminotransferase (EC 2.6.1.66)
	gene1345	0.26	Recombination related cluster	DNA polymerase X family
	gene4355	0.26	Clustering-based subsystems	Succinyl-CoA synthetase, alpha subunit-related enzymes
	gene2600	0.26	Monosaccharides	Mannonate dehydratase (EC 4.2.1.8)
	gene1910	0.26	Oxidative stress	Glutaredoxin
	gene2739	0.26	Metabolism of Aromatic Compounds	Monoamine oxidase (1.4.3.4)
	gene2730	0.26	Cofactors, Vitamins, Prosthetic Groups, Pigments	Molybdopterin biosynthesis enzyme
	gene644	0.26	Protein degradation	ATP-dependent protease La (EC 3.4.21.53) Type I
	gene4973	0.26	Fermentation	Xylulose-5-phosphate phosphoketolase (EC 4.1.2.9)
	gene501	0.25	Protein degradation	Aminopeptidase C (EC 3.4.22.40)
	gene3770	0.25	Organic sulfur assimilation	Putative glutathione transporter,solute-binding component
	gene3960	0.25	Nitrogen Metabolism	Response regulator NasT
	gene4654	0.25	Di- and oligosaccharides	Trehalose-6-phosphate phosphatase (EC 3.1.3.12)
	gene4835	0.25	Protein degradation	Uncharacterized protein, similar to the N-terminal domain of Lon protease
	gene3660	0.25	Pyruvate kinase associated cluster	protein of unknown function DUF882
	gene2938	0.25	Oxidative stress	Nicotinamidase (EC 3.5.1.19)
	gene478	0.25	Di- and oligosaccharides	Alpha,alpha-trehalose-phosphate synthase [UDP-forming] (EC 2.4.1.15)
	gene3647	0.25	Protein export	Protein export cytoplasm protein SecA ATPase RNA helicase (TC 3.A.5.1.1)
	gene4688	0.25	RNA processing and modification	tRNA-guanine transglycosylase (EC 2.4.2.29)
	gene2744	0.25	Carbohydrates	Monooxygenase component C
	gene3741	0.25	Nitrogen Metabolism	putative cytochrome P450 hydroxylase
	gene155	0.24	Branched-chain amino acids	3-ketoacyl-CoA thiolase (EC 2.3.1.16)
	gene4142	0.24	Regulation and Cell signaling	Sensor histidine kinase ChvG (EC 2.7.3.-)
	gene4104	0.24	Detoxification	S-(hydroxymethyl)glutathione dehydrogenase (EC 1.1.1.284)
	gene2297	0.24	Metabolism of central aromatic intermediates	Isoquinoline 1-oxidoreductase alpha subunit (EC 1.3.99.16)
	gene1986	0.24	Biosynthesis of galactoglycans and related lipopolysacharides	Glycosyl transferase, group 1
	gene4520	0.24	Transcription	Transcription elongation factor GreA
	gene3546	0.24	Clustering-based subsystems	Probable carboxyvinyl-carboxyphosphonate phosphorylmutase (EC 2.7.8.23)
	gene1120	0.24	Alanine, serine, and glycine	Cysteine desulfurase (EC 2.8.1.7), SufS subfamily
	gene1013	0.24	RNA processing and modification	COG1355, Predicted dioxygenase
	gene12	0.24	Branched-chain amino acids	(R)-citramalate synthase (EC 2.3.1.182)
	gene2657	0.24	Resistance to antibiotics and toxic compounds	Metal-dependent hydrolases of the beta-lactamase superfamily III

Table D.2 continued from previous page

month	gene	$\log_2$Fold Change	level1	level4
	gene1998	0.24	Protein biosynthesis	Glycyl-tRNA synthetase (EC 6.1.1.14)
	gene3919	0.23	Miscellaneous	Quinone oxidoreductase (EC 1.6.5.5)
	gene3256	0.23	Miscellaneous	Phosphocarrier protein kinase/phosphorylase, nitrogen regulation associated
	gene2231	0.23	Purines	Inosine-5'-monophosphate dehydrogenase (EC 1.1.1.205)
	gene313	0.23	Central carbohydrate metabolism	Acetoin dehydrogenase E1 component alpha-subunit (EC 1.2.4.-)
	gene3047	0.23	Oxidative stress	Organic hydroperoxide resistance protein
	gene4988	0.23	Clustering-based subsystems	YlxP-like protein
	gene2341	0.23	Monosaccharides	L-fuconolactone hydrolase
	gene3234	0.23	Phosphorus Metabolism	Phosphate ABC transporter, periplasmic phosphate-binding protein PstS (TC 3.A.1.7.1)
	gene2594	0.23	Oxidative stress	Manganese superoxide dismutase (EC 1.15.1.1)
	gene3829	0.23	Miscellaneous	Putative stomatin/prohibitin-family membrane protease subunit aq_911
	gene1631	0.23	Clustering-based subsystems	FIG146085: $3'$-to-$5'$ oligoribonuclease A, Bacillus type
	gene4452	0.23	Plant-Prokaryote DOE project	Thiamin biosynthesis protein ThiC
	gene1434	0.23	Electron accepting reactions	Electron transfer flavoprotein-ubiquinone oxidoreductase (EC 1.5.5.1)
	gene4941	0.23	Purines	Xanthine dehydrogenase iron-sulfur subunit (EC 1.17.1.4)
	gene635	0.23	DNA repair	ATP-dependent DNA ligase (EC 6.5.1.1) LigC
	gene2302	0.23	Potassium metabolism	Kef-type K+ transport systems (NAD-binding component fused to domain related to exopolyphosphatase)
	gene4410	0.23	Oxidative stress	Superoxide dismutase [Fe] (EC 1.15.1.1)
	gene2298	0.22	Metabolism of central aromatic intermediates	Isoquinoline 1-oxidoreductase beta subunit (EC 1.3.99.16)
	gene2650	0.22	Resistance to antibiotics and toxic compounds	Mercuric ion reductase (EC 1.16.1.1)
	gene339	0.22	Resistance to antibiotics and toxic compounds	Acriflavin resistance protein
	gene2244	0.22	Bacteriophage integration/excision/lysogeny	Integrase
	gene458	0.22	Sulfur Metabolism	Alpha-galactosidase precursor (EC 3.2.1.22)
	gene642	0.22	Protein degradation	ATP-dependent protease HslV (EC 3.4.25.-)
	gene2305	0.22	Clustering-based subsystems	KH domain RNA binding protein YlqC
	gene3123	0.22	Resistance to antibiotics and toxic compounds	Peptidase M48, Ste24p precursor
	gene4056	0.22	Oxidative stress	RNA polymerase sigma factor
	gene4712	0.22	Clustering-based subsystems	Two component transcriptional regulator VraR
	gene270	0.22	Respiration	ABC transporter involved in cytochrome c biogenesis, CcmB subunit
	gene3153	0.22	Folate and pterines	Periplasmic molybdate-binding domain
	gene1259	0.22	Monosaccharides	Deoxyribose-phosphate aldolase (EC 4.1.2.4)
	gene2833	0.21	Nucleosides and Nucleotides	N-methylhydantoinase A (EC 3.5.2.14)
	gene565	0.21	Arginine; urea cycle, polyamines	Argininosuccinate synthase (EC 6.3.4.5)
	gene3600	0.21	One-carbon Metabolism	Propionyl-CoA carboxylase beta chain (EC 6.4.1.3)
	gene3229	0.21	Phosphorus Metabolism	PhnB protein
	gene2799	0.21	Arginine; urea cycle, polyamines	N-acetyl-gamma-glutamyl-phosphate reductase (EC 1.2.1.38)
	gene244	0.21	Clustering-based subsystems	50S ribosomal protein acetyltransferase
	gene1990	0.21	Biosynthesis of galactoglycans and related lipopolysacharides	Glycosyltransferase
	gene2094	0.21	Histidine Metabolism	Histidinol-phosphatase [alternative form] (EC 3.1.3.15)
	gene1402	0.21	Putative asociate of RNA polymerase sigma-54 factor rpoN	DNA-directed RNA polymerase specialized sigma subunit, sigma24-like
	gene1706	0.21	Protein folding	Foldase protein PrsA precursor (EC 5.2.1.8)
	gene2546	0.21	Protein biosynthesis	Lysyl-tRNA synthetase (class I) (EC 6.1.1.6)

Table D.2 continued from previous page

month	gene	$\log_2$Fold Change	level1	level4
	gene1226	0.21	Nucleosides and Nucleotides	D-hydantoinase (EC 3.5.2.2)
	gene922	0.21	Aminosugars	Chitin catabolic cascade sensor histidine kinase ChiS
	gene4994	0.21	Membrane Transport	Zinc ABC transporter, ATP-binding protein ZnuC
	gene2307	0.21	Potassium metabolism	Kup system potassium uptake protein
	gene3959	0.21	Bacteriocins, ribosomally synthesized antibacterial peptides	Response regulator LiaR
	gene476	0.21	Tetrapyrroles	Alpha-ribazole-5'-phosphate phosphatase (EC 3.1.3.73)
	gene2143	0.21	Plant-Prokaryote DOE project	Hydroxymethylpyrimidine ABC transporter, substrate-binding component
	gene4542	0.20	Di- and oligosaccharides	Transcriptional activator of maltose regulon, MalT
	gene2823	0.20	Quorum sensing and biofilm formation	N-acyl homoserine lactone hydrolase
	gene4454	0.20	Folate and pterines	Thiamin-phosphate pyrophosphorylase (EC 2.5.1.3)
	gene361	0.20	Purines	Adenine deaminase (EC 3.5.4.2)
	gene1977	0.20	Polysaccharides	Glycogen phosphorylase (EC 2.4.1.1)
	gene54	0.20	Organic acids	2-dehydro-3-deoxyglucarate aldolase (EC 4.1.2.20)
	gene3756	0.20	Polysaccharides	putative esterase
	gene284	0.19	Cytochrome biogenesis	ABC-type multidrug transport system, permease component
	gene3681	0.19	Metabolism of central aromatic intermediates	Protocatechuate 3,4-dioxygenase beta chain (EC 1.13.11.3)
	gene3906	0.19	Central carbohydrate metabolism	Pyruvate,phosphate dikinase (EC 2.7.9.1)
	gene3122	0.19	Clustering-based subsystems	Peptidase M23B precursor
	gene4621	0.19	Protein biosynthesis	Translation elongation factor Ts
	gene3227	0.19	Peripheral pathways for catabolism of aromatic compounds	Phenylpropionate dioxygenase and related ring-hydroxylating dioxygenases, large terminal subunit
	gene2142	0.19	Cofactors, Vitamins, Prosthetic Groups, Pigments	Hydroxymethylpyrimidine ABC transporter, ATPase component
	gene557	0.19	Arginine; urea cycle, polyamines	Arginine pathway regulatory protein ArgR, repressor of arg regulon
	gene3130	0.19	Protein processing and modification	Peptide methionine sulfoxide reductase MsrB (EC 1.8.4.12)
	gene638	0.19	Protein degradation	ATP-dependent hsl protease ATP-binding subunit HslU
	gene149	0.19	Branched-chain amino acids	3-hydroxyisobutyrate dehydrogenase (EC 1.1.1.31)
	gene4617	0.19	Protein biosynthesis	Translation elongation factor G-related protein
	gene773	0.19	Biotin	Biotin synthesis protein bioH
	gene2797	0.19	Aminosugars	N-Acetyl-D-glucosamine ABC transport system, sugar-binding protein
	gene1853	0.19	Central carbohydrate metabolism	Gluconolactonase (EC 3.1.1.17)
	gene3989	0.19	RNA processing and modification	Ribonuclease Z (EC 3.1.26.11)
	gene3769	0.19	Organic sulfur assimilation	Putative glutathione transporter, permease component
	gene432	0.19	Oxidative stress	Alkyl hydroperoxide reductase subunit C-like protein
	gene203	0.19	Folate and pterines	4-hydroxyphenylpyruvate dioxygenase (EC 1.13.11.27)
	gene4700	0.19	Aromatic amino acids and derivatives	Tryptophan 2-monooxygenase (EC 1.13.12.3)
	gene4058	0.18	Transcription	RNA polymerase sigma factor RpoD
	gene1318	0.18	ABC transporters	Dipeptide transport system permease protein DppC (TC 3.A.1.5.2)
	gene3135	0.18	Capsular and extracellular polysacchrides	Peptidoglycan N-acetylglucosamine deacetylase (EC 3.5.1.-)
	gene1710	0.18	DNA repair	Formamidopyrimidine-DNA glycosylase (EC 3.2.2.23)
	gene504	0.18	Protein degradation	Aminopeptidase YpdF (MP-, MA-, MS-, AP-, NP- specific)
	gene3807	0.18	Miscellaneous	Putative oxidoreductase YncB
	gene166	0.18	Metabolism of central aromatic intermediates	3-oxoadipate CoA-transferase subunit A (EC 2.8.3.6)
	gene2856	0.18	Clustering-based subsystems	NAD-dependent epimerase/dehydratase
	gene4713	0.18	Regulation and Cell signaling	Two-component nitrogen fixation transcriptional regulator FixJ

Table D.2 continued from previous page

month	gene	$\log_2$Fold Change	level1	level4
	gene4375	0.18	Sulfatases and sulfatase modifying factor 1 (and a hypothetical)	Sulfatase modifying factor 1 precursor (C-alpha-formyglycine- generating enzyme 1)
	gene620	0.18	Regulation and Cell signaling	ATP-dependent Clp protease proteolytic subunit (EC 3.4.21.92)
	gene4860	0.18	Pyrimidines	Uracil phosphoribosyltransferase (EC 2.4.2.9)
	gene1566	0.18	DNA recombination	FIG000859: hypothetical protein
	gene1285	0.18	Folate and pterines	Dihydrofolate reductase (EC 1.5.1.3)
	gene4944	0.17	Purines	Xanthine dehydrogenase, molybdenum binding subunit (EC 1.17.1.4)
	gene2776	0.17	Cell Wall and Capsule	Multimodular transpeptidase-transglycosylase (EC 2.4.1.129) (EC 3.4.-.-)
	gene3431	0.17	Aromatic amino acids and derivatives	Predicted 2-keto-4-pentenoate hydratase/2-oxohepta-3-ene-1,7-dioic acid hydratase
	gene1250	0.17	Proline and 4-hydroxyproline	Delta-1-pyrroline-5-carboxylate dehydrogenase (EC 1.5.1.12)
	gene795	0.17	Electron accepting reactions	Butyryl-CoA dehydrogenase (EC 1.3.99.2)
	gene424	0.17	Phosphorus Metabolism	Alkaline phosphatase synthesis transcriptional regulatory protein PhoP
	gene799	0.17	Regulation and Cell signaling	C4-type zinc finger protein, DksA/TraR family
	gene4095	0.17	Stress Response	RsbR, positive regulator of sigma-B
	gene1006	0.17	Folate and pterines	COG0488: ATPase components of ABC transporters with duplicated ATPase domains
	gene161	0.17	Fatty acids	3-oxoacyl-[acyl-carrier protein] reductase (EC 1.1.1.100)
	gene2109	0.17	Lysine, threonine, methionine, and cysteine	Homoserine O-acetyltransferase (EC 2.3.1.31)
	gene3125	0.17	Protein biosynthesis	Peptide chain release factor 1
	gene145	0.17	Fermentation	3-hydroxybutyryl-CoA dehydrogenase (EC 1.1.1.157)
	gene1708	0.17	Folate and pterines	Folylpolyglutamate synthase (EC 6.3.2.17)
	gene3241	0.17	Phosphorus Metabolism	Phosphate transport ATP-binding protein PstB (TC 3.A.1.7.1)
	gene4518	0.17	Clustering-based subsystems	Transcription accessory protein (S1 RNA-binding domain)
	gene2811	0.16	Arginine; urea cycle, polyamines	N-acetylglutamate synthase (EC 2.3.1.1)
	gene3024	0.16	Quinone cofactors	O-succinylbenzoate-CoA synthase (EC 4.2.1.-)
	gene2104	0.16	Folate and pterines	Homogentisate 1,2-dioxygenase (EC 1.13.11.5)
	gene4477	0.16	Branched-chain amino acids	Threonine dehydratase (EC 4.3.1.19)
	gene1599	0.16	Plant-Prokaryote DOE project	FIG017823: ATPase, MoxR family
	gene1594	0.16	Two related proteases	FIG006542: Phosphoesterase
	gene1246	0.16	Detoxification	DedA protein
	gene314	0.16	Central carbohydrate metabolism	Acetoin dehydrogenase E1 component beta-subunit (EC 1.2.4.-)
	gene29	0.16	Pyridoxine	1-deoxy-D-xylulose 5-phosphate synthase (EC 2.2.1.7)
	gene2964	0.16	Metabolism of Aromatic Compounds	Nitrilotriacetate monooxygenase component B (EC 1.14.13.-)
	gene897	0.16	Clustering-based subsystems	Cell division trigger factor (EC 5.2.1.8)
	gene3129	0.16	Protein processing and modification	Peptide methionine sulfoxide reductase MsrA (EC 1.8.4.11)
	gene1918	0.16	Oxidative stress	Glutathione peroxidase (EC 1.11.1.9)
	gene3796	0.16	Regulation and Cell signaling	Putative metal chaperone, involved in Zn homeostasis, GTPase of COG0523 family
	gene3315	0.16	Purines	Phosphoribosylformylglycinamidine synthase, PurS subunit (EC 6.3.5.3)
	gene3062	0.15	Potassium metabolism	Osmosensitive K+ channel histidine kinase KdpD (EC 2.7.3.-)
	gene1847	0.15	Central carbohydrate metabolism	Gluconate dehydratase (EC 4.2.1.39)
	gene2790	0.15	Sugar alcohols	Myo-inositol 2-dehydrogenase 1 (EC 1.1.1.18)
	gene4892	0.15	Clustering-based subsystems	Uridylate kinase (EC 2.7.4.-)
	gene598	0.15	Protein biosynthesis	Aspartyl-tRNA(Asn) amidotransferase subunit A (EC 6.3.5.6)
	gene4690	0.15	RNA processing and modification	tRNA-i(6)A37 methylthiotransferase
	gene349	0.15	Branched-chain amino acids	Acyl-CoA dehydrogenase, short-chain specific (EC 1.3.99.2)
	gene3242	0.15	Phosphorus Metabolism	Phosphate transport regulator (distant homolog of PhoU)

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month	gene	log$_2$Fold Change	level1	level4
	gene490	0.15	Purines	Amidophosphoribosyltransferase (EC 2.4.2.14)
	gene847	0.15	Clustering-based subsystems	Carboxyl-terminal protease (EC 3.4.21.102)
	gene309	0.15	Branched-chain amino acids	Acetoacetyl-CoA synthetase (EC 6.2.1.16)
	gene163	0.15	Fatty acids	3-oxoacyl-[acyl-carrier-protein] synthase, KASII (EC 2.3.1.41)
	gene731	0.15	Di- and oligosaccharides	Beta-glucosidase (EC 3.2.1.21)
	gene4209	0.15	Protein biosynthesis	Signal recognition particle, subunit Ffh SRP54 (TC 3.A.5.1.1)
	gene1496	0.14	DNA repair	Excinuclease ABC subunit A
	gene4714	0.14	Regulation and Cell signaling	Two-component oxygen-sensor histidine kinase FixL
	gene738	0.14	Metabolism of central aromatic intermediates	Beta-ketoadipate enol-lactone hydrolase (EC 3.1.1.24)
	gene30	0.14	Isoprenoid/cell wall biosynthesis: PREDICTED UNDECAPRENYL DIPHOSPHATE PHOSPHATASE	1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate synthase (EC 1.17.7.1)
	gene1457	0.14	Fatty acids	Enoyl-[acyl-carrier-protein] reductase [NADH] (EC 1.3.1.9)
	gene193	0.14	Isoprenoids	4-hydroxy-3-methylbut-2-enyl diphosphate reductase (EC 1.17.1.2)
	gene744	0.14	Resistance to antibiotics and toxic compounds	Beta-lactamase class C and other penicillin binding proteins
	gene2482	0.14	Protein biosynthesis	LSU ribosomal protein L25p
	gene4778	0.14	Quinone cofactors	Ubiquinone biosynthesis monooxygenase UbiB
	gene4675	0.14	RNA processing and modification	tRNA nucleotidyltransferase (EC 2.7.7.21) (EC 2.7.7.25)
	gene3667	0.14	RNA processing and modification	Protein RtcB
	gene4538	0.14	Transcription	Transcription-repair coupling factor
	gene3038	0.14	ABC transporters	Oligopeptide transport system permease protein OppC (TC 3.A.1.5.1)
	gene599	0.14	Protein biosynthesis	Aspartyl-tRNA(Asn) amidotransferase subunit B (EC 6.3.5.6)
	gene564	0.14	Arginine; urea cycle, polyamines	Argininosuccinate lyase (EC 4.3.2.1)
	gene933	0.14	Osmotic stress	Choline-sulfatase (EC 3.1.6.6)
	gene4169	0.14	Lysine, threonine, methionine, and cysteine	Serine acetyltransferase (EC 2.3.1.30)
	gene370	0.14	Biotin	Adenosylmethionine-8-amino-7-oxononanoate aminotransferase (EC 2.6.1.62)
	gene333	0.13	Arginine; urea cycle, polyamines	Acetylglutamate kinase (EC 2.7.2.8)
	gene2865	0.13	Oxidative stress	NAD-dependent protein deacetylase of SIR2 family
	gene896	0.13	Clustering-based subsystems	Cell division transporter, ATP-binding protein FtsE (TC 3.A.5.1.1)
	gene4487	0.13	Pyrimidines	Thymidylate kinase (EC 2.7.4.9)
	gene985	0.13	Resistance to antibiotics and toxic compounds	Cobalt-zinc-cadmium resistance protein
	gene2311	0.13	NAD and NADP	Kynurenine formamidase, bacterial (EC 3.5.1.9)
	gene3308	0.13	Purines	Phosphoribosylaminoimidazole-succinocarboxamide synthase (EC 6.3.2.6)
	gene4171	0.13	Alanine, serine, and glycine	Serine hydroxymethyltransferase (EC 2.1.2.1)
	gene1459	0.13	Fermentation	Enoyl-CoA hydratase (EC 4.2.1.17)
	gene1283	0.13	Lysine, threonine, methionine, and cysteine	Dihydrodipicolinate synthase (EC 4.2.1.52)
	gene2444	0.13	Alanine, serine, and glycine	Low-specificity L-threonine aldolase (EC 4.1.2.5)
	gene1754	0.12	Central carbohydrate metabolism	Fructose-bisphosphate aldolase class I (EC 4.1.2.13)
	gene4492	0.12	TldD cluster	TldD protein, part of proposed TldE/TldD proteolytic complex (PMID 12029038)
	gene248	0.12	Monosaccharides	6-phosphofructokinase (EC 2.7.1.11)
	gene1196	0.12	Pyridoxine	D-3-phosphoglycerate dehydrogenase (EC 1.1.1.95)
	gene595	0.12	Lysine, threonine, methionine, and cysteine	Aspartate-semialdehyde dehydrogenase (EC 1.2.1.11)
	gene4773	0.11	Protein biosynthesis	Tyrosyl-tRNA synthetase (EC 6.1.1.1)
	gene3037	0.11	ABC transporters	Oligopeptide transport system permease protein OppB (TC 3.A.1.5.1)

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month	gene	$\log_2$Fold Change	level1	level4
	gene4470	0.11	Respiration	Thiol:disulfide oxidoreductase related to ResA
	gene4036	0.11	Protein biosynthesis	Ribosome recycling factor
	gene3559	0.11	Phosphorus Metabolism	Probable low-affinity inorganic phosphate transporter
	gene4909	0.11	Protein biosynthesis	Valyl-tRNA synthetase (EC 6.1.1.9)
	gene4673	0.11	RNA processing and modification	tRNA dihydrouridine synthase B (EC 1.-.-.-)
	gene1335	0.11	DNA replication	DNA polymerase I (EC 2.7.7.7)
	gene4173	0.11	Stress Response	Serine phosphatase RsbU, regulator of sigma subunit
	gene329	0.11	Fatty acids	Acetyl-coenzyme A carboxyl transferase beta chain (EC 6.4.1.2)
	gene1348	0.11	DNA replication	DNA primase (EC 2.7.7.-)
	gene4206	0.11	Protein processing and modification	Signal peptidase I (EC 3.4.21.89)
	gene4619	0.11	Protein biosynthesis	Translation elongation factor P
	gene3225	0.10	Protein biosynthesis	Phenylalanyl-tRNA synthetase alpha chain (EC 6.1.1.20)
	gene236	0.10	Detoxification	5-nucleotidase SurE (EC 3.1.3.5)
	gene882	0.10	Clustering-based subsystems	Cell division protein FtsH (EC 3.4.24.-)
	gene2293	0.10	Protein biosynthesis	Isoleucyl-tRNA synthetase (EC 6.1.1.5)
	gene2675	0.10	Protein biosynthesis	Methionyl-tRNA synthetase (EC 6.1.1.10)
	gene3380	0.10	Purines	Polyphosphate kinase (EC 2.7.4.1)
	gene1125	0.09	Protein biosynthesis	Cysteinyl-tRNA synthetase (EC 6.1.1.16)
	gene334	0.09	Arginine; urea cycle, polyamines	Acetylornithine aminotransferase (EC 2.6.1.11)
	gene28	0.09	Isoprenoid/cell wall biosynthesis: PREDICTED UNDECAPRENYL DIPHOSPHATE PHOSPHATASE	1-deoxy-D-xylulose 5-phosphate reductoisomerase (EC 1.1.1.267)
	gene2632	0.08	Isoprenoid/cell wall biosynthesis: PREDICTED UNDECAPRENYL DIPHOSPHATE PHOSPHATASE	Membrane-associated zinc metalloprotease
	gene1084	0.08	Pyrimidines	CTP synthase (EC 6.3.4.2)
	gene2990	0.05	NA	NO HIERARCHY
June	gene1338	-0.07	DNA replication	DNA polymerase III beta subunit (EC 2.7.7.7)
	gene1204	-0.09	Cell Wall and Capsule	D-alanyl-D-alanine carboxypeptidase (EC 3.4.16.4)
	gene4799	-0.09	Cell Wall and Capsule	UDP-N-acetylmuramoylalanine–D-glutamate ligase (EC 6.3.2.9)
	gene254	-0.10	Riboflavin, FMN, FAD	6,7-dimethyl-8-ribityllumazine synthase (EC 2.5.1.9)
	gene3254	-0.10	Cell Wall and Capsule	Phospho-N-acetylmuramoyl-pentapeptide-transferase (EC 2.7.8.13)
	gene2691	-0.12	Branched-chain amino acids	Methylglutaconyl-CoA hydratase (EC 4.2.1.18)
	gene1337	-0.12	Clustering-based subsystems	DNA polymerase III alpha subunit (EC 2.7.7.7)
	gene4800	-0.12	Cell Wall and Capsule	UDP-N-acetylmuramoylalanyl-D-glutamate–2,6-diaminopimelate ligase (EC 6.3.2.13)
	gene2903	-0.12	Electron donating reactions	NADH-ubiquinone oxidoreductase chain K (EC 1.6.5.3)
	gene4838	-0.13	Biosynthesis of galactoglycans and related lipopolysacharides	Undecaprenyl-phosphate galactosephosphotransferase (EC 2.7.8.6)
	gene4794	-0.13	Capsular and extracellular polysacchrides	UDP-N-acetylglucosamine 2-epimerase (EC 5.1.3.14)
	gene3947	-0.14	DNA replication	Replicative DNA helicase (EC 3.6.1.-)
	gene1887	-0.15	Nitrogen Metabolism	Glutamate-ammonia-ligase adenylyltransferase (EC 2.7.7.42)
	gene4234	-0.15	DNA repair	Single-stranded-DNA-specific exonuclease RecJ (EC 3.1.-.-)
	gene4707	-0.15	Respiration	tungsten-containing formate dehydrogenase beta subunit
	gene1333	-0.15	DNA repair	DNA mismatch repair protein MutL
	gene2764	-0.15	Resistance to antibiotics and toxic compounds	Multicopper oxidase
	gene2902	-0.16	Electron donating reactions	NADH-ubiquinone oxidoreductase chain J (EC 1.6.5.3)
	gene4733	-0.16	Regulation and Cell signaling	Two-component system response regulator QseB
	gene2904	-0.16	Electron donating reactions	NADH-ubiquinone oxidoreductase chain L (EC 1.6.5.3)
	gene2784	-0.16	Cell Wall and Capsule	Muramoyltetrapeptide carboxypeptidase (EC 3.4.17.13)

Table D.2 continued from previous page

month	gene	$\log_2$Fold Change	level1	level4
	gene4167	-0.16	Cell Division and Cell Cycle	Septum site-determining protein MinD
	gene1922	−0.17	Oxidative stress	Glutathione S-transferase family protein
	gene3314	−0.17	Purines	Phosphoribosylformylglycinamidine synthase, glutamine amidotransferase subunit (EC 6.3.5.3)
	gene1279	−0.17	Stress Response	diguanylate cyclase/phosphodiesterase (GGDEF & EAL domains) with PAS/PAC sensor(s)
	gene4797	−0.17	Cell Wall and Capsule	UDP-N-acetylmuramate–alanine ligase (EC 6.3.2.8)
	gene2038	-0.17	Respiration	Heme A synthase, cytochrome oxidase biogenesis protein Cox15-CtaA
	gene1725	−0.17	Respiration	Formate dehydrogenase-O, major subunit (EC 1.2.1.2)
	gene2905	−0.18	Electron donating reactions	NADH-ubiquinone oxidoreductase chain M (EC 1.6.5.3)
	gene3675	−0.18	Clustering-based subsystems	Protein YicC
	gene1548	−0.18	Iron acquisition and metabolism	Ferric iron ABC transporter, iron-binding protein
	gene2906	−0.19	Electron donating reactions	NADH-ubiquinone oxidoreductase chain N (EC 1.6.5.3)
	gene1817	−0.19	Protein secretion system, Type II	General secretion pathway protein G
	gene1552	−0.19	Oxidative stress	Ferric uptake regulation protein FUR
	gene1707	−0.19	Folate and pterines	FolM Alternative dihydrofolate reductase 1
	gene1357	−0.19	DNA repair	DNA repair protein RadA
	gene4252	−0.19	DNA repair	SOS-response repressor and protease LexA (EC 3.4.21.88)
	gene1298	−0.19	Pyrimidines	Dihydroorotate dehydrogenase (EC 1.3.3.1)
	gene4137	−0.19	Selenoproteins	Selenide,water dikinase (EC 2.7.9.3)
	gene1342	−0.19	DNA polymerase III epsilon cluster	DNA polymerase III epsilon subunit (EC 2.7.7.7)
	gene4772	-0.20	Biosynthesis of galactoglycans and related lipopolysacharides	Tyrosine-protein kinase Wzc (EC 2.7.10.2)
	gene2702	-0.20	RNA processing and modification	Methylthioadenosine deaminase
	gene191	-0.20	Isoprenoids	4-diphosphocytidyl-2-C-methyl-D-erythritol kinase (EC 2.7.1.148)
	gene3439	-0.20	Phosphorus Metabolism	Predicted ATPase related to phosphate starvation-inducible protein PhoH
	gene2133	-0.20	Regulation and Cell signaling	Hydrolase, alpha/beta fold family functionally coupled to Phosphoribulokinase
	gene2098	-0.20	DNA replication	Holliday junction DNA helicase RuvA
	gene683	-0.21	Siderophores	Bacillibactin synthetase component F (EC 2.7.7.-)
	gene3338	-0.21	Isoprenoids	Phytoene dehydrogenase (EC 1.14.99.-)
	gene1172	-0.21	Respiration	Cytochrome c551/c552
	gene116	-0.21	Fatty acids	2,4-dienoyl-CoA reductase [NADPH] (EC 1.3.1.34)
	gene75	-0.22	Gram-Negative cell wall components	2-Keto-3-deoxy-D-manno-octulosonate-8-phosphate synthase (EC 2.5.1.55)
	gene4174	-0.22	Clustering-based subsystems	Serine protease (EC 3.4.21.-)
	gene511	-0.22	Electron accepting reactions	Anaerobic dimethyl sulfoxide reductase chain A (EC 1.8.99.-)
	gene4796	-0.22	Cell Wall and Capsule	UDP-N-acetylglucosamine–N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase (EC 2.4.1.227)
	gene1569	-0.22	Clustering-based subsystems	FIG001571: Hypothetical protein
	gene4782	-0.22	Gram-Negative cell wall components	UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase (EC 3.5.1.-)
	gene4695	-0.22	RNA processing and modification	tRNA(Cytosine32)-2-thiocytidine synthetase
	gene3868	-0.22	Siderophores	Pyoverdine chromophore precursor synthetase PvdL
	gene891	-0.22	Cell Division and Cell Cycle	Cell division protein FtsZ (EC 3.4.24.-)
	gene1225	-0.23	Capsular and extracellular polysacchrides	D-glycero-D-manno-heptose 1,7-bisphosphate phosphatase (EC 3.1.1.-)
	gene430	-0.23	Sulfur Metabolism	Alkyl hydroperoxide reductase protein C (EC 1.6.4.-)
	gene1112	-0.23	Lysine, threonine, methionine, and cysteine	Cystathionine beta-lyase (EC 4.4.1.8)
	gene1928	-0.23	Clustering-based subsystems	Glutathione synthetase (EC 6.3.2.3)

Table D.2 continued from previous page

month	gene	$\log_2$Fold Change	level1	level4
	gene2994	-0.23	Siderophores	Non-ribosomal peptide synthetase modules, pyoverdine
	gene3239	-0.24	Phosphorus Metabolism	Phosphate starvation-inducible ATPase PhoH with RNA binding motif
	gene885	-0.24	Cell Division and Cell Cycle	Cell division protein FtsK
	gene4728	-0.24	Protein and nucleoprotein secretion system, Type IV	Two-component sensor PilS
	gene2326	-0.24	Monosaccharides	L-arabonate dehydratase (EC 4.2.1.25)
	gene4219	-0.24	Proline and 4-hydroxyproline	Similar to eukaryotic Peptidyl prolyl 4-hydroxylase, alpha subunit (EC 1.14.11.2)
	gene2112	-0.25	Regulation and Cell signaling	HPr kinase/phosphorylase (EC 2.7.1.-) (EC 2.7.4.-)
	gene1606	-0.25	Fatty acids	FIG022199: FAD-binding protein
	gene1863	-0.25	Capsular and extracellular polysacchrides	Glucose-1-phosphate cytidylyltransferase (EC 2.7.7.33)
	gene2995	-0.25	Siderophores	Non-ribosomal peptide synthetase modules, pyoverdine
	gene3870	-0.25	Siderophores	Pyoverdine sidechain non-ribosomal peptide synthetase PvdD
	gene3121	-0.25	Protein degradation	Peptidase B (EC 3.4.11.23)
	gene307	-0.25	Fermentation	Acetoacetyl-CoA reductase (EC 1.1.1.36)
	gene4388	-0.25	Sulfur Metabolism	Sulfide dehydrogenase [flavocytochrome C] flavoprotein chain precursor (EC 1.8.2.-)
	gene3263	-0.25	Central carbohydrate metabolism	Phosphoenolpyruvate synthase (EC 2.7.9.2)
	gene158	-0.25	Fatty acids	3-oxoacyl-[ACP] reductase (EC 1.1.1.100)
	gene892	-0.25	Cell Division and Cell Cycle	Cell division protein MraZ
	gene4478	-0.25	Branched-chain amino acids	Threonine dehydratase biosynthetic (EC 4.3.1.19)
	gene1549	-0.26	Iron acquisition and metabolism	Ferric iron ABC transporter, permease protein
	gene1668	-0.26	Flagellar motility in Prokaryota	Flagellar hook-length control protein FliK
	gene3928	-0.26	DNA repair	RecA protein
	gene1195	-0.26	One-carbon Metabolism	cytosolic long-chain acyl-CoA thioester hydrolase family protein
	gene2123	-0.26	Respiration	Hydrogenase-4 component B (EC 1.-.-.-)
	gene3894	-0.26	Central carbohydrate metabolism	Pyruvate carboxyl transferase subunit A (EC 6.4.1.1)
	gene518	-0.26	Cell Wall and Capsule	Anhydro-N-acetylmuramic acid kinase (EC 2.7.1.-)
	gene772	-0.26	Biotin	Biotin synthesis protein bioC
	gene1815	-0.26	Protein secretion system, Type II	General secretion pathway protein E
	gene2256	-0.27	Fatty Acids, Lipids, and Isoprenoids	Intracellular PHB depolymerase (EC 3.1.1.-)
	gene2550	-0.27	Putative asociate of RNA polymerase sigma-54 factor rpoN	macromolecule metabolism
	gene1031	-0.27	Cold shock	Cold shock protein CspA
	gene3006	-0.27	Bacterial cytostatics, differentiation factors and antibiotics	NRPS module 4 PG-Ser-Gly-Thr
	gene1914	-0.27	Oxidative stress	Glutaredoxin 3 (Grx3)
	gene4515	-0.27	Membrane Transport	TPR repeat containing exported protein
	gene1046	-0.28	Urate degradation	conserved hypothetical membrane protein, paralogue of Y20848
	gene404	-0.28	Monosaccharides	Alcohol dehydrogenase, zinc-containing
	gene1752	-0.29	Central carbohydrate metabolism	Fructose-1,6-bisphosphatase, type I (EC 3.1.3.11)
	gene909	-0.29	Protein folding	Chaperone protein HscB
	gene4823	-0.29	Clustering-based subsystems	Uncharacterized protein ImpJ/VasE
	gene4589	-0.29	Regulation and Cell signaling	Transcriptional regulatory protein basR/pmrA
	gene3709	-0.29	Capsular and extracellular polysacchrides	PTS system, mannose-specific IIA component (EC 2.7.1.69)
	gene1141	-0.29	Respiration	Cytochrome c heme lyase subunit CcmL
	gene1352	-0.29	DNA repair	DNA recombination protein RmuC
	gene4052	-0.29	Translation	RNA polymerase associated protein RapA (EC 3.6.1.-)
	gene1148	-0.30	Electron accepting reactions	Cytochrome c oxidase polypeptide IV(EC 1.9.3.1)

Table D.2 continued from previous page

month	gene	$\log_2$Fold Change	level1	level4
	gene4744	-0.30	Protein secretion system, Type II	Type II/IV secretion system ATPase TadZ/CpaE, associated with Flp pilus assembly
	gene4162	-0.30	Regulation and Cell signaling	Sensory histidine kinase QseC
	gene4806	-0.30	Gram-Negative cell wall components	Uncharacterized ABC transporter, auxiliary component YrbC
	gene1111	-0.30	Lysine, threonine, methionine, and cysteine	Cys regulon transcriptional activator CysB
	gene811	-0.30	Capsular and extracellular polysacchrides	capsular polysaccharide biosynthesis protein
	gene673	-0.31	Protein degradation	ATPase, AFG1 family
	gene1814	-0.31	Protein secretion system, Type II	General secretion pathway protein D
	gene1947	-0.31	Phospholipids	Glycerol-3-phosphate acyltransferase (EC 2.3.1.15)
	gene4742	-0.31	Membrane Transport	Type I secretion outer membrane protein, TolC precursor
	gene3082	-0.31	Clustering-based subsystems	Outer membrane protein YfgL, lipoprotein component of the protein assembly complex (forms a complex with YaeT, YfiO, and NlpB)
	gene1643	-0.31	Potassium metabolism	FKBP-type peptidyl-prolyl cis-trans isomerase SlyD (EC 5.2.1.8)
	gene780	-0.31	Clustering-based subsystems	Bis(5'-nucleosyl)-tetraphosphatase, symmetrical (EC 3.6.1.41)
	gene797	-0.32	NAD and NADP	C-terminal domain of CinA type S homolog
	gene4166	-0.32	Cell Division and Cell Cycle	Septum site-determining protein MinC
	gene1894	-0.32	Glutamine, glutamate, aspartate, asparagine; ammonia assimilation	glutamine synthetase family protein
	gene2246	-0.32	DNA Metabolism	Integration host factor alpha subunit
	gene2274	-0.32	Transcription	Iron-sulfur cluster regulator IscR
	gene3513	-0.32	Flagellar motility in Prokaryota	Predicted signal transduction protein
	gene4486	-0.32	Pyrimidines	Thymidine phosphorylase (EC 2.4.2.4)
	gene2952	-0.32	Nitrogen Metabolism	Nitrate/nitrite sensor protein (EC 2.7.3.-)
	gene1613	-0.32	Clustering-based subsystems	FIG037127: Two-component system sensor protein
	gene1339	-0.32	DNA replication	DNA polymerase III chi subunit (EC 2.7.7.7)
	gene135	-0.32	Gram-Negative cell wall components	3-deoxy-D-manno-octulosonate 8-phosphate phosphatase (EC 3.1.3.45)
	gene1505	-0.32	DNA repair	Exodeoxyribonuclease V beta chain (EC 3.1.11.5)
	gene1629	-0.33	Fatty acids	FIG143263: Glycosyl transferase
	gene3161	-0.33	Protein folding	Periplasmic thiol:disulfide oxidoreductase DsbB, required for DsbA reoxidation
	gene1034	-0.33	Cold shock	Cold shock protein CspD
	gene4980	-0.33	RNA processing and modification	YciO family
	gene3907	-0.33	Central carbohydrate metabolism	Pyruvate:ferredoxin oxidoreductase, alpha subunit (EC 1.2.7.1)
	gene321	-0.33	Peripheral pathways for catabolism of aromatic compounds	Acetyl-CoA C-acyltransferase (EC 2.3.1.16)
	gene456	-0.33	Capsular and extracellular polysacchrides	Alpha-D-GlcNAc alpha-1,2-L-rhamnosyltransferase (EC 2.4.1.-)
	gene1025	-0.33	Cell Wall and Capsule	COG3178: Predicted phosphotransferase related to Ser/Thr protein kinases
	gene3979	-0.33	RNA processing and modification	Ribonuclease E inhibitor RraA
	gene3440	-0.34	Protein secretion system, Type II	Predicted ATPase with chaperone activity, associated with Flp pilus assembly
	gene266	-0.34	Clustering-based subsystems	ABC transporter ATP-binding protein USSDB6B
	gene1037	-0.34	Cold shock	Cold shock protein CspG
	gene4743	-0.34	Protein secretion system, Type II	Type II/IV secretion system ATP hydrolase TadA/VirB11/CpaF, TadA subfamily
	gene1516	-0.34	RNA processing and modification	Exoribonuclease II (EC 3.1.13.1)
	gene3423	-0.34	Potassium metabolism	POTASSIUM/PROTON ANTIPORTER ROSB
	gene4455	-0.34	Detoxification	Thiamin-phosphate pyrophosphorylase-like protein
	gene3871	-0.34	Siderophores	Pyoverdine sidechain non-ribosomal peptide synthetase PvdI

Table D.2 continued from previous page

month	gene	$\log_2$Fold Change	level1	level4
	gene4641	-0.34	Tricarboxylate transporter	TRAP-type C4-dicarboxylate transport system, periplasmic component
	gene3318	-0.34	Purines	Phosphoribosylglycinamide formyltransferase 2 (EC 2.1.2.-)
	gene160	-0.35	Fatty acids	3-oxoacyl-[ACP] synthase (EC 2.3.1.41) FabV like
	gene880	-0.35	Cell Division and Cell Cycle	Cell division protein FtsA
	gene1241	-0.35	Fatty Acids, Lipids, and Isoprenoids	D(-)-3-hydroxybutyrate oligomer hydrolase (EC 3.1.1.22)
	gene4163	-0.35	CO2 fixation	Sensory subunit of low CO2-induced protein complex, putative
	gene3548	-0.35	Gram-Negative cell wall components	Probable component of the lipoprotein assembly complex (forms a complex with YaeT, YfgL, and NlpB)
	gene1816	-0.35	Protein secretion system, Type II	General secretion pathway protein F
	gene1729	-0.36	Respiration	Formate hydrogenlyase subunit 4
	gene4656	-0.36	Tricarboxylate transporter	Tricarboxylate transport membrane protein TctA
	gene4789	-0.36	Gram-Negative cell wall components	UDP-glucose:(heptosyl) LPS alpha1,3-glucosyltransferase WaaG (EC 2.4.1.-)
	gene2399	-0.36	Central carbohydrate metabolism	Leucine-, isoleucine-, valine-, threonine-, and alanine-binding protein
	gene866	-0.36	Tetrapyrroles	CblY, a non-orthologous displasment for Alpha-ribazole-5'-phosphate phosphatase
	gene3357	-0.36	RNA processing and modification	Poly(A) polymerase (EC 2.7.7.19)
	gene3185	-0.36	Stress Response	Phage shock protein C
	gene1948	-0.36	Capsular and extracellular polysacchrides	Glycerol-3-phosphate cytidylyltransferase (EC 2.7.7.39)
	gene1696	-0.36	Stress Response	Flavohemoprotein (Hemoglobin-like protein) (Flavohemoglobin) (Nitric oxide dioxygenase) (EC 1.14.12.17)
	gene3909	-0.36	Central carbohydrate metabolism	Pyruvate:ferredoxin oxidoreductase, gamma subunit (EC 1.2.7.1)
	gene4780	-0.36	Gram-Negative cell wall components	UDP-2,3-diacylglucosamine hydrolase (EC 3.6.1.-)
	gene809	-0.37	Capsular and extracellular polysacchrides	Capsular polysaccharide ABC transporter, permease protein KpsM
	gene803	-0.37	Regulation and Cell signaling	CAMP phosphodiesterases class-II:Metallo-beta-lactamase superfamily
	gene4491	-0.37	Protein degradation	TldD family protein, Beta/Gamma-proteobacterial subgroup
	gene535	-0.37	Gram-Negative cell wall components	Arabinose 5-phosphate isomerase (EC 5.3.1.13)
	gene572	-0.37	Folate and pterines	Aromatic-amino-acid aminotransferase (EC 2.6.1.57)
	gene1933	-0.37	Potassium metabolism	Glutathione-regulated potassium-efflux system protein KefB
	gene2985	-0.37	Nitrogen Metabolism	Nitrous oxide reductase maturation transmembrane protein NosY
	gene2754	-0.37	Protein and nucleoprotein secretion system, Type IV	MSHA biogenesis protein MshM
	gene4360	-0.37	Arginine; urea cycle, polyamines	Succinylglutamic semialdehyde dehydrogenase (EC 1.2.1.71)
	gene4584	-0.38	Central carbohydrate metabolism	Transcriptional regulator, LysR family, in glycolate utilization operon
	gene4093	-0.38	Clustering-based subsystems	rRNA small subunit methyltransferase H
	gene3262	-0.38	Phosphorus Metabolism	Phosphoenolpyruvate phosphomutase (EC 5.4.2.9)
	gene3908	-0.38	Central carbohydrate metabolism	Pyruvate:ferredoxin oxidoreductase, beta subunit (EC 1.2.7.1)
	gene2785	-0.38	Cell Wall and Capsule	Murein-DD-endopeptidase (EC 3.4.99.-)
	gene2241	-0.38	Clustering-based subsystems	Integral membrane protein CcmA involved in cell shape determination
	gene4640	-0.38	Tricarboxylate transporter	TRAP-type C4-dicarboxylate transport system, large permease component
	gene4180	-0.38	One-carbon Metabolism	Serine-pyruvate aminotransferase/archaeal aspartate aminotransferase
	gene887	-0.39	Cell Division and Cell Cycle	Cell division protein FtsN
	gene2511	-0.39	Protein biosynthesis	LSU ribosomal protein L8e (L2p)
	gene1506	-0.39	DNA repair	Exodeoxyribonuclease V gamma chain (EC 3.1.11.5)
	gene3375	-0.39	Gram-Negative cell wall components	Polymyxin resistance protein ArnT, undecaprenyl phosphate-alpha-L-Ara4N transferase
	gene625	-0.39	DNA repair	ATP-dependent DNA helicase Rep
	gene126	-0.39	RNA processing and modification	23S rRNA (Uracil-5-) -methyltransferase rumA (EC 2.1.1.-)

Table D.2 continued from previous page

month	gene	log$_2$Fold Change	level1	level4
	gene1571	-0.39	Cell Division and Cell Cycle	FIG001721: Predicted N6-adenine-specific DNA methylase
	gene1623	-0.40	Lysine Biosynthesis	FIG138056: a glutathione-dependent thiol reductase
	gene2388	-0.40	Clustering-based subsystems	Large exoproteins involved in heme utilization or adhesion
	gene3540	-0.40	Programmed Cell Death and Toxin-antitoxin Systems	Preprotein translocase subunit SecG (TC 3.A.5.1.1)
	gene4112	-0.40	RNA processing and modification	S-adenosylmethionine:tRNA ribosyltransferase-isomerase (EC 5.-.-.-)
	gene3603	-0.40	Lipoic acid	Proposed lipoate regulatory protein YbeD
	gene4979	-0.40	Miscellaneous	YciL protein
	gene4928	-0.40	Protein secretion system, Type II	Von Willebrand factor type A domain protein, associated with Flp pilus assembly
	gene3021	-0.41	Detoxification	Nudix-like NDP and NTP phosphohydrolase YmfB
	gene4468	-0.41	Protein folding	Thiol:disulfide interchange protein DsbC
	gene813	-0.41	Capsular and extracellular polysacchrides	Capsular polysaccharide biosynthesis/export periplasmic protein WcbC
	gene4226	-0.41	DNA repair	Similar to phosphoglycolate phosphatase, clustered with ubiquinone biosynthesis SAM-dependent O-methyltransferase
	gene4140	-0.41	Selenoproteins	Selenophosphate-dependent tRNA 2-selenouridine synthase
	gene1576	-0.41	Fatty acids	FIG002571: 4-hydroxybenzoyl-CoA thioesterase domain protein
	gene1711	-0.41	Electron accepting reactions	Formate dehydrogenase -O, gamma subunit (EC 1.2.1.2)
	gene4805	-0.41	Gram-Negative cell wall components	Uncharacterized ABC transporter, ATP-binding protein YrbF
	gene426	-0.41	Regulation and Cell signaling	Alkanesulfonate utilization operon LysR-family regulator CbI
	gene2124	-0.41	Respiration	Hydrogenase-4 component E (EC 1.-.-.-)
	gene1585	-0.41	Plant-Prokaryote DOE project	FIG004454: RNA binding protein
	gene4540	-0.42	Lysine, threonine, methionine, and cysteine	Transcriptional activator MetR
	gene3466	-0.42	Gram-Negative cell wall components	Predicted hydrolase of the metallo-beta-lactamase superfamily, clustered with KDO2-Lipid A biosynthesis genes
	gene1504	-0.42	DNA repair	Exodeoxyribonuclease V alpha chain (EC 3.1.11.5)
	gene343	-0.42	Fatty acids	Acyl carrier protein (ACP1)
	gene1860	-0.42	Central carbohydrate metabolism	Glucose ABC transport system, periplasmic sugar-binding protein
	gene537	-0.42	Monosaccharides	Arabinose-proton symporter
	gene3763	-0.42	Respiration	Putative formate dehydrogenase iron-sulfur subunit (EC 1.2.1.2)
	gene3599	-0.43	Organic acids	Propionate–CoA ligase (EC 6.2.1.17)
	gene2750	-0.43	Protein and nucleoprotein secretion system, Type IV	MSHA biogenesis protein MshE
	gene4583	-0.43	Detoxification	Transcriptional regulator, LysR family, in formaldehyde detoxification operon
	gene3890	-0.43	Phosphorus Metabolism	Pyrophosphate-specific outer membrane porin OprO
	gene1589	-0.44	Oxidative stress	FIG005121: SAM-dependent methyltransferase (EC 2.1.1.-)
	gene649	-0.44	RNA processing and modification	ATP-dependent RNA helicase DbpA
	gene444	-0.44	Protein processing and modification	Alpha-1,4-N-acetylgalactosamine transferase PglJ (EC 2.4.1.-)
	gene295	-0.44	Clustering-based subsystems	ABC-type transport system involved in resistance to organic solvents, periplasmic component USSDB6C
	gene1777	-0.44	Putative GGDEF domain protein related to agglutinin secretion	GAF domain/GGDEF domain/EAL domain protein
	gene2259	-0.44	Siderophores	iron aquisition yersiniabactin synthesis enzyme (Irp1,polyketide synthetase)
	gene1056	-0.44	Resistance to antibiotics and toxic compounds	Copper resistance protein B
	gene1798	-0.45	Arginine; urea cycle, polyamines	Gamma-glutamyl-putrescine oxidase (EC1.4.3.-)
	gene1098	-0.45	Arginine; urea cycle, polyamines	Cyanophycin synthase II
	gene4753	-0.45	Protein and nucleoprotein secretion system, Type IV	Type IV fimbrial assembly, ATPase PilB

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month	gene	$\log_2$Fold Change	level1	level4
	gene4752	-0.45	Protein and nucleoprotein secretion system, Type IV	Type IV fimbrial assembly protein PilC
	gene652	-0.45	RNA processing and modification	ATP-dependent RNA helicase RhlB
	gene1691	-0.45	Flagellar motility in Prokaryota	Flagellum-specific ATP synthase FliI
	gene3193	-0.46	Phages, Prophages	Phage terminase large subunit
	gene1704	-0.46	Folate and pterines	Folate transporter 3
	gene3677	-0.46	Protein translocation across cytoplasmic membrane	Protein-export membrane protein SecD (TC 3.A.5.1.1)
	gene4902	-0.46	ATP synthases	V-type ATP synthase subunit A (EC 3.6.3.14)
	gene1683	-0.46	Flagellar motility in Prokaryota	Flagellar synthesis regulator FleN
	gene4028	-0.46	RNA processing and modification	Ribosomal small subunit pseudouridine synthase A (EC 4.2.1.70)
	gene2193	-0.46	Protein secretion system, Type VI	IcmF-related protein
	gene4645	-0.46	Tricarboxylate transporter	TRAP-type uncharacterized transport system, fused permease component
	gene1818	-0.46	Protein secretion system, Type II	General secretion pathway protein H
	gene1168	-0.46	Respiration	Cytochrome c4
	gene641	-0.46	Protein degradation	ATP-dependent protease domain protein (EC 3.4.21.-)
	gene947	-0.47	Clustering-based subsystems	Chromosome partitioning protein ParA
	gene888	-0.47	Cell Division and Cell Cycle	Cell division protein FtsQ
	gene3840	-0.47	Translation	Putative TEGT family carrier/transport protein
	gene3275	-0.47	Osmotic stress	Phosphoglycerol transferase I (EC 2.7.8.20)
	gene4745	-0.47	Protein secretion system, Type II	Type II/IV secretion system protein TadC, associated with Flp pilus assembly
	gene2266	-0.47	Iron acquisition and metabolism	Iron-regulated protein A precursor
	gene125	-0.48	RNA processing and modification	23S rRNA (guanosine-2'-O-) -methyltransferase rlmB (EC 2.1.1.-)
	gene4374	-0.48	Sulfatases and sulfatase modifying factor 1 (and a hypothetical)	Sulfatase
	gene2705	-0.48	Isoprenoids	Mevalonate kinase (EC 2.7.1.36)
	gene3723	-0.48	Peripheral pathways for catabolism of aromatic compounds	putative 3-hydroxyphenylpropionic acid porine
	gene1155	-0.49	Respiration	Cytochrome c-552 precursor
	gene124	-0.49	Clustering-based subsystems	21 kDa hemolysin precursor
	gene2835	-0.49	Protein and nucleoprotein secretion system, Type IV	N-methyltransferase (EC 2.1.1.-)
	gene2531	-0.49	Fatty acids	Lysophospholipid acyltransferase
	gene3376	-0.49	Gram-Negative cell wall components	Polymyxin resistance protein PmrJ, predicted deacetylase
	gene1637	-0.49	Fatty acids	FIGfam138462: Acyl-CoA synthetase, AMP-(fatty) acid ligase
	gene4844	-0.49	Shiga toxin cluster	unknown protein encoded within prophage CP-933V
	gene3991	-0.49	Nucleosides and Nucleotides	Ribonucleotide reductase of class Ia (aerobic), beta subunit (EC 1.17.4.1)
	gene159	-0.49	Fatty acids	3-oxoacyl-[ACP] synthase
	gene1588	-0.50	TldD cluster	FIG005080: Possible exported protein
	gene3568	-0.50	Resistance to antibiotics and toxic compounds	Probable transcription regulator protein of MDR efflux pump cluster
	gene2562	-0.50	Central carbohydrate metabolism	Malate:quinone oxidoreductase (EC 1.1.5.4)
	gene2070	-0.50	Capsular and extracellular polysacchrides	Heteropolysaccharide repeat unit export protein
	gene4903	-0.50	ATP synthases	V-type ATP synthase subunit B (EC 3.6.3.14)
	gene93	-0.50	Clustering-based subsystems	2-octaprenyl-3-methyl-6-methoxy-1,4-benzoquinol hydroxylase (EC 1.14.13.-)
	gene1144	-0.50	Electron accepting reactions	Cytochrome c oxidase (B(O/a)3-type) chain II (EC 1.9.3.1)
	gene3747	-0.50	DNA Metabolism	Putative deoxyribonuclease YjjV
	gene1165	-0.50	Nitrogen Metabolism	Cytochrome c-type protein NapC

Table D.2 continued from previous page

month	gene	$\log_2$Fold Change	level1	level4
	gene4358	-0.50	Arginine; urea cycle, polyamines	Succinylarginine dihydrolase (EC 3.5.3.23)
	gene3662	-0.50	Clustering-based subsystems	Protein of unknown function Smg
	gene1236	-0.51	Monosaccharides	D-xylose proton-symporter XylE
	gene3952	-0.51	Nitrogen Metabolism	Respiratory nitrate reductase beta chain (EC 1.7.99.4)
	gene398	-0.51	Alanine, serine, and glycine	Alanine racemase, catabolic (EC 5.1.1.1)
	gene1170	-0.51	Nitrogen Metabolism	Cytochrome c551 NirM
	gene3085	-0.51	Iron acquisition and metabolism	Outer membrane receptor proteins, mostly Fe transport
	gene2818	-0.51	Cell Wall and Capsule	N-acetylmuramoyl-L-alanine amidase (EC 3.5.1.28) AmpD
	gene3782	-0.51	Fatty acids	Putative histidine ammonia-lyase protein
	gene1652	-0.51	Flagellar motility in Prokaryota	Flagellar biosynthesis protein FlhB
	gene2280	-0.51	Protein degradation	Isoaspartyl dipeptidase (EC 3.4.19.5)
	gene3990	-0.51	Nucleosides and Nucleotides	Ribonucleotide reductase of class Ia (aerobic), alpha subunit (EC 1.17.4.1)
	gene2187	-0.51	Electron donating reactions	Hypothetical transmembrane protein coupled to NADH-ubiquinone oxidoreductase chain 5 homolog
	gene4826	-0.51	Clustering-based subsystems	Uncharacterized protein with LysM domain, COG1652
	gene4554	-0.51	Monosaccharides	Transcriptional regulator FrcR for fructose utilization, ROK family
	gene1648	-0.52	Flagellar motility in Prokaryota	Flagellar basal-body rod protein FlgC
	gene4906	-0.52	ATP synthases	V-type ATP synthase subunit K (EC 3.6.3.14)
	gene431	-0.52	Sulfur Metabolism	Alkyl hydroperoxide reductase protein F (EC 1.6.4.-)
	gene1699	-0.52	Protein secretion system, Type II	Flp pilus assembly protein TadB
	gene4711	-0.52	Protein and nucleoprotein secretion system, Type IV	Twitching motility protein PilT
	gene4230	-0.52	Protein secretion system, Type II	Similar to TadZ/CpaE, associated with Flp pilus assembly
	gene1313	-0.52	DNA repair	DinG family ATP-dependent helicase CPE1197
	gene364	-0.53	Detoxification	Adenosine (5')-pentaphospho-(5")-adenosine pyrophosphohydrolase (EC 3.6.1.-)
	gene1651	-0.53	Flagellar motility in Prokaryota	Flagellar biosynthesis protein FlhA
	gene245	-0.53	Clustering-based subsystems	50S ribosomal subunit maturation GTPase RbgA (B. subtilis YlqF)
	gene648	-0.53	RNA processing and modification	ATP-dependent RNA helicase Bcep18194_A5658
	gene3657	−0.54	Hypothetical in Lysine biosynthetic cluster	Protein of unknown function DUF484
	gene3188	−0.54	Phages, Prophages	Phage tail fiber protein
	gene1602	-0.54	Fatty acids	FIG018329: 1-acyl-sn-glycerol-3-phosphate acyltransferase
	gene1143	-0.54	Electron accepting reactions	Cytochrome c oxidase (B(O/a)3-type) chain I (EC 1.9.3.1)
	gene1586	-0.54	DNA polymerase III epsilon cluster	FIG004599: Hypothetical protein
	gene1608	-0.54	Clustering-based subsystems	FIG026291: Hypothetical periplasmic protein
	gene2478	-0.54	Protein biosynthesis	LSU ribosomal protein L23e (L14p)
	gene3650	-0.54	DNA uptake, competence	Protein involved in catabolism of external DNA
	gene4761	-0.55	Protein and nucleoprotein secretion system, Type IV	Type IV pilus biogenesis protein PilM
	gene2878	-0.55	Electron donating reactions	NAD(P)H-quinone oxidoreductase chain 1
	gene2288	-0.55	Central carbohydrate metabolism	Isocitrate dehydrogenase phosphatase (EC 2.7.11.5)/kinase (EC 3.1.3.-)
	gene3757	-0.55	Clustering-based subsystems	Putative esterase, FIGfam005057
	gene1775	-0.55	Dormancy and Sporulation	Fused spore maturation proteins A and B
	gene3111	-0.55	Central carbohydrate metabolism	Particulate methane monooxygenase C-subunit (EC 1.14.13.25)
	gene562	-0.56	Arginine; urea cycle, polyamines	Arginine/ornithine ABC transporter, periplasmic arginine/ornithine binding protein
	gene338	-0.56	Central carbohydrate metabolism	Aconitate hydratase 2 (EC 4.2.1.3)
	gene1161	-0.56	Respiration	Cytochrome c-type biogenesis protein Ccs1/ResB
	gene3416	-0.56	Potassium metabolism	Potassium uptake protein TrkH
	gene3049	-0.56	Clustering-based subsystems	Organic solvent tolerance protein precursor

Table D.2 continued from previous page

month	gene	$\log_2$Fold Change	level1	level4
	gene1429	-0.57	Putative asociate of RNA polymerase sigma-54 factor rpoN	ECF sigma factor
	gene1219	-0.57	Monosaccharides	D-Galactonate repressor DgoR
	gene1438	-0.57	Electron donating reactions	Electron transport complex protein RnfB
	gene1605	-0.57	Fatty acids	FIG021862: membrane protein, exporter
	gene3691	-0.57	Protein degradation	Pseudolysin, extracellular zinc protease (EC 3.4.24.26)
	gene4128	-0.58	Secretion	Secreted alkaline metalloproteinase (EC 3.4.24.-), PrtA/B/C/G homolog
	gene1528	-0.58	Gram-Negative cell wall components	Fe(2+)/alpha-ketoglutarate-dependent dioxygenase LpxO
	gene698	-0.58	Clustering-based subsystems	BatD
	gene2446	-0.58	Gram-Negative cell wall components	LPS-assembly lipoprotein RlpB precursor (Rare lipoprotein B)
	gene3862	-0.58	Arginine; urea cycle, polyamines	Putrescine utilization regulator
	gene870	-0.59	Resistance to antibiotics and toxic compounds	Cd(II)/Pb(II)-responsive transcriptional regulator
	gene3933	-0.59	DNA repair	Recombinational DNA repair protein RecT (prophage associated)
	gene2045	-0.59	Nitrogen Metabolism	Heme d1 biosynthesis protein NirJ
	gene1675	-0.60	Flagellar motility in Prokaryota	Flagellar motor switch protein FliN
	gene4587	-0.60	Clustering-based subsystems	Transcriptional regulator, MerR family, associated with photolyase
	gene3146	-0.60	Di- and oligosaccharides	Periplasmic alpha-amylase (EC 3.2.1.1)
	gene3	-0.60	Fatty acids	(3R)-hydroxymyristoyl-[ACP] dehydratase (EC 4.2.1.-)
	gene651	-0.60	RNA processing and modification	ATP-dependent RNA helicase PA3950
	gene1642	-0.61	Protein folding	FKBP-type peptidyl-prolyl cis-trans isomerase slpA (EC 5.2.1.8)
	gene2556	-0.61	Type III, Type IV, Type VI, ESAT secretion systems	Major pilus subunit of type IV secretion complex (VirB2)
	gene3463	-0.61	Di- and oligosaccharides	Predicted glucose transporter in maltodextrin utilization gene cluster
	gene1149	-0.61	Electron accepting reactions	Cytochrome c oxidase subunit CcoN (EC 1.9.3.1)
	gene2493	-0.61	Protein biosynthesis	LSU ribosomal protein L32e
	gene1066	-0.61	Tetrapyrroles	Coproporphyrinogen III oxidase, oxygen-independent (EC 1.3.99.22)
	gene2954	-0.61	Nitrogen Metabolism	Nitric oxide -responding transcriptional regulator Dnr (Crp/Fnr family)
	gene4200	-0.62	Periplasmic Stress	Sigma factor RpoE negative regulatory protein RseB precursor
	gene3182	-0.62	Phages, Prophages	Phage portal protein
	gene2968	-0.62	Nitrogen Metabolism	Nitrite reductase associated c-type cytochorome NirN
	gene1984	-0.62	Gram-Negative cell wall components	Glycosyl transferase in large core OS assembly cluster
	gene90	-0.63	Organic acids	2-methylcitrate dehydratase FeS dependent (EC 4.2.1.79)
	gene4765	-0.63	Protein and nucleoprotein secretion system, Type IV	Type IV pilus biogenesis protein PilQ
	gene4349	-0.63	Electron donating reactions	Succinate dehydrogenase cytochrome b558 subunit
	gene4932	-0.63	Regulation and Cell signaling	WhiB-type transcription regulator
	gene1827	-0.64	Isoprenoids	Geranyl-CoA carboxylase carboxyl transferase subunit
	gene1655	-0.64	Flagellar motility in Prokaryota	Flagellar biosynthesis protein FliL
	gene3372	-0.64	Gram-Negative cell wall components	Polymyxin resistance protein ArnA_DH, UDP-glucuronic acid decarboxylase (EC 4.1.1.-)
	gene3482	-0.64	Organic acids	Predicted Lactate-responsive regulator, IclR family
	gene4684	-0.64	RNA processing and modification	tRNA uridine 5-carboxymethylaminomethyl modification enzyme GidA
	gene1562	-0.65	RNA processing and modification	Fibrillarin
	gene4040	-0.65	NAD and NADP	Ribosyl nicotinamide transporter, PnuC-like
	gene540	-0.65	DNA replication	Archaeal DNA polymerase II large subunit (EC 2.7.7.7)
	gene1698	-0.65	Protein secretion system, Type II	Flp pilus assembly protein RcpC/CpaB
	gene1658	-0.65	Flagellar motility in Prokaryota	Flagellar biosynthesis protein FliQ
	gene3484	-0.66	Di- and oligosaccharides	Predicted maltose transporter MalT

Table D.2 continued from previous page

month	gene	$\log_2$Fold Change	level1	level4
	gene1624	-0.66	Protein degradation	FIG138315: Putative alpha helix protein
	gene4014	-0.66	RNA processing and modification	Ribosomal large subunit pseudouridine synthase E (EC 4.2.1.70)
	gene1717	-0.66	Respiration	Formate dehydrogenase N gamma subunit (EC 1.2.1.2)
	gene2542	-0.66	Regulation and Cell signaling	LysR family transcriptional regulator YneJ
	gene4299	-0.66	Protein biosynthesis	SSU ribosomal protein S26e
	gene4620	-0.66	Protein biosynthesis	Translation elongation factor P-related protein
	gene3283	-0.66	Triacylglycerols	Phospholipase A1 precursor (EC 3.1.1.32, EC 3.1.1.4)
	gene3486	-0.66	Monosaccharides	Predicted mannose transporter, GGP family
	gene3542	-0.66	DNA replication	Primosomal replication protein N
	gene3268	-0.66	Gram-Negative cell wall components	Phosphoethanolamine transferase EptA specific for the 1 phosphate group of core-lipid A
	gene4766	-0.67	Protein secretion system, Type II	Type IV prepilin peptidase TadV/CpaA
	gene1353	-0.67	DNA repair	DNA recombination-dependent growth factor C
	gene1404	-0.67	Transcription	DNA-directed RNA polymerase subunit A' (EC 2.7.7.6)
	gene4768	-0.68	Protein secretion system, Type VI	Type VI secretion lipoprotein/VasD
	gene3069	-0.68	Gram-Negative cell wall components	Outer membrane lipoprotein SmpA, a component of the essential YaeT outer-membrane protein assembly complex
	gene2671	-0.68	Lysine, threonine, methionine, and cysteine	Methionine aminotransferase, PLP-dependent
	gene1670	-0.68	Flagellar motility in Prokaryota	Flagellar M-ring protein FliF
	gene4754	-0.69	Protein and nucleoprotein secretion system, Type IV	Type IV fimbrial biogenesis protein FimT
	gene1678	-0.69	Flagellar motility in Prokaryota	Flagellar protein FlbD
	gene1674	-0.69	Flagellar motility in Prokaryota	Flagellar motor switch protein FliM
	gene3676	-0.69	Quinone cofactors	Protein YigP (COG3165) clustered with ubiquinone biosynthetic genes
	gene1653	-0.69	Flagellar motility in Prokaryota	Flagellar biosynthesis protein FlhF
	gene1639	-0.69	Electron accepting reactions	FixO3 cytochrome-c oxidase subunit
	gene1649	-0.69	Flagellar motility in Prokaryota	Flagellar basal-body rod protein FlgF
	gene4746	-0.69	Protein secretion system, Type II	Type II/IV secretion system secretin RcpA/CpaC, associated with Flp pilus assembly
	gene2447	-0.70	Gram-Negative cell wall components	LptA, protein essential for LPS transport across the periplasm
	gene4762	-0.70	Protein and nucleoprotein secretion system, Type IV	Type IV pilus biogenesis protein PilN
	gene1697	-0.71	Protein secretion system, Type II	Flp pilus assembly protein CpaD
	gene2491	-0.71	Protein biosynthesis	LSU ribosomal protein L31e
	gene2876	-0.71	Central carbohydrate metabolism	NAD(P)-dependent glyceraldehyde 3-phosphate dehydrogenase archaeal (EC 1.2.1.59)
	gene1673	-0.72	Flagellar motility in Prokaryota	Flagellar motor switch protein FliG
	gene4205	-0.72	NA	Sigma-fimbriae usher protein
	gene1611	-0.72	Clustering-based subsystems	FIG034602: Probable transmembrane protein
	gene2768	-0.72	Clustering-based subsystems	Multidrug efflux transporter [USSDB4C]
	gene4332	-0.72	Stress Response	Starvation lipoprotein Slp paralog
	gene1405	-0.74	Transcription	DNA-directed RNA polymerase subunit A" (EC 2.7.7.6)
	gene4760	-0.74	Protein and nucleoprotein secretion system, Type IV	Type IV pilus biogenesis protein PilE
	gene4825	-0.75	Clustering-based subsystems	Uncharacterized protein similar to VCA0109
	gene1323	-0.75	Toxins and superantigens	diptheria toxin resistance protein required for diphthamide biosynthesis (Saccharomyces)-like 1
	gene1799	-0.75	Arginine; urea cycle, polyamines	Gamma-glutamyl-putrescine synthetase (EC 6.3.1.11)
	gene4755	-0.75	Protein and nucleoprotein secretion system, Type IV	Type IV fimbrial biogenesis protein PilV
	gene2451	-0.75	Protein biosynthesis	LSU ribosomal protein L10Ae (L1p)

Table D.2 continued from previous page

month	gene	$\log_2$Fold Change	level1	level4
	gene3646	-0.75	Protein translocation across cytoplasmic membrane	Protein export cytoplasm chaperone protein (SecB, maintains protein to be exported in unfolded state)
	gene3549	-0.76	Respiration	Probable cytochrome c2
	gene1667	-0.76	Flagellar motility in Prokaryota	Flagellar hook-basal body complex protein FliE
	gene3678	-0.76	Protein translocation across cytoplasmic membrane	Protein-export membrane protein SecF (TC 3.A.5.1.1)
	gene4764	-0.76	Protein and nucleoprotein secretion system, Type IV	Type IV pilus biogenesis protein PilP
	gene1406	-0.77	Transcription	DNA-directed RNA polymerase subunit B (EC 2.7.7.6)
	gene4759	-0.77	Protein and nucleoprotein secretion system, Type IV	Type IV pilin PilA
	gene4763	-0.77	Protein and nucleoprotein secretion system, Type IV	Type IV pilus biogenesis protein PilO
	gene4305	-0.78	Protein biosynthesis	SSU ribosomal protein S30e
	gene3524	-0.78	Aminosugars	Predicted transcriptional regulator of N-Acetylglucosamine utilization, LacI family
	gene3761	-0.79	Peripheral pathways for catabolism of aromatic compounds	putative facilitator of salicylate uptake
	gene85	-0.79	Monosaccharides	2-ketogluconate utilization repressor PtxS
	gene1679	-0.79	Flagellar motility in Prokaryota	Flagellar protein FlgJ [peptidoglycan hydrolase] (EC 3.2.1.-)
	gene2851	-0.79	Miscellaneous	Na+/H+ antiporter NhaB
	gene3954	-0.80	Nitrogen Metabolism	Respiratory nitrate reductase gamma chain (EC 1.7.99.4)
	gene1276	-0.81	Isoprenoids	Digeranylgeranylglycerophospholipid reductase
	gene1659	-0.82	Flagellar motility in Prokaryota	Flagellar biosynthesis protein FliR
	gene2276	-0.82	Siderophores	Iron-sulfur protein in siderophore [Alcaligin] cluster
	gene3076	-0.82	Gram-Negative cell wall components	Outer membrane protein Imp, required for envelope biogenesis
	gene4463	-0.82	Cofactors, Vitamins, Prosthetic Groups, Pigments	Thiazole biosynthetic enzyme Thi4
	gene3951	-0.82	Nitrogen Metabolism	Respiratory nitrate reductase alpha chain (EC 1.7.99.4)
	gene184	-0.83	Folate and pterines	4-amino-4-deoxy-L-arabinose transferase and related glycosyltransferases of PMT family
	gene881	-0.83	Cell Division and Cell Cycle	Cell division protein FtsB
	gene4737	-0.83	Electron accepting reactions	Type cbb3 cytochrome oxidase biogenesis protein CcoG, involved in Cu oxidation
	gene1647	-0.83	Flagellar motility in Prokaryota	Flagellar basal-body rod protein FlgB
	gene3561	-0.83	Cell wall of Mycobacteria	PROBABLE MULTIFUNCTIONAL MYCOCEROSIC ACID SYNTHASE MEMBRANE-ASSOCIATED MAS
	gene4359	-0.84	Arginine; urea cycle, polyamines	Succinylglutamate desuccinylase (EC 3.5.1.96)
	gene1502	-0.84	DNA repair	Exodeoxyribonuclease I (EC 3.1.11.1)
	gene3221	-0.84	Aromatic amino acids and derivatives	Phenylalanine hydroxylase transcriptional activator PhhR
	gene4102	-0.85	Oxidative stress	Rubredoxin-NAD(+) reductase (EC 1.18.1.1)
	gene3921	-0.85	Electron donating reactions	Quinone-reactive Ni/Fe-hydrogenase small chain precursor (EC 1.12.5.1)
	gene2753	-0.85	Protein and nucleoprotein secretion system, Type IV	MSHA biogenesis protein MshL
	gene2445	-0.85	Clustering-based subsystems	LppC putative lipoprotein
	gene674	-0.85	Quorum sensing and biofilm formation	Autoinducer 2 (AI-2) ABC transport system, periplasmic AI-2 binding protein LsrB
	gene18	-0.85	Electron donating reactions	[Ni/Fe] hydrogenase, group 1, large subunit
	gene527	-0.86	Programmed Cell Death and Toxin-antitoxin Systems	Antiholin-like protein LrgA
	gene1893	-0.86	Glutamine, glutamate, aspartate, asparagine; ammonia assimilation	Glutamine synthetase (EC 6.3.1.2)
	gene886	-0.86	Cell Division and Cell Cycle	Cell division protein FtsL

Table D.2 continued from previous page

month	gene	$\log_2$Fold Change	level1	level4
	gene1665	-0.86	Flagellar motility in Prokaryota	Flagellar hook-associated protein FlgL
	gene2972	-0.87	Oxidative stress	Nitrite-sensitive transcriptional repressor NsrR
	gene3184	-0.87	Stress Response	Phage shock protein B
	gene1689	-0.87	Flagellar motility in Prokaryota	Flagellin protein FlaB
	gene1664	-0.87	Flagellar motility in Prokaryota	Flagellar hook-associated protein FlgK
	gene4109	-0.87	Arginine; urea cycle, polyamines	S-adenosylmethionine decarboxylase proenzyme (EC 4.1.1.50), prokaryotic class 1A
	gene2355	-0.88	Lysine, threonine, methionine, and cysteine	L-pipecolate dehydrogenase (EC 1.5.99.3)
	gene1331	-0.88	DNA repair	DNA helicase IV
	gene3623	-0.88	Protein degradation	Proteasome subunit alpha (EC 3.4.25.1), archaeal
	gene1151	-0.88	Electron accepting reactions	Cytochrome c oxidase subunit CcoP (EC 1.9.3.1)
	gene1748	-0.88	Monosaccharides	Fructose repressor FruR, LacI family
	gene2983	-0.89	Nitrogen Metabolism	Nitrous oxide reductase maturation protein NosR
	gene1176	-0.89	Respiration	Cytochrome C553 (soluble cytochrome f)
	gene1612	-0.89	Clustering-based subsystems	FIG035830: Two-component system regulatory protein
	gene3953	-0.89	Nitrogen Metabolism	Respiratory nitrate reductase delta chain (EC 1.7.99.4)
	gene2756	-0.89	Protein and nucleoprotein secretion system, Type IV	MSHA pilin protein MshA
	gene1441	-0.90	Electron donating reactions	Electron transport complex protein RnfG
	gene4758	-0.90	Protein and nucleoprotein secretion system, Type IV	Type IV fimbrial biogenesis protein PilY1
	gene2628	-0.91	Clustering-based subsystems	Membrane lipoprotein lipid attachment site containing protein USSDB6D
	gene380	-0.91	Detoxification	ADP compounds hydrolase NudE (EC 3.6.1.-)
	gene2893	-0.91	Fermentation	NADH-dependent butanol dehydrogenase A (EC 1.1.1.-)
	gene1701	-0.91	Protein secretion system, Type II	Flp pilus assembly protein, pilin Flp
	gene4203	-0.92	NA	Sigma-fimbriae chaperone protein
	gene2792	-0.92	Aminosugars	N-acetyl glucosamine transporter, NagP
	gene2752	-0.92	Protein and nucleoprotein secretion system, Type IV	MSHA biogenesis protein MshI
	gene1372	-0.92	Regulation and Cell signaling	DNA transformation protein TfoX
	gene4508	-0.92	Membrane Transport	TonB-dependent hemin , ferrichrome receptor
	gene1440	-0.92	Electron donating reactions	Electron transport complex protein RnfD
	gene4459	-0.93	Plant-Prokaryote DOE project	Thiamine biosynthesis protein thiI
	gene1645	-0.94	Flagellar motility in Prokaryota	Flagellar basal-body P-ring formation protein FlgA
	gene3910	-0.96	Biosynthesis of phenylpropanoids	Quercetin 3-O-methyltransferase 1 (EC 2.1.1.76)
	gene3828	-0.96	CO2 fixation	putative sodium-dependent bicarbonate transporter
	gene928	-0.97	Tetrapyrroles	Chlorophyll a synthase ChlG (EC 2.5.1.62)
	gene1439	-0.97	Electron donating reactions	Electron transport complex protein RnfC
	gene636	-0.99	DNA repair	ATP-dependent helicase DinG/Rad3
	gene1690	-0.99	Flagellar motility in Prokaryota	Flagellin protein FlaG
	gene2436	-0.99	Fatty acids	Long-chain fatty acid transport protein
	gene4756	-0.99	Protein and nucleoprotein secretion system, Type IV	Type IV fimbrial biogenesis protein PilW
	gene3632	-0.99	Protein degradation	Proteasome subunit beta (EC 3.4.25.1), archaeal
	gene1403	-0.99	Transcription	DNA-directed RNA polymerase subunit A (EC 2.7.7.6)
	gene293	-0.99	Putative GGDEF domain protein related to agglutinin secretion	ABC-type protease exporter, membrane fusion protein (MFP) family component PrtE/AprE
	gene3596	-1.00	Regulation and Cell signaling	Prophage Clp protease-like protein
	gene4843	-1.00	Stress Response	Universal stress protein family COG0589

Table D.3: Variation in the expression of soil microbial genes between years (2017 vs. 2016), based on RefSeq bacterial functional annotation (DESeq2, adjusted $P < 0.05$).

year	gene	$\log_2$Fold Change	function.
2017	fun54566	5.91	pesticidal protein
	fun56087	4.27	phycobilisome Linker polypeptide/CpcD/allophycocyanin linker domain-containing protein
	fun36858	4.27	MULTISPECIES: DUF3181 domain-containing protein
	fun68754	4.19	Tic22-like family
	fun58704	4.00	PsaD
	fun58641	3.97	protoheme ferro-lyase (ferrochelatase)
	fun63368	3.68	Ribulose bisphosphate carboxylase large chain 2
	fun55980	3.66	photosystem I reaction centre subunit III
	fun5147	3.66	aromatic alcohol reductase
	fun55975	3.64	photosystem I reaction center subunit X-like protein
	fun66484	3.62	Spy protein
	fun56014	3.60	photosystem II protein D1 1
	fun56505	3.59	plasmid segregation actin-type ATPase ParM
	fun56077	3.58	phycobiliprotein lyase
	fun15946	3.56	DUF4101 domain-containing protein
	fun15394	3.51	DUF3155 domain-containing protein
	fun56037	3.49	photosystem II reaction center protein Ycf12
	fun10073	3.46	cofactor assembly of complex C subunit B
	fun28782	3.46	Manganese-stabilising protein / photosystem II polypeptide
	fun16156	3.45	DUF4278 domain-containing protein
	fun15405	3.35	DUF3172 domain-containing protein
	fun10825	3.34	cyanoexosortase A
	fun8911	3.34	cellulose synthase subunit
	fun56039	3.34	photosystem II reaction center X protein
	fun69186	3.33	TonB-dependent outer membrane Receptor/Oar-like protein
	fun43747	3.32	MULTISPECIES: PHA accumulation regulator DNA-binding-like
	fun56081	3.26	phycobilisome degradation protein nblA
	fun16198	3.26	DUF4327 domain-containing protein
	fun56046	3.24	photosystem one PsaX
	fun30048	3.19	microcyclamide/patellamide family RiPP
	fun14504	3.19	DUF1825 domain-containing protein
	fun36139	3.18	MULTISPECIES: DUF1257 domain-containing protein
	fun56011	3.17	photosystem II phosphoprotein
	fun58692	3.15	PrpF, AcnD-accessory
	fun15223	3.15	DUF2949 domain-containing protein
	fun68448	3.14	thiazoline oxidase
	fun44242	3.13	MULTISPECIES: photosystem II reaction center protein PsbN
	fun57454	3.12	primase P4
	fun15004	3.11	DUF2605 domain-containing protein
	fun14495	3.10	DUF1816 domain-containing protein
	fun56018	3.07	photosystem II protein Psb27
	fun14977	3.06	DUF2555 domain-containing protein
	fun37535	3.05	MULTISPECIES: DUF760 domain-containing protein
	fun36847	3.04	MULTISPECIES: DUF3155 domain-containing protein
	fun30221	3.04	Mo-dependent nitrogenase

Table D.3 continued from previous page

year	gene	$\log_2$Fold Change	function.
	fun55969	3.02	photosystem I reaction center subunit PsaK
	fun14507	2.98	DUF1830 domain-containing protein
	fun14181	2.97	DUF129 domain-containing protein
	fun32504	2.97	MULTISPECIES: allophycocyanin subunit beta
	fun54627	2.94	PHA accumulation regulator DNA-binding-like protein
	fun36759	2.94	MULTISPECIES: DUF2997 domain-containing protein
	fun56027	2.93	photosystem II reaction center protein K
	fun44244	2.92	MULTISPECIES: photosystem II reaction center protein T
	fun54602	2.92	Pfpi family intracellular protease
	fun32942	2.91	MULTISPECIES: apocytochrome f
	fun56089	2.90	phycobilisome protein
	fun8060	2.90	carbon dioxide-concentrating mechanism protein CcmK
	fun50777	2.89	NblA-related protein
	fun56104	2.87	phycoerythrin alpha chain
	fun44266	2.87	MULTISPECIES: phycocyanin subunit beta
	fun14501	2.87	DUF1822 domain-containing protein
	fun56103	2.85	phycocyanobilin:ferredoxin oxidoreductase
	fun62162	2.83	RbcX protein
	fun41543	2.82	MULTISPECIES: menaquinol-cytochrome c reductase iron-sulfur protein
	fun14610	2.81	DUF1995 domain-containing protein
	fun4066	2.81	aluminum resistance family protein
	fun14350	2.80	DUF1565 domain-containing protein, partial
	fun56082	2.80	phycobilisome degradation protein NblA
	fun63671	2.79	RNA polymerase sigma subunit RpsC/SigC
	fun68753	2.79	Tic22 family protein
	fun23171	2.78	high light inducible protein
	fun9786	2.78	CO2 hydration protein
	fun14245	2.78	DUF1400 domain-containing protein
	fun55968	2.76	photosystem I reaction center subunit IX, partial
	fun16622	2.76	DUF561 domain-containing protein
	fun13896	2.73	DRTGG domain protein
	fun3746	2.70	allophycocyanin
	fun16209	2.69	DUF4335 domain-containing protein
	fun11169	2.68	cytochrome b559 subunit alpha, partial
	fun63367	2.68	Ribulose bisphosphate carboxylase large chain 1
	fun14141	2.68	DUF1230 domain-containing protein
	fun55967	2.68	Photosystem I reaction center subunit IX
	fun15534	2.68	DUF3370 domain-containing protein
	fun14225	2.66	DUF1350 domain-containing protein
	fun15593	2.65	DUF3464 domain-containing protein
	fun68858	2.64	TIGR02652 family protein
	fun44206	2.64	MULTISPECIES: photosystem I protein PsaD
	fun16679	2.63	DUF760 domain-containing protein
	fun26456	2.62	isomerase YbhH
	fun51379	2.61	nitrogenase
	fun36450	2.58	MULTISPECIES: DUF2184 domain-containing protein
	fun15120	2.58	DUF2808 domain-containing protein
	fun11185	2.57	cytochrome b6-f complex subunit 5
	fun62918	2.56	Rho termination factor domain protein
	fun15141	2.56	DUF2839 domain-containing protein

Table D.3 continued from previous page

year	gene	$\log_2$Fold Change	function.
	fun56031	2.56	photosystem II reaction center protein PsbH
	fun56019	2.54	photosystem II protein PsbQ
	fun15691	2.53	DUF3611 domain-containing protein
	fun55944	2.52	photosystem I iron-sulfur center protein PsaC
	fun15742	2.52	DUF3747 domain-containing protein
	fun15412	2.52	DUF3181 domain-containing protein
	fun14613	2.52	DUF1997 domain-containing protein
	fun57682	2.50	ProQ activator of osmoprotectant transporter prop
	fun16235	2.50	DUF4359 domain-containing protein
	fun62921	2.49	Rho termination protein
	fun15753	2.49	DUF3769 domain-containing protein
	fun16204	2.49	DUF4330 domain-containing protein
	fun55932	2.48	photosystem I assembly protein Ycf4
	fun11565	2.47	cytochrome c6
	fun62820	2.46	resuscitation-promoting factor RpfE
	fun55956	2.46	photosystem I reaction center protein subunit XI
	fun19456	2.45	flavohemoglobin
	fun56028	2.45	photosystem II reaction center protein L
	fun73411	2.45	WecB/TagA/CpsF family exopolysaccharide biosynthesis protein
	fun6078	2.44	autotransporter subunit beta
	fun14494	2.44	DUF1815 domain-containing protein
	fun13255	2.44	DNA nickase
	fun10580	2.44	CpcD phycobilisome linker domain protein
	fun15257	2.44	DUF2996 domain-containing protein
	fun10843	2.43	cyanoporin
	fun44258	2.43	MULTISPECIES: phycobilisome degradation protein NblA
	fun15681	2.43	DUF3593 domain-containing protein
	fun52364	2.40	Orange carotenoid-binding protein
	fun29822	2.39	methylitaconate delta2-delta3-isomerase
	fun56008	2.39	photosystem II manganese-stabilizing protein PsbO
	fun3736	2.39	Allergen V5/Tpx-1 related protein
	fun17896	2.38	exosortase O
	fun15262	2.38	DUF3007 domain-containing protein
	fun1219	2.37	30S ribosomal protein Ycf65
	fun43728	2.36	MULTISPECIES: pesticin
	fun16676	2.35	DUF751 domain-containing protein
	fun28838	2.34	mannose-6-phosphate isomerase type 2
	fun52363	2.33	Orange carotenoid protein
	fun56084	2.33	phycobilisome linker polypeptide
	fun40309	2.32	MULTISPECIES: iron-sulfur cluster repair di-iron protein
	fun15448	2.32	DUF3252 domain-containing protein
	fun56025	2.32	photosystem II reaction center protein I
	fun15245	2.30	DUF2973 domain-containing protein
	fun63836	2.30	RNase HII
	fun1455	2.30	4Fe-4S ferredoxin, iron-sulfur-binding protein
	fun18718	2.29	ferrous iron transporter C
	fun6901	2.28	bifunctional DNA primase/polymerase famiily protein
	fun30941	2.28	multi-copper polyphenol oxidoreductase laccase
	fun37980	2.27	MULTISPECIES: exported hypothetical protein
	fun46189	2.26	MULTISPECIES: rubsico expression protein CbbX

Table D.3 continued from previous page

year	gene	$\log_2$Fold Change	function.
	fun3748	2.26	allophycocyanin alpha-B subunit apoprotein
	fun44236	2.26	MULTISPECIES: photosystem II reaction center protein I
	fun15293	2.25	DUF3038 domain-containing protein
	fun62483	2.25	relaxase/mobilization nuclease
	fun70428	2.25	TrkA-N domain protein
	fun63896	2.24	Rne/Rng family ribonuclease
	fun57729	2.23	protease PfpI family
	fun9282	2.23	chlorophyll synthase ChlG
	fun38208	2.23	MULTISPECIES: ferredoxin:protochlorophyllide reductase (ATP-dependent) subunit N
	fun56036	2.23	photosystem II reaction center protein T
	fun15929	2.22	DUF4079 domain-containing protein
	fun26617	2.22	KaiA family protein
	fun24494	2.22	integral membrane protein, partial
	fun26639	2.21	KAP family P-loop domain-containing protein
	fun56006	2.21	Photosystem II manganese-stabilizing polypeptide
	fun56091	2.20	phycobilisome rod-core linker polypeptide CpcG
	fun55964	2.20	photosystem I reaction center subunit IV
	fun38880	2.17	MULTISPECIES: GfdT protein
	fun12552	2.17	diflavin flavoprotein A
	fun66642	2.17	stearoyl-CoA 9-desaturase, partial
	fun56079	2.17	phycobilisome core-membrane linker protein
	fun55979	2.16	photosystem I reaction center subunit XII
	fun55966	2.16	photosystem I reaction center subunit IX
	fun55103	2.16	pheophorbide a oxygenase
	fun49823	2.15	N-ethylmaleimide reductase NemA
	fun34141	2.13	MULTISPECIES: cation-transporting ATPase
	fun63757	2.13	RNA polymerase, sigma subunit, ECF family
	fun55946	2.12	photosystem I P700 chlorophyll a apoprotein A2
	fun56032	2.11	photosystem II reaction center protein PsbM
	fun44203	2.10	MULTISPECIES: photosystem I iron-sulfur center protein PsaC
	fun56030	2.10	photosystem II reaction center protein Psb28
	fun51412	2.09	nitrous oxidase accessory protein NosD
	fun55945	2.09	photosystem I P700 chlorophyll a apoprotein A1
	fun38206	2.08	MULTISPECIES: ferredoxin:protochlorophyllide reductase (ATP-dependent) iron-sulfur ATP-binding protein
	fun22061	2.08	glycyl radical enzyme
	fun3751	2.08	allophycocyanin subunit alpha apoprotein
	fun15298	2.08	DUF3040 domain-containing protein, partial
	fun56098	2.07	phycocyanin subunit alpha
	fun14724	2.06	DUF218 domain-containing protein
	fun50272	2.05	NAD(P)H-quinone oxidoreductase subunit M
	fun37290	2.04	MULTISPECIES: DUF4327 domain-containing protein
	fun35135	2.02	MULTISPECIES: cytochrome b6-f complex subunit IV
	fun56013	2.02	photosystem II protein D1
	fun55940	2.01	photosystem I core protein PsaA
	fun3752	2.00	allophycocyanin subunit beta
	fun44201	2.00	MULTISPECIES: photosystem I core protein PsaA
	fun53324	1.99	PBS lyase HEAT domain protein repeat-containing protein
	fun55999	1.98	photosystem II D2 protein (photosystem q(a) protein)
	fun11445	1.97	cytochrome c-550
	fun55942	1.96	photosystem I core protein PsaB

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year	gene	$\log_2$Fold Change	function.
	fun46728	1.96	MULTISPECIES: sodium-dependent bicarbonate transport family permease
	fun72664	1.95	universal stress family protein
	fun14320	1.95	DUF1517 domain-containing protein
	fun55973	1.94	photosystem I reaction center subunit VIII
	fun26175	1.94	IS701 family transposase ISMtsp19
	fun44165	1.93	MULTISPECIES: phosphorylase
	fun21078	1.92	glutamine amidotransferase class-I
	fun55992	1.92	photosystem II core protein PsbZ
	fun51478	1.91	NmrA-like protein
	fun46215	1.91	MULTISPECIES: S-layer protein
	fun55960	1.90	Photosystem I reaction center subunit II
	fun56099	1.88	phycocyanin subunit beta
	fun25599	1.88	IS3 family transposase ISNmu3
	fun7322	1.88	bleomycin hydrolase
	fun8268	1.87	carotene isomerase
	fun51376	1.86	nitrogen regulatory protein P-II family
	fun11442	1.86	cytochrome C subunit protein
	fun27494	1.85	lignostilbene-alpha,beta-dioxygenase-like protein
	fun23807	1.84	hydrogenase 4, membrane subunit
	fun38844	1.84	MULTISPECIES: general secretion pathway protein GspD
	fun20528	1.83	geranylgeranyl pyrophosphate synthase, partial
	fun21219	1.82	Glutathione S-transferase zeta class
	fun62821	1.82	Resuscitation-promoting factor RpfE
	fun24835	1.82	iron uptake system component EfeO
	fun18624	1.82	ferredoxin:protochlorophyllide reductase (ATP-dependent) iron-sulfur ATP-binding protein
	fun44219	1.82	MULTISPECIES: photosystem II 44 kDa subunit reaction center protein
	fun42424	1.81	MULTISPECIES: NAD(P)H-quinone oxidoreductase subunit 3
	fun52859	1.79	oxidoreductase LLM family
	fun11188	1.78	cytochrome b6-f complex subunit IV
	fun67076	1.78	sucrose synthase
	fun11338	1.77	cytochrome c oxidase accessory protein CcoG, partial
	fun23628	1.75	HSR1-like GTP-binding protein
	fun31893	1.75	MULTISPECIES: 7Fe ferredoxin
	fun1765	1.74	6-pyruvoyl tetrahydropterin synthase and hypothetical protein
	fun27247	1.74	large Ala/Glu-rich protein
	fun29544	1.73	methane monooxygenase/ammonia monooxygenase subunit C, partial
	fun12953	1.73	dissimilatory-type sulfite reductase subunit alpha
	fun35125	1.72	MULTISPECIES: cytochrome b559 subunit beta
	fun3132	1.72	adenylate/guanylate cyclase with GAF and PAS/PAC sensors
	fun3424	1.71	alcohol dehydrogenase AdhP, partial
	fun62819	1.71	resuscitation-promoting factor rpfE
	fun15498	1.70	DUF3318 domain-containing protein
	fun50260	1.69	NAD(P)H-quinone oxidoreductase subunit 4
	fun61782	1.68	pyrroloquinoline-quinone synthase PqqC
	fun3998	1.68	alpha/beta family hydrolase
	fun59512	1.68	putative carboxytransferase
	fun63380	1.68	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit
	fun6277	1.66	bacterioferritin-associated ferredoxin-like protein
	fun53100	1.66	PAC2 family protein, partial
	fun13079	1.65	DNA binding domain protein, excisionase family

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year	gene	$\log_2$Fold Change	function.
	fun56026	1.65	photosystem II reaction center protein J
	fun54835	1.64	phage tail length tape measure protein, partial
	fun12990	1.64	ditF protein
	fun52396	1.64	organic solvent ABC transporter substrate-bindng protein
	fun16706	1.63	DUF820 domain-containing protein
	fun21615	1.63	glycoside hydrolase family 24
	fun14153	1.63	DUF1257 domain-containing protein
	fun8600	1.61	cell division protein DivIVA, partial
	fun7919	1.61	capsule synthesis protein, CapA
	fun55990	1.60	photosystem II chlorophyll-binding protein CP47
	fun56637	1.60	poly-beta-hydroxybutyrate polymerase, partial
	fun1542	1.60	5-oxo-L-prolinase
	fun56022	1.59	photosystem II q(b) protein
	fun50267	1.59	NAD(P)H-quinone oxidoreductase subunit I
	fun10989	1.59	cystathionine beta-synthase, partial
	fun33332	1.59	MULTISPECIES: atrA protein
	fun19389	1.59	flavin reductase FMN-binding protein
	fun8228	1.58	carboxysome shell carbonic anhydrase
	fun11184	1.57	cytochrome b6-f complex subunit 4
	fun18474	1.57	fe2+ zn2+ uptake regulation protein
	fun23229	1.56	HipA-like protein
	fun11206	1.56	cytochrome BD quinol oxidase subunit I
	fun26153	1.55	IS66 family transposase ISStau5
	fun55985	1.55	photosystem II 44 kDa subunit reaction center protein
	fun11449	1.55	cytochrome C-551
	fun30400	1.53	molybdenum ABC transporter periplasmic molybdate-binding protein
	fun38116	1.53	MULTISPECIES: FdrA family protein
	fun67601	1.51	Suppressor of fused domain protein
	fun28502	1.50	magnesium-protoporphyrin IX monomethyl ester (oxidative) cyclase
	fun66872	1.49	subtilisin family serine protease
	fun16713	1.49	DUF849 domain-containing protein
	fun64142	1.49	S-layer domain-containing protein
	fun63633	1.48	RNA polymerase sigma factor SigC
	fun42380	1.48	MULTISPECIES: NAD(+) diphosphatase
	fun41537	1.48	MULTISPECIES: menaquinol-cytochrome c reductase
	fun18041	1.47	extradiol ring-cleavage dioxygenase, partial
	fun12821	1.47	dinitrogenase reductase
	fun6004	1.47	atrA protein
	fun29542	1.46	methane monooxygenase/ammonia monooxygenase subunit B, partial
	fun29232	1.45	membrane-bound ClpP-class protease
	fun26218	1.44	ISAs1 family transposase ISAzs12
	fun11168	1.44	cytochrome b559 subunit alpha
	fun19607	1.43	FMN-dependent oxidoreductase nitrilotriacetate monooxygenase family
	fun28289	1.42	LytR family transcriptional regulator, partial
	fun22168	1.42	GPR1/FUN34/yaaH family protein
	fun55937	1.42	photosystem I chlorophyll a apoprotein A2
	fun40105	1.42	MULTISPECIES: InaA protein
	fun982	1.41	3-methyl-2-oxobutanoate dehydrogenase (2-methylpropanoyl-transferring)
	fun10053	1.41	coenzyme PQQ biosynthesis protein A
	fun58294	1.40	protein p60 precursor

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year	gene	$\log_2$Fold Change	function.
	fun49849	1.40	N-methylhydantoinase A, partial
	fun4957	1.40	aquaporin Z, partial
	fun18625	1.40	ferredoxin:protochlorophyllide reductase (ATP-dependent) subunit B
	fun40194	1.38	MULTISPECIES: intein-containing DNA gyrase subunit A
	fun13353	1.38	DNA polymerase IV, partial
	fun34969	1.38	MULTISPECIES: cupredoxin domain-containing protein
	fun73944	1.37	zf-TFIIB domain containing protein
	fun50263	1.37	NAD(P)H-quinone oxidoreductase subunit F
	fun37178	1.37	MULTISPECIES: DUF4168 domain-containing protein
	fun24268	1.36	InaA protein
	fun14980	1.36	DUF2561 domain-containing protein
	fun34558	1.36	MULTISPECIES: class E sortase
	fun33181	1.35	MULTISPECIES: ATP synthase F0F1 subunit A
	fun69889	1.34	transglycosylase-like protein
	fun14781	1.34	DUF2249 domain-containing protein
	fun55309	1.34	phosphatidylinositol 3-and 4-kinase
	fun15980	1.33	DUF4135 domain-containing protein
	fun64442	1.33	Secreted acid phosphatase
	fun49846	1.33	N-methylhydantoinase (ATP-hydrolyzing) A 2
	fun70233	1.32	trehalose biosynthesis protein
	fun24921	1.32	iron-sulfur cluster repair di-iron protein
	fun4916	1.32	apocytochrome f
	fun63373	1.31	ribulose bisphosphate carboxylase small subunit, partial
	fun8755	1.30	cell wall assembly protein
	fun56023	1.29	photosystem II q(b) protein, partial
	fun64360	1.29	SCP-like protein
	fun57341	1.27	Predicted transcriptional regulators
	fun67670	1.27	Survival protein SurE
	fun25825	1.27	IS5 family transposase ISAzo24
	fun51410	1.26	nitrosocyanin
	fun67412	1.26	sulfite reductase, ferredoxin dependent
	fun29528	1.26	metallothionein
	fun59542	1.25	putative cellulose-binding protein
	fun51960	1.25	nucleotidyltransferase/DNA polymerase
	fun71429	1.24	type II glyceraldehyde-3-phosphate dehydrogenase
	fun28466	1.24	magnesium chelatase ATPase subunit D
	fun19381	1.24	flavin reductase domain-containing FMN-binding protein
	fun63324	1.24	ribosome modulation factor
	fun63668	1.24	RNA polymerase sigma subunit ECF family
	fun41644	1.22	MULTISPECIES: methicillin resistance protein
	fun25898	1.22	IS5 family transposase ISMch2
	fun19649	1.21	folate/biopterin family MFS transporter
	fun44903	1.21	MULTISPECIES: protein serine/threonine phosphatase with GAF(s) sensor(s)
	fun44476	1.21	MULTISPECIES: polyhydroxyalkanoic acid synthase
	fun57956	1.21	protein FdrA
	fun26729	1.21	ketosteroid isomerase-like enzyme
	fun47540	1.20	MULTISPECIES: thiamine pyrophosphate-requiring protein
	fun30523	1.20	molybdopterin converting factor, large subunit
	fun68578	1.20	thiolase family protein, partial
	fun20159	1.20	galactarate dehydratase, partial

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year	gene	$\log_2$Fold Change	function.
	fun11181	1.20	cytochrome b6-f complex iron-sulfur subunit
	fun49600	1.20	mycothiol-dependent maleylpyruvate isomerase
	fun8514	1.19	CDP-alcohol phosphatidyltransferase family protein
	fun68412	1.19	thiamine pyrophosphate-requiring protein
	fun18070	1.19	F0F1 ATP synthase subunit B, partial
	fun20114	1.19	G5 domain-containing protein
	fun18626	1.18	ferredoxin:protochlorophyllide reductase (ATP-dependent) subunit N
	fun29134	1.18	membrane protease FtsH catalytic subunit
	fun56486	1.18	plasmid replication initiator protein
	fun30661	1.18	monooxygenase component MmoB/DmpM
	fun37966	1.18	MULTISPECIES: exosortase system-associated protein, TIGR04073 family
	fun39950	1.18	MULTISPECIES: hydrogenase assembly protein HypC
	fun50305	1.18	NADH (or F420H2) dehydrogenase, subunit C
	fun28583	1.17	malate synthase G, partial
	fun53175	1.17	paraslipin, partial
	fun43457	1.17	MULTISPECIES: PEP-utilizing protein mobile subunit
	fun11374	1.17	cytochrome C oxidase subunit 1, partial
	fun70698	1.17	Trp biosynthesis associated, protein, Oprn/Chp
	fun72688	1.17	universal stress protein, partial
	fun42987	1.16	MULTISPECIES: nucleotide sugar-1-phosphate transferase
	fun16164	1.16	DUF4287 domain-containing protein
	fun37662	1.16	MULTISPECIES: electron transfer flavoprotein domain protein
	fun49566	1.16	mycofactocin precursor
	fun63387	1.16	ribulose-bisphosphate carboxylase large subunit
	fun9880	1.16	cobalamin biosynthesis protein CobB
	fun51026	1.15	nicotinamide mononucleotide adenylyltransferase
	fun52834	1.15	Oxidoreductase FAD-binding domain-containing protein
	fun21335	1.15	Glycerol-3-phosphate ABC transporter, periplasmic glycerol-3-phosphate-binding protein
	fun1346	1.15	4-hydroxybutyrate coenzyme A transferase
	fun73255	1.15	Vitamin K epoxide reductase
	fun67519	1.13	sulfurtransferase TusE
	fun61909	1.13	pyruvate-flavodoxin oxidoreductase
	fun67496	1.13	sulfur relay protein DsrC
	fun7346	1.13	blue-copper protein
	fun9750	1.12	Cna B domain protein
	fun36489	1.12	MULTISPECIES: DUF2267 domain-containing protein
	fun50415	1.12	NADH-dependent flavin oxidoreductase
	fun49568	1.11	mycofactocin radical SAM maturase, partial
	fun32201	1.10	MULTISPECIES: acyl-CoA reductase
	fun61885	1.10	pyruvate oxidase, partial
	fun14219	1.10	DUF1344 domain-containing protein
	fun50959	1.10	Ni/Fe hydrogenase
	fun36406	1.10	MULTISPECIES: DUF2071 domain-containing protein
	fun69880	1.10	transglycosylase domain-containing protein
	fun70502	1.09	tRNA 2-methylthio-N6-isopentenyl adenosine(37) hydroxylase MiaE-like protein
	fun42627	1.09	MULTISPECIES: NDMA-dependent methanol dehydrogenase
	fun23591	1.09	HscA chaperone
	fun47266	1.08	MULTISPECIES: sulfur carrier protein ThiS
	fun45948	1.08	MULTISPECIES: ribulose-bisphosphate carboxylase large subunit
	fun5896	1.08	ATP-independent chaperone

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year	gene	$\log_2$Fold Change	function.
	fun68786	1.07	TIGR00303 family protein
	fun2474	1.07	acetyl-CoA carboxylase, partial
	fun3958	1.06	alpha-mannosidase, partial
	fun70054	1.06	transport-associated protein
	fun26254	1.06	ISAzo13 family transposase ISLsp4
	fun64222	1.06	SagB-type dehydrogenase
	fun49562	1.05	mycofactocin biosynthesis chaperone MftB
	fun16764	1.05	DUF928 domain-containing protein
	fun62809	1.05	resuscitation-promoting factor RpfA
	fun48342	1.05	MULTISPECIES: trypsin
	fun50217	1.05	NAD(P)H nitroreductase
	fun36176	1.05	MULTISPECIES: DUF1344 domain-containing protein
	fun273	1.05	2-aminophenol 1,6-dioxygenase subunit beta
	fun20682	1.05	global nitrogen regulator NtcA
	fun12165	1.04	decaheme c-type cytochrome, OmcA/MtrC family
	fun22015	1.04	glycosyltransferase family protein
	fun72903	1.04	UTRA domain-containing protein
	fun72099	1.03	type VII secretion protein EsxI
	fun51166	1.03	nitrate reductase subunit alpha
	fun10681	1.03	crotonyl-CoA reductase
	fun38873	1.03	MULTISPECIES: geranylgeranyl reductase
	fun56635	1.03	poly-beta-hydroxybutyrate polymerase subunit
	fun56153	1.03	phytochrome-like protein cph2
	fun63372	1.03	ribulose bisphosphate carboxylase small subunit
	fun26408	1.03	isoaspartyl peptidase
	fun50649	1.03	NADP-dependent alcohol dehydrogenase
	fun21658	1.02	glycoside hydrolase family 65
	fun22936	1.02	hemerythrin HHE cation binding domain-containing protein
	fun37285	1.02	MULTISPECIES: DUF4307 domain-containing protein
	fun51168	1.02	nitrate reductase subunit beta
	fun24636	1.02	intersectin-EH binding protein Ibp1
	fun477	1.02	2-oxoacid:acceptor oxidoreductase subunit gamma
	fun49699	1.02	N-acetylglucosamine specific PTS subunit
	fun9954	1.02	cobalt transporter CbiM
	fun72996	1.02	valine dehydrogenase
	fun37754	1.02	MULTISPECIES: endopeptidase
	fun40039	1.01	MULTISPECIES: hydroxyneurosporene methyltransferase
	fun34940	1.01	MULTISPECIES: crotonyl-CoA reductase
	fun987	1.01	3-methyl-2-oxobutanoate dehydrogenase subunit VorB
	fun37115	1.01	MULTISPECIES: DUF4031 domain-containing protein
	fun49585	1.01	mycothiol acetyltransferase
	fun41959	1.00	MULTISPECIES: molybdopterin synthase
	fun51245	1.00	nitric-oxide reductase large subunit
	fun2796	1.00	acyl-CoA carboxylase subunit epsilon
	fun24038	1.00	Hydroxymethylpyrimidine ABC transporter substrate-binding component
	fun43135	1.00	MULTISPECIES: Orn/DAP/Arg decarboxylase 2
	fun42169	1.00	MULTISPECIES: mycofactocin biosynthesis chaperone MftB
	fun11258	1.00	cytochrome C biogenesis protein CcmC
	fun49870	0.99	N-succinyl-L,L-diaminopimelate aminotransferase
	fun35751	0.99	MULTISPECIES: DksA/TraR family C4-type zinc finger protein

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year	gene	$\log_2$Fold Change	function.
	fun43969	0.98	MULTISPECIES: phosphatidylinositol mannoside acyltransferase
	fun64862	0.98	sensory rhodopsin transducer
	fun70741	0.98	trypsin domain-containing protein
	fun60144	0.98	putative Iron-sulphur cluster assembly protein,contains Rieske (2Fe-2S) domain (fragment, part 2)
	fun51207	0.98	nitrate/sulfite reductase
	fun2341	0.98	acetoin utilization protein AcuB
	fun63412	0.98	Rieske (2Fe-2S) iron-sulfur domain protein protein
	fun11699	0.97	Cytosine-specific methyltransferase (fragment)
	fun39037	0.97	MULTISPECIES: glutamate mutase
	fun26269	0.97	ISAzo13 family transposase, partial
	fun10051	0.97	coenzyme F420:NADP oxidoreductase
	fun57622	0.97	Propeptide PepSY amd peptidase M4
	fun159	0.97	15-cis-phytoene desaturase
	fun26263	0.97	ISAzo13 family transposase ISStau11
	fun44176	0.97	MULTISPECIES: phosphosugar isomerase
	fun18687	0.97	Ferritin Dps family protein (fragment)
	fun50430	0.97	NADH-quinone oxidoreductase chain B
	fun989	0.96	3-methyl-2-oxobutanoate hydroxymethyltransferase, partial
	fun43556	0.96	MULTISPECIES: peptidase M75 family protein
	fun65198	0.96	SH3 domain-containing-like protein 1
	fun36770	0.95	MULTISPECIES: DUF3017 domain-containing protein
	fun49567	0.95	mycofactocin radical SAM maturase
	fun50800	0.95	NDMA-dependent methanol dehydrogenase
	fun12369	0.94	deoxyribose-phosphate aldolase/phospho-2-dehydro-3-deoxyheptonate aldolase
	fun32083	0.94	MULTISPECIES: acetyl-coenzyme A synthetase
	fun59085	0.94	purine/cytosine permease
	fun3784	0.94	alpha-1 2-mannosidase
	fun67051	0.94	succinyldiaminopimelate transaminase, partial
	fun31186	0.94	MULTISPECIES: 12-oxophytodienoate reductase
	fun66951	0.93	succinate dehydrogenase subunit B
	fun1407	0.93	4-oxalomesaconate tautomerase
	fun39715	0.93	MULTISPECIES: heterodisulfide reductase subunit B
	fun37234	0.93	MULTISPECIES: DUF4245 domain-containing protein
	fun31264	0.93	MULTISPECIES: 2-hydroxycyclohexanecarboxyl-CoA dehydrogenase
	fun28710	0.93	maltose/cellobiose ABC transporter ATP-binding subunit
	fun23845	0.92	hydrogenase HoxE
	fun39386	0.92	MULTISPECIES: glyoxalase/bleomycin resistance/dioxygenase family protein
	fun46665	0.92	MULTISPECIES: SLC13 family permease
	fun70862	0.92	twin arginine-targeting protein translocase TatB
	fun50157	0.92	NAD(FAD)-dependent dehydrogenase
	fun8375	0.92	cation transport regulator
	fun1514	0.92	5-methylcytosine restriction system protein
	fun41504	0.92	MULTISPECIES: membrane assembly protein AsmA
	fun42172	0.92	MULTISPECIES: mycofactocin radical SAM maturase
	fun62630	0.91	respiratory nitrate reductase subunit gamma
	fun57826	0.91	protein containing cell-wall hydrolase domain
	fun49575	0.91	mycofactocin system protein MftB
	fun23830	0.91	hydrogenase expression protein HypD
	fun55829	0.90	phosphoribulokinase
	fun26129	0.90	IS66 family transposase ISCysp4

Table D.3 continued from previous page

year	gene	$\log_2$Fold Change	function.
	fun45094	0.90	MULTISPECIES: PTS lactose transporter subunit IIC
	fun9526	0.90	citrate (pro-3S)-lyase
	fun58885	0.90	PTS lactose transporter subunit IIC
	fun4818	0.89	antigen
	fun30579	0.89	molybdopterin oxidoreductase, partial
	fun73523	0.89	xanthine dehydrogenase molybdenum-binding
	fun56996	0.89	polysaccharide pyruvyl transferase
	fun49564	0.88	mycofactocin biosynthesis peptidyl-dipeptidase MftE
	fun472	0.88	2-oxoacid:acceptor oxidoreductase
	fun5131	0.88	Armadillo/beta-catenin-like repeat protein
	fun36471	0.88	MULTISPECIES: DUF2231 domain-containing protein
	fun2120	0.88	ABC-1 domain-containing protein
	fun9029	0.88	chaperone protein dnaK2
	fun41230	0.88	MULTISPECIES: M18 family aminopeptidase
	fun73989	0.88	zinc finger, UBP-type
	fun51012	0.88	nickel-type superoxide dismutase maturation protease
	fun14578	0.88	DUF1931 domain-containing protein
	fun72884	0.88	UsfY protein
	fun19671	0.87	form I ribulose bisphosphate carboxylase large subunit
	fun51310	0.87	nitroalkane oxidase
	fun66994	0.87	succinate-semialdehyde dehydrogenase, partial
	fun16338	0.87	DUF4446 domain-containing protein
	fun36024	0.87	MULTISPECIES: DoxX family membrane protein
	fun54445	0.87	periplasmic serine protease, Do/DeqQ family
	fun68700	0.87	threonyl/alanyl tRNA synthetase SAD
	fun61600	0.87	pyridoxal biosynthesis lyase PdxS, partial
	fun5396	0.86	aspartate oxidase
	fun22768	0.86	helix-hairpin-helix protein
	fun62816	0.86	Resuscitation-promoting factor RpfB
	fun66052	0.85	solanesyl diphosphate synthase
	fun20334	0.85	gas vesicle synthesis GvpLGvpF
	fun47888	0.85	MULTISPECIES: toxin-antitoxin system HicB family antitoxin
	fun45107	0.85	MULTISPECIES: PTS mannose transporter subunit IIA
	fun62323	0.85	redox-sensing transcriptional repressor Rex, partial
	fun35369	0.85	MULTISPECIES: D-beta-D-heptose 1-phosphate adenosyltransferase
	fun49058	0.85	MULTISPECIES: vanillate O-demethylase oxidoreductase VanB
	fun23064	0.85	heparin-binding hemagglutinin
	fun57392	0.85	preprotein translocase SecA
	fun58497	0.84	protein usg
	fun63879	0.84	RND superfamily protein-like exporter
	fun6618	0.84	beta-glucosidase, partial
	fun10859	0.84	cyclic 2,3-phosphoglycerate synthetase
	fun69793	0.84	transcriptional regulators, TraR/DksA family protein
	fun23817	0.84	hydrogenase assembly protein HupF
	fun10948	0.84	cyclopropane fatty acid synthase
	fun53888	0.84	peptidase S1 and S6, chymotrypsin/Hap
	fun58836	0.84	PTS fructose transporter subunit IIBC
	fun14718	0.84	DUF2171 domain-containing protein
	fun70230	0.83	trehalose 6-phosphate phosphorylase
	fun68831	0.83	TIGR02234 family membrane protein

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year	gene	$\log_2$Fold Change	function.
	fun72897	0.83	UspA domain-containing protein
	fun11664	0.83	cytochrome-c oxidase, partial
	fun28521	0.83	magnetic particle membrane specific GTPase P16
	fun36295	0.83	MULTISPECIES: DUF1727 domain-containing protein
	fun4826	0.83	antioxidant protein
	fun16766	0.83	DUF929 domain-containing protein
	fun42170	0.83	MULTISPECIES: mycofactocin biosynthesis peptidyl-dipeptidase MftE
	fun64217	0.83	SAF domain-containing protein
	fun5987	0.83	ATPase-like, ParA/MinD
	fun11148	0.83	cytochrome b subunit of the bc complex
	fun44691	0.83	MULTISPECIES: preprotein translocase subunit TatA
	fun6659	0.83	beta-ketoacyl synthase, partial
	fun22859	0.83	heme iron utilization protein
	fun49771	0.82	N-acetyltransferase GCN5
	fun9299	0.82	cholesterol esterase
	fun69906	0.82	translation elongation factor
	fun49296	0.82	MULTISPECIES: YHS domain-containing protein
	fun68891	0.82	TIGR03118 family protein, partial
	fun16259	0.82	DUF4385 domain-containing protein
	fun36844	0.82	MULTISPECIES: DUF3151 domain-containing protein
	fun63470	0.82	ring-hydroxylating dioxygenase
	fun12984	0.81	disulfide oxidoreductase
	fun45489	0.81	MULTISPECIES: pyruvate:ferredoxin (flavodoxin) oxidoreductase
	fun37217	0.81	MULTISPECIES: DUF4229 domain-containing protein
	fun50319	0.81	NADH dehydrogenase FAD-containing subunit
	fun54446	0.81	periplasmic serine protease, DO/DeqQ family protein
	fun44716	0.81	MULTISPECIES: primosome assembly protein PriA
	fun55886	0.81	phosphotransferase system enzyme IIA component
	fun9550	0.81	citrate synthase 2
	fun55310	0.81	phosphatidylinositol alpha-mannosyltransferase
	fun27622	0.81	lipid kinase YegS
	fun12827	0.80	dinucleotide-binding enzyme
	fun15406	0.80	DUF3175 domain-containing protein
	fun72734	0.80	uracil-DNA glycosylase-associated domain / uracil-DNA glycosylase family 6
	fun514	0.80	2-oxoglutarate ferredoxin oxidoreductase subunit alpha, partial
	fun32700	0.80	MULTISPECIES: aminofutalosine synthase MqnE
	fun24450	0.80	Insertion element protein
	fun19986	0.80	fumarate lyase
	fun28132	0.80	lycopene cyclase family protein
	fun5743	0.80	ATP-dependent DNA helicase RecG, partial
	fun64089	0.79	S-adenosyl methyltransferase
	fun1847	0.79	aa3-type cytochrome c oxidase subunit IV
	fun51472	0.79	NmrA family transcriptional regulator
	fun26029	0.79	IS607 family transposase
	fun39212	0.79	MULTISPECIES: glycine oxidase ThiO
	fun30864	0.79	MspA protein
	fun32051	0.78	MULTISPECIES: acetyl-/propionyl-CoA carboxylase subunit alpha
	fun24237	0.78	IMP dehydrogenase, partial
	fun14762	0.78	DUF2231 domain-containing protein
	fun9924	0.78	cobalamin-independent synthase MetE-like protein

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year	gene	$\log_2$Fold Change	function.
	fun45011	0.78	MULTISPECIES: protoporphyrinogen oxidase
	fun42784	0.78	MULTISPECIES: nitrite reductase
	fun42130	0.78	MULTISPECIES: Mur ligase
	fun47240	0.78	MULTISPECIES: sulfite reductase subunit A
	fun15799	0.78	DUF3854 domain-containing protein
	fun13947	0.78	DsrE family protein
	fun13910	0.78	drug resistance transporter
	fun28572	0.78	malate dehydrogenase (oxaloacetate-decarboxylating)
	fun14797	0.77	DUF2267 domain-containing protein
	fun36566	0.77	MULTISPECIES: DUF2505 domain-containing protein
	fun37446	0.77	MULTISPECIES: DUF4916 domain-containing protein
	fun2694	0.77	AcrR family transcriptional regulator
	fun21230	0.77	glutathione transferase GstA
	fun58778	0.77	pterin dehydratase
	fun40549	0.77	MULTISPECIES: IS630 family transposase ISAzs37
	fun24599	0.77	intein-containing radical SAM protein
	fun72340	0.77	UDP pyrophosphate phosphatase
	fun20031	0.77	fusaric acid resistance protein
	fun20877	0.77	glucose-6-phosphate dehydrogenase assembly protein OpcA
	fun8748	0.77	cell wall anchor
	fun53125	0.77	pantothenate synthetase
	fun28008	0.77	LPS biosynthesis choline kinase
	fun52424	0.77	Orn/Lys/Arg decarboxylase major region
	fun70687	0.77	tRNA/tmRNA/rRNA uracil-C5-methylase
	fun44932	0.77	MULTISPECIES: protein translocase TatA
	fun14444	0.76	DUF1729 domain-containing protein
	fun5118	0.76	argininosuccinate lyase, partial
	fun12943	0.76	dipZ protein
	fun32553	0.76	MULTISPECIES: alpha-hydroxy-acid oxidizing enzyme
	fun37093	0.76	MULTISPECIES: DUF397 domain-containing protein
	fun35129	0.76	MULTISPECIES: cytochrome b6
	fun41421	0.76	MULTISPECIES: mannose-6-phosphate isomerase, class I
	fun23846	0.76	hydrogenase HoxU
	fun16021	0.76	DUF4168 domain-containing protein
	fun70733	0.76	TrwC relaxase
	fun47673	0.76	MULTISPECIES: TIGR00300 family protein
	fun51162	0.75	nitrate reductase molybdenum cofactor assembly chaperone
	fun51148	0.75	nitrate oxidoreductase subunit beta, partial
	fun67113	0.75	sugar ABC transporter
	fun70839	0.75	tungsten formylmethanofuran dehydrogenase subunit C
	fun48798	0.75	MULTISPECIES: ubiquinol-cytochrome c reductase
	fun52944	0.75	Oxoglutarate decarboxylase
	fun66377	0.75	spore protein SP21
	fun7289	0.74	BioY protein
	fun2423	0.74	acetyl-/propionyl-CoA carboxylase subunit alpha
	fun15436	0.74	DUF3235 domain-containing protein
	fun60733	0.74	putative phosphoribosyltransferase
	fun69053	0.74	Tol biopolymer transport system periplasmic protein
	fun11649	0.74	cytochrome ubiquinol oxidase subunit II
	fun4726	0.74	anti-sigma 24 factor

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year	gene	$\log_2$Fold Change	function.
	fun26253	0.74	ISAzo13 family transposase ISKra3
	fun66326	0.74	spore coat protein
	fun26704	0.74	ketohydroxyglutarate aldolase
	fun30246	0.74	Mobile element protein
	fun14252	0.73	DUF1416 domain-containing protein
	fun26918	0.73	L-cysteine:1D-myo-inositol 2-amino-2-deoxy-alpha-D-glucopyranoside ligase
	fun1849	0.73	AAA ATPase central domain protein
	fun10400	0.73	conserved protein of unknown function BmrU
	fun40183	0.72	MULTISPECIES: integration host factor
	fun41726	0.72	MULTISPECIES: methylmalonyl Co-A mutase-associated GTPase MeaB
	fun493	0.72	2-oxoglutarate dehydrogenase E1
	fun61918	0.72	pyruvate:ferredoxin (flavodoxin) oxidoreductase
	fun56997	0.72	polysaccharide pyruvyl transferase CsaB
	fun4074	0.72	amicyanin
	fun29845	0.72	methylmalonyl-CoA mutase small subunit
	fun26438	0.72	isocitrate dehydrogenase, partial
	fun66924	0.72	succinate dehydrogenase cytochrome B subunit, b558 family
	fun86	0.72	1-phosphofructokinase
	fun47755	0.72	MULTISPECIES: TIGR03842 family LLM class F420-dependent oxidoreductase
	fun11648	0.71	cytochrome ubiquinol oxidase subunit I, partial
	fun481	0.71	2-oxoacid:ferredoxin oxidoreductase subunit beta, partial
	fun56633	0.71	poly-beta-hydroxybutyrate polymerase
	fun34706	0.71	MULTISPECIES: coenzyme F420 biosynthesis-associated protein
	fun11071	0.71	cysteine–1-D-myo-inosityl 2-amino-2-deoxy-alpha-D-glucopyranoside ligase
	fun39652	0.71	MULTISPECIES: heme-copper oxidase subunit III
	fun49576	0.71	mycofactocin system transcriptional regulator
	fun50766	0.71	natural resistance-associated macrophage protein
	fun30337	0.71	molecular chaperone Hsp20
	fun8538	0.71	CDP-glycerol–poly(glycerophosphate) glycerophosphotransferase
	fun70142	0.71	transposon DNA-invertase
	fun53882	0.71	peptidase S01 family protein
	fun67027	0.71	succinyl-CoA synthetase subunit alpha
	fun14960	0.71	DUF2530 domain-containing protein
	fun48803	0.71	MULTISPECIES: ubiquinol-cytochrome c reductase cytochrome b subunit
	fun61867	0.71	pyruvate formate-lyase
	fun15191	0.70	DUF29 domain-containing protein
	fun56527	0.70	plastocyanin-like protein
	fun29546	0.70	methane/phenol/toluene hydroxylase
	fun4577	0.70	anaerobic glycerol-3-phosphate dehydrogenase subunit B
	fun52343	0.70	OpgC protein
	fun6347	0.70	barbiturase
	fun14546	0.70	DUF1876 domain-containing protein
	fun74012	0.70	zinc permease
	fun22625	0.70	heat-shock protein A
	fun61798	0.70	pyruvate dehydrogenase (acetyl-transferring)
	fun7410	0.70	branched-chain alpha-keto acid dehydrogenase subunit E2, partial
	fun55834	0.69	phosphorylase
	fun67080	0.69	sucrose-6F-phosphate phosphohydrolase
	fun28443	0.69	maf protein
	fun37188	0.69	MULTISPECIES: DUF4177 domain-containing protein

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year	gene	$\log_2$Fold Change	function.
	fun40270	0.69	MULTISPECIES: iron transporter
	fun11553	0.69	cytochrome C554
	fun7172	0.69	binding-protein-dependent transporters inner membrane component
	fun63352	0.69	ribosylglycohydrolase
	fun49400	0.69	MULTISPECIES: zinc-binding dehydrogenase
	fun3919	0.69	alpha-ketoglutarate decarboxylase, partial
	fun51373	0.69	nitrogen regulatory protein P-II 1
	fun66973	0.69	succinate–CoA ligase (ADP-forming)
	fun57597	0.69	prolyl-tRNA editing protein
	fun9705	0.69	Clp protease adaptor protein ClpS
	fun2457	0.69	acetyl-CoA carboxylase carboxyltransferase subunit alpha/beta
	fun25938	0.68	IS5 family transposase ISPaen3
	fun58107	0.68	protein meaA
	fun24812	0.68	iron reductase
	fun6920	0.68	bifunctional Fe-S cluster assembly protein NifU/tRNA 2-thiouridine(34) synthase MnmA
	fun20390	0.68	GDP-mannose pyrophosphorylase
	fun2475	0.68	acetyl-CoA carboxyltransferase
	fun36094	0.68	MULTISPECIES: DUF1116 domain-containing protein
	fun37491	0.68	MULTISPECIES: DUF5130 domain-containing protein
	fun20677	0.67	GlnR family transcriptional regulator
	fun2998	0.67	addiction module antidote protein
	fun61976	0.67	quercetin 2,3-dioxygenase
	fun55753	0.67	phosphoribosyl transferase
	fun66960	0.67	succinate dehydrogenase, partial
	fun69	0.67	1-Cys peroxiredoxin
	fun52401	0.67	organic solvent resistance ABC transporter substrate-binding protein
	fun74140	0.67	Zn-dependent oxidoreductase NADPH:quinone reductase
	fun15548	0.67	DUF3386 domain-containing protein
	fun50099	0.67	NAD-dependent formate dehydrogenase iron-sulfur protein
	fun15174	0.67	DUF2877 domain-containing protein
	fun22651	0.67	heat-shock protein SP21
	fun74141	0.67	Zn-dependent oxidoreductase, NADPH:quinone reductase
	fun45605	0.66	MULTISPECIES: redox-sensing transcriptional repressor Rex
	fun8312	0.66	catalase-peroxidase, partial
	fun38649	0.66	MULTISPECIES: fructose-bisphosphate aldolase class I
	fun19774	0.66	formimidoyltetrahydrofolate cyclodeaminase
	fun67404	0.66	sulfite reductase subunit A
	fun12637	0.66	dihydrodipicolinate synthase
	fun62142	0.66	raiA ribosome-associated inhibitor A
	fun36538	0.66	MULTISPECIES: DUF2383 domain-containing protein
	fun30452	0.66	molybdenum cofactor synthesis protein
	fun72469	0.66	UDP-N-acetylglucosamine 2-epimerase
	fun32284	0.66	MULTISPECIES: adenosylmethionine decarboxylase
	fun36198	0.66	MULTISPECIES: DUF1416 domain-containing protein
	fun32925	0.66	MULTISPECIES: antitoxin VapB
	fun39873	0.66	MULTISPECIES: HrcA family transcriptional regulator
	fun72670	0.66	universal stress protein A
	fun51916	0.66	nucleotide sugar-1-phosphate transferase
	fun2508	0.66	acetyl-coenzyme A synthetase, partial
	fun47117	0.66	MULTISPECIES: SUF system Fe-S cluster assembly regulator

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year	gene	$\log_2$Fold Change	function.
	fun38688	0.65	MULTISPECIES: fumarate reductase/succinate dehydrogenase flavoprotein subunit
	fun64988	0.65	serine peptidase
	fun35625	0.65	MULTISPECIES: dihydrofolate synthase
	fun2856	0.65	acyl-CoA hydratase
	fun23656	0.65	HtrA protease/chaperone protein
	fun38949	0.65	MULTISPECIES: glucan export ABC transporter ATP-binding protein
	fun42181	0.65	MULTISPECIES: mycothiol conjugate amidase Mca
	fun15495	0.65	DUF3311 domain-containing protein
	fun67488	0.65	sulfur globule protein precursor
	fun34617	0.65	MULTISPECIES: co-chaperone YbbN
	fun21769	0.65	glycosyl hydrolase family 32
	fun47736	0.65	MULTISPECIES: TIGR03085 family protein
	fun34939	0.65	MULTISPECIES: crotonyl-CoA carboxylase/reductase
	fun5508	0.64	ATP F0F1 synthase subunit I
	fun14825	0.64	DUF2294 domain-containing protein
	fun64592	0.64	secretion protein EspR
	fun15633	0.64	DUF3515 domain-containing protein
	fun49571	0.64	mycofactocin system glycosyltransferase
	fun3097	0.64	adenosylmethionine-8-amino-7-oxononanoate aminotransferase
	fun14484	0.64	DUF1802 domain-containing protein
	fun13682	0.63	DNA-directed RNA polymerase subunit beta”
	fun13903	0.63	drug exporter-like protein of the RND superfamily
	fun2670	0.63	ACP S-malonyltransferase
	fun214	0.63	1D-myo-inositol 2-acetamido-2-deoxy-alpha-D-glucopyranoside deacetylase
	fun26274	0.63	ISKra4 family transposase
	fun23704	0.63	hyaluronate lyase
	fun43880	0.63	MULTISPECIES: phenylacetic acid degradation protein PaaN
	fun73449	0.63	X-Pro dipeptidase
	fun8534	0.63	CDP-glycerol glycerophosphotransferase family protein
	fun28587	0.63	malate–CoA ligase subunit beta
	fun19705	0.63	formate dehydrogenase H subunit alpha, selenocysteine-containing
	fun62038	0.63	quinoline 2-oxidoreductase
	fun14266	0.63	DUF1440 domain-containing protein
	fun33337	0.63	MULTISPECIES: auracyanin-B
	fun16086	0.63	DUF4229 domain-containing protein
	fun68890	0.63	TIGR03118 family protein
	fun16114	0.62	DUF4245 domain-containing protein
	fun20087	0.62	futalosine hydrolase
	fun4576	0.62	anaerobic glycerol-3-phosphate dehydrogenase subunit A
	fun61764	0.62	pyrroloquinoline quinone biosynthesis protein B
	fun50756	0.62	NarK family nitrate/nitrite MFS transporter
	fun414	0.62	2-ketoisovalerate ferredoxin oxidoreductase subunit alpha
	fun12074	0.62	daunorubicin ABC transporter ATPase
	fun4728	0.62	anti-sigma B factor antagonist
	fun18011	0.62	extracellular solute-binding protein
	fun60443	0.62	putative NADH-quinone oxidoreductase subunit F 2
	fun71405	0.61	type IA DNA topoisomerase
	fun18604	0.61	ferredoxin-NADP reductase
	fun23607	0.61	Hsp20/alpha crystallin family protein
	fun2203	0.61	ABC-type sugar transport system, periplasmic component

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year	gene	$\log_2$Fold Change	function.
	fun495	0.61	2-oxoglutarate dehydrogenase E1 component, partial
	fun20515	0.61	geranyl transferase
	fun43324	0.61	MULTISPECIES: PA-phosphatase
	fun8144	0.61	carboxyl transferase
	fun10520	0.61	copper-transporting ATPase
	fun51892	0.61	nucleotidase
	fun62152	0.61	rare lipoprotein A
	fun42848	0.61	MULTISPECIES: NmrA family transcriptional regulator
	fun23663	0.61	HtrA2 peptidase
	fun33120	0.61	MULTISPECIES: aspartate 1-decarboxylase
	fun12192	0.61	DegP-like serine endoprotease
	fun5545	0.61	ATP synthase F0 subunit alpha
	fun62807	0.61	resuscitation-promoting factor
	fun13686	0.61	DNA-directed RNA polymerase subunit gamma
	fun42651	0.61	MULTISPECIES: neutral zinc metallopeptidase
	fun37052	0.61	MULTISPECIES: DUF3817 domain-containing protein
	fun11604	0.61	cytochrome o ubiquinol oxidase
	fun46558	0.61	MULTISPECIES: SigB/SigF/SigG family RNA polymerase sigma factor
	fun30509	0.61	molybdopterin biosynthesis protein MoeZ
	fun16782	0.61	DUF948 domain-containing protein
	fun14569	0.61	DUF1918 domain-containing protein
	fun58473	0.60	protein translocase TatA
	fun18386	0.60	FdrA family protein
	fun65202	0.60	SH3 type 3 domain-containing protein
	fun40249	0.60	MULTISPECIES: iron ABC transporter ATP-binding protein
	fun26386	0.60	ISNCY family transposase ISPlas1
	fun26402	0.60	isoamylase
	fun17112	0.60	elongation factor G-like protein EF-G2
	fun59338	0.60	putative aminotransferase
	fun15021	0.60	DUF2630 domain-containing protein
	fun72256	0.60	ubiquinol-cytochrome c reductase
	fun34536	0.60	MULTISPECIES: citrate synthase/methylcitrate synthase
	fun66914	0.60	succinate dehydrogenase and fumarate reductase iron-sulfur protein
	fun37902	0.60	MULTISPECIES: exo-alpha-sialidase
	fun57607	0.60	propanediol dehydratase large subunit
	fun36820	0.60	MULTISPECIES: DUF3105 domain-containing protein
	fun68597	0.60	thioredoxin domain-containing protein, partial
	fun14995	0.59	DUF2587 domain-containing protein
	fun10679	0.59	crotonyl-CoA carboxylase/reductase
	fun42626	0.59	MULTISPECIES: NDMA-dependent alcohol dehydrogenase
	fun60744	0.59	putative PhoU
	fun30580	0.59	molybdopterin synthase
	fun18432	0.59	Fe-S oxidoreductase, partial
	fun27652	0.59	lipoamide dehydrogenase
	fun44397	0.59	MULTISPECIES: plasmid stabilization protein
	fun22634	0.59	heat-shock protein Hsp20
	fun65377	0.59	sigma factor regulator FecR
	fun13194	0.58	DNA ligase (NAD(+)) LigA, partial
	fun24549	0.58	integration host factor MihF
	fun8498	0.58	CdaR family transcriptional regulator

Table D.3 continued from previous page

year	gene	$\log_2$Fold Change	function.
	fun47303	0.58	MULTISPECIES: superoxide dismutase, Ni
	fun5317	0.58	AsnC family protein
	fun5338	0.58	Asp23/Gls24 family envelope stress response protein
	fun14953	0.58	DUF2520 domain-containing protein
	fun65029	0.58	serine protease HtrA
	fun9035	0.58	chaperone protein HtpG
	fun9671	0.58	class IV aminotransferase
	fun73829	0.58	YibE/F family protein
	fun15186	0.58	DUF2892 domain-containing protein
	fun30478	0.58	molybdenum-pterin-binding protein
	fun63760	0.58	RNA polymerase, sigma-24 subunit, ECF subfamily
	fun32001	0.58	MULTISPECIES: acetate–CoA ligase
	fun30314	0.58	molecular chaperone
	fun53863	0.58	peptidase M75 family protein
	fun17495	0.57	erythromycin esterase
	fun66261	0.57	sphingosine kinase
	fun13331	0.57	DNA polymerase III subunit gamma/tau, partial
	fun4374	0.57	aminoglycoside resistance protein
	fun16329	0.57	DUF4439 domain-containing protein
	fun52791	0.57	oxaloacetate decarboxylase subunit alpha
	fun63027	0.57	riboflavin deaminase
	fun18805	0.57	fibronectin/fibrinogen-binding protein
	fun26424	0.57	isochorismate synthase
	fun43559	0.57	MULTISPECIES: peptidase S1
	fun8080	0.57	carbon monoxide dehydrogenase medium subunit
	fun12780	0.57	dimethylallyltranstransferase
	fun559	0.57	2-phosphoglycerate kinase
	fun50758	0.57	NarK/NasA family nitrate transporter
	fun67591	0.57	superoxide dismutase, Ni
	fun10503	0.57	copper-binding protein
	fun64381	0.57	SDR family mycofactocin-dependent oxidoreductase
	fun53578	0.57	peptidase A2
	fun72877	0.57	uroporphyrinogen-III C-methyltransferase, partial
	fun22672	0.57	heavy metal transport/detoxification protein
	fun36789	0.57	MULTISPECIES: DUF3052 domain-containing protein
	fun544	0.57	2-oxoisovalerate dehydrogenase subunit alpha
	fun15656	0.57	DUF3558 domain-containing protein
	fun46286	0.57	MULTISPECIES: SDR family mycofactocin-dependent oxidoreductase
	fun26695	0.56	ketoacyl reductase
	fun7336	0.56	blue (type 1) copper domain-containing protein
	fun64369	0.56	scramblase
	fun2283	0.56	acetamidase/formamidase family protein
	fun20349	0.56	GatB/YqeY domain-containing protein
	fun29356	0.56	mesaconyl-CoA isomerase
	fun60470	0.56	putative nitrite reductase
	fun56526	0.56	plastocyanin
	fun17243	0.56	endonuclease IV
	fun39222	0.56	MULTISPECIES: glycine–tRNA ligase
	fun33763	0.56	MULTISPECIES: biotin attachment protein
	fun17637	0.56	ethylmalonyl-CoA mutase

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year	gene	$\log_2$Fold Change	function.
	fun36062	0.56	MULTISPECIES: dual-specificity RNA methyltransferase RlmN
	fun36849	0.56	MULTISPECIES: DUF3159 domain-containing protein
	fun72260	0.56	ubiquinol-cytochrome c reductase cytochrome b subunit
	fun18012	0.55	extracellular solute-binding protein family 1
	fun54237	0.55	Peptidoglycan-binding LysM
	fun32558	0.55	MULTISPECIES: alpha-ketoglutarate decarboxylase
	fun63167	0.55	ribonucleotide reductase
	fun1006	0.55	3-methylitaconate isomerase
	fun68844	0.55	TIGR02449 family protein
	fun36156	0.55	MULTISPECIES: DUF1290 domain-containing protein
	fun5968	0.55	ATPase P
	fun29826	0.55	methylmalonate-semialdehyde dehydrogenase (CoA acylating), partial
	fun34533	0.55	MULTISPECIES: citrate synthase
	fun12753	0.55	dihydroxyacetone kinase subunit L
	fun72257	0.55	ubiquinol-cytochrome C reductase
	fun22055	0.55	glycosyltransferase/methyltransferase
	fun23103	0.55	heterodisulfide reductase
	fun10058	0.55	coenzyme PQQ precursor peptide PqqA
	fun14625	0.55	DUF202 domain-containing protein
	fun20048	0.55	fused acetyl/propionyl-CoA carboxylase subunit alpha/methylmalonyl-CoA decarboxylase subunit alpha
	fun22832	0.55	heme b synthase
	fun7884	0.55	capsule biosynthesis protein CapD
	fun29582	0.54	methicillin resistance protein
	fun59798	0.54	putative enzyme
	fun15389	0.54	DUF3151 domain-containing protein
	fun60979	0.54	putative S1B family peptidase
	fun73254	0.54	vitamin K epoxide reductase
	fun63029	0.54	riboflavin kinase
	fun14442	0.54	DUF1727 domain-containing protein
	fun15297	0.54	DUF3040 domain-containing protein
	fun46966	0.54	MULTISPECIES: STAS/SEC14 domain-containing protein
	fun14830	0.54	DUF2304 domain-containing protein
	fun14787	0.54	DUF2255 domain-containing protein
	fun70254	0.54	trehalose-6-phosphate synthase, partial
	fun20734	0.54	glucan export ABC transporter ATP-binding protein
	fun20069	0.54	fused response regulator/phosphatase
	fun20732	0.54	glucan endo-1,6-beta-glucosidase
	fun8181	0.54	carboxylic ester hydrolase
	fun48933	0.54	MULTISPECIES: undecaprenyl/decaprenyl-phosphate alpha-N-acetylglucosaminyl 1-phosphate transferase
	fun26736	0.54	ketosynthase chain-length factor
	fun11194	0.54	cytochrome bc complex cytochrome b subunit
	fun49588	0.54	mycothiol conjugate amidase Mca
	fun37429	0.54	MULTISPECIES: DUF4870 domain-containing protein
	fun15402	0.54	DUF3168 domain-containing protein
	fun73617	0.54	XshC-Cox1 family protein
	fun49594	0.54	mycothiol synthase
	fun30126	0.54	mitomycin antibiotics/polyketide fumonisin biosynthesis protein
	fun67484	0.53	sulfur carrier protein ThiS
	fun63662	0.53	RNA polymerase sigma factor, RpoD/SigA family
	fun62031	0.53	quinolinate phosphoribosyl transferase

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year	gene	$\log_2$Fold Change	function.
	fun15255	0.53	DUF2993 domain-containing protein
	fun61776	0.53	pyrroloquinoline quinone precursor peptide PqqA
	fun24323	0.53	inhibition of morphological differentiation protein
	fun23541	0.53	HoxN/HupN/NixA family nickel/cobalt transporter
	fun57257	0.53	PRC-barrel domain-containing protein
	fun557	0.53	2-phospho-L-lactate transferase
	fun7926	0.53	carbamoyl phosphate synthase
	fun12484	0.53	diacylglycerol O-acyltransferase
	fun19600	0.53	FMN-dependent NADH-azoreductase
	fun28510	0.53	magnesium-transporting ATPase
	fun39472	0.53	MULTISPECIES: GuaB3 family IMP dehydrogenase-related protein
	fun36782	0.53	MULTISPECIES: DUF3040 domain-containing protein
	fun21043	0.53	glutamate-pyruvate aminotransferase
	fun56387	0.52	pimeloyl-CoA synthetase
	fun14667	0.52	DUF2090 domain-containing protein
	fun11614	0.52	cytochrome oxidase
	fun21368	0.52	glycerophosphatase
	fun14436	0.52	DUF1707 domain-containing protein
	fun20064	0.52	fused N-dimethylarginine dimethylaminohydrolase/saccharopine dehydrogenase domain-containing protein
	fun70439	0.52	tRNA (5-methylaminomethyl-2-thiouridylate)-methyltransferase
	fun7238	0.52	biotin attachment protein
	fun45720	0.52	MULTISPECIES: resuscitation-promoting factor
	fun68888	0.52	TIGR03089 family protein
	fun16034	0.52	DUF4177 domain-containing protein
	fun36825	0.52	MULTISPECIES: DUF3117 domain-containing protein
	fun74039	0.52	zinc-binding dehydrogenase
	fun27249	0.52	large conductance mechanosensitive channel protein
	fun67991	0.52	Tellurite resistance TerB
	fun37702	0.52	MULTISPECIES: elongation factor G-like protein EF-G2
	fun37993	0.52	MULTISPECIES: extracellular solute-binding protein
	fun29932	0.52	MexE family multidrug efflux RND transporter periplasmic adaptor subunit
	fun3918	0.52	alpha-ketoglutarate decarboxylase
	fun9791	0.52	CoA ester lyase
	fun72668	0.52	universal stress protein
	fun14054	0.52	DUF1059 domain-containing protein
	fun50965	0.52	Ni/Fe hydrogenase subunit gamma
	fun8975	0.52	CHAD domain-containing protein
	fun15731	0.52	DUF3710 domain-containing protein
	fun63764	0.52	RNA polymerase, sigma-24 subunit, RpoE
	fun8468	0.52	CBS domain-containing protein
	fun49162	0.52	MULTISPECIES: WYL domain-containing protein
	fun12986	0.52	disulfide reductase
	fun61768	0.52	pyrroloquinoline quinone biosynthesis protein PqqB
	fun50541	0.52	NADH-ubiquinone oxidoreductase subunit M
	fun44866	0.52	MULTISPECIES: protein meaA
	fun49023	0.52	MULTISPECIES: uroporphyrinogen-III C-methyltransferase
	fun36497	0.52	MULTISPECIES: DUF2277 domain-containing protein
	fun36604	0.51	MULTISPECIES: DUF2587 domain-containing protein
	fun55825	0.51	phosphoribosyltransferase
	fun12992	0.51	dithiol-disulfide isomerase

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year	gene	$\log_2$Fold Change	function.
	fun67504	0.51	sulfurase
	fun17007	0.51	electron transfer flavoprotein
	fun805	0.51	3-carboxy-cis,cis-muconate cycloisomerase
	fun22074	0.51	glyoxalase family protein
	fun15810	0.51	DUF3866 domain-containing protein

Table D.4: Variation in the expression of soil microbial genes between months (September vs. June), based on RefSeq bacterial functional annotation (DESeq2, adjusted $P < 0.05$).

month	gene	$\log_2$Fold Change	function.
September	fun33370	5.90	MULTISPECIES: avidin
	fun34096	5.80	MULTISPECIES: caspase-like domain-containing protein
	fun40014	5.33	MULTISPECIES: hydroxyacid oxidoreductase
	fun42819	5.31	MULTISPECIES: nitrogenase molybdenum-iron protein alpha chain
	fun44137	5.26	MULTISPECIES: phosphoribosyl-ATP pyrophosphatase 2
	fun25139	5.22	IS1380 family transposase ISBj1
	fun26085	5.17	IS630 family transposase ISRj1
	fun40555	5.13	MULTISPECIES: IS66 family insertion sequence hypothetical protein
	fun33354	5.10	MULTISPECIES: autoinducer-binding protein
	fun25323	5.09	IS21 family transposase IS1631
	fun45627	4.82	MULTISPECIES: regulator PrlF
	fun53637	4.75	peptidase C39, partial
	fun62546	4.65	replication initiation protein RepC, partial
	fun45961	4.58	MULTISPECIES: Rieske iron-sulfur protein
	fun33504	4.46	MULTISPECIES: beta-1,6-glucan synthase
	fun25834	4.34	IS5 family transposase ISBj2
	fun14248	4.30	DUF1403 domain-containing protein, partial
	fun43475	4.26	MULTISPECIES: peptidase C14, caspase catalytic subunit p20
	fun40360	4.23	MULTISPECIES: IS1380 family transposase ISBdi2
	fun36985	4.10	MULTISPECIES: DUF3551 domain-containing protein
	fun56087	4.09	phycobilisome Linker polypeptide/CpcD/allophycocyanin linker domain-containing protein
	fun44088	4.01	MULTISPECIES: phosphonate C-P lyase system protein PhnG
	fun41330	3.88	MULTISPECIES: malonate carrier protein
	fun44385	3.74	MULTISPECIES: plasmid partitioning protein RepB
	fun25835	3.73	IS5 family transposase ISBj2_B
	fun40641	3.63	MULTISPECIES: isoprenyl transferase 2
	fun5127	3.60	arginyltransferase, partial
	fun40282	3.58	MULTISPECIES: iron-dependent peroxidase
	fun16843	3.57	E3 ubiquitin–protein ligase
	fun44097	3.53	MULTISPECIES: phosphonate metabolism transcriptional regulator PhnF
	fun38424	3.51	MULTISPECIES: flagellar protein
	fun62162	3.50	RbcX protein
	fun46480	3.49	MULTISPECIES: serine–glyoxylate aminotransferase
	fun41763	3.37	MULTISPECIES: MexW/MexI family multidrug efflux RND transporter permease subunit
	fun44384	3.35	MULTISPECIES: plasmid partitioning protein RepA

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month	gene	$\log_2$Fold Change	function.
	fun36645	3.34	MULTISPECIES: DUF2735 domain-containing protein
	fun29566	3.32	methanol oxidase
	fun35067	3.32	MULTISPECIES: cysteine biosynthesis protein
	fun51391	3.28	nitrogenase reductase
	fun51386	3.28	nitrogenase iron-molybdenum cofactor biosynthesis protein NifE
	fun37322	3.26	MULTISPECIES: DUF4375 domain-containing protein
	fun38814	3.23	MULTISPECIES: GcrA-like regulator
	fun43973	3.22	MULTISPECIES: phosphatidylserine decarboxylase proenzyme
	fun32170	3.14	MULTISPECIES: acyl transferase
	fun17519	3.10	EscI/YscI/HrpB family type III secretion system inner rod protein
	fun37303	3.10	MULTISPECIES: DUF4339 domain-containing protein
	fun33714	3.06	MULTISPECIES: bifunctional tRNA (adenosine(37)-N6)-threonylcarbamoyltransferase complex ATPase subunit type 1 TsaE/phosphotransferase
	fun37921	3.04	MULTISPECIES: exodeoxyribosylnuclease
	fun47224	3.04	MULTISPECIES: sulfate/thiosulfate import ATP-binding protein CysA
	fun35068	3.02	MULTISPECIES: cysteine biosynthesis protein CysZ
	fun34091	3.02	MULTISPECIES: carotenoid biosynthesis protein
	fun35401	3.02	MULTISPECIES: D-mycarose 3-C-methyltransferase
	fun68754	3.00	Tic22-like family
	fun55470	2.91	phosphoglucomutase, alpha-D-glucose phosphate-specific
	fun44286	2.91	MULTISPECIES: pigment protein
	fun58067	2.91	protein kinase domain with FHA domain
	fun43043	2.90	MULTISPECIES: O-succinylbenzoic acid–CoA ligase
	fun34095	2.89	MULTISPECIES: caspase (peptidase)
	fun2392	2.88	acetone carboxylase gamma subunit
	fun28259	2.87	lysylphosphatidylglycerol synthetase family protein, partial
	fun48421	2.87	MULTISPECIES: two-component system response regulator protein-glutamate methylesterase
	fun35233	2.84	MULTISPECIES: cytochrome C-binding protein
	fun8293	2.84	caspase-like domain-containing protein, partial
	fun44832	2.82	MULTISPECIES: protein FixA
	fun62819	2.82	resuscitation-promoting factor rpfE
	fun50679	2.79	NADPH dehydrogenase, partial
	fun68365	2.78	thiamine pyrophosphate dependent decarboxylase pyruvate decarboxylase
	fun36228	2.78	MULTISPECIES: DUF1488 domain-containing protein
	fun40209	2.73	MULTISPECIES: intracellular septation protein
	fun36271	2.72	MULTISPECIES: DUF1636 domain-containing protein
	fun33204	2.71	MULTISPECIES: ATP synthase subunit b 2
	fun35270	2.69	MULTISPECIES: cytochrome c6
	fun35120	2.68	MULTISPECIES: cytochrome b/c1
	fun48480	2.67	MULTISPECIES: type I phosphodiesterase/nucleotide pyrophosphatase
	fun38780	2.67	MULTISPECIES: gamma-glutamyltranspeptidase
	fun41032	2.65	MULTISPECIES: lipolytic enzyme
	fun46979	2.63	MULTISPECIES: steroid monooxygenase
	fun35206	2.58	MULTISPECIES: cytochrome c oxidase subunit 1
	fun34037	2.56	MULTISPECIES: carbon-phosphorus lyase complex subunit PhnI
	fun35310	2.54	MULTISPECIES: cytochrome-c3 hydrogenase
	fun31661	2.53	MULTISPECIES: 4-diphosphocytidyl-2C-methyl-D-erythritol kinase
	fun36438	2.52	MULTISPECIES: DUF2158 domain-containing protein
	fun12990	2.50	ditF protein
	fun51390	2.49	nitrogenase molybdenum-iron protein subunit beta

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month	gene	$\log_2$Fold Change	function.
	fun50545	2.48	NADH-ubiquinone oxidoreductase-related protein
	fun37532	2.42	MULTISPECIES: DUF736 domain-containing protein
	fun9230	2.39	chitooligosaccharide synthase NodC
	fun33350	2.39	MULTISPECIES: autoinducer synthase
	fun62543	2.37	replication initiation protein RepC
	fun8745	2.31	cell surface receptor IPT/TIG domain-containing protein, partial
	fun36842	2.31	MULTISPECIES: DUF3147 domain-containing protein
	fun14247	2.30	DUF1403 domain-containing protein
	fun8123	2.30	carbon-phosphorus lyase complex subunit PhnI
	fun47849	2.28	MULTISPECIES: TonB system transport protein ExbD
	fun28971	2.27	MBL fold hydrolase, partial
	fun44617	2.26	MULTISPECIES: potassium-transporting ATPase subunit F
	fun18319	2.25	fatty acid oxidation complex alpha-subunit
	fun29629	2.25	methionine synthase II (cobalamin-independent)
	fun8382	2.25	cation transporter E1-E2 family ATPase
	fun17799	2.24	exonuclease VII large subunit
	fun36697	2.24	MULTISPECIES: DUF2848 domain-containing protein
	fun47598	2.22	MULTISPECIES: thiol:disulfide interchange protein TlpA
	fun32476	2.22	MULTISPECIES: alkyl/aryl-sulfatase
	fun62821	2.21	Resuscitation-promoting factor RpfE
	fun19058	2.21	flagellar biosynthesis protein FlgC
	fun34004	2.20	MULTISPECIES: carbohydrate porin
	fun52890	2.17	oxidoreductase short-chain dehydrogenase/reductase family
	fun7309	2.14	BJP family subclass B3 metallo-beta-lactamase
	fun15740	2.12	DUF374 domain-containing protein, partial
	fun55653	2.11	phosphonate C-P lyase system protein PhnH
	fun36705	2.11	MULTISPECIES: DUF2865 domain-containing protein
	fun24148	2.10	Ig domain protein, group 2 domain protein
	fun37199	2.08	MULTISPECIES: DUF4189 domain-containing protein
	fun37204	2.06	MULTISPECIES: DUF4194 domain-containing protein
	fun43436	2.06	MULTISPECIES: penicillin-binding protein, partial
	fun29547	2.06	methane/Phenol/Toluene hydroxylase
	fun54153	2.06	peptide-binding protein, partial
	fun36368	2.05	MULTISPECIES: DUF1929 domain-containing protein
	fun18507	2.04	FecR domain-containing protein
	fun18100	2.04	F420-0:Gamma-glutamyl ligase
	fun14044	2.03	DUF1036 domain-containing protein, partial
	fun47699	2.03	MULTISPECIES: TIGR02186 family protein
	fun51387	2.02	nitrogenase iron-molybdenum cofactor biosynthesis protein NifN
	fun49059	2.02	MULTISPECIES: vanillate O-demethylase oxygenase
	fun60544	2.00	putative oxidoreductase molybdopterin-binding subunit/oxidoreductase iron-sulfur subunit
	fun41865	1.99	MULTISPECIES: molecular chaperone
	fun16020	1.99	DUF4167 domain-containing protein, partial
	fun36622	1.98	MULTISPECIES: DUF2628 domain-containing protein
	fun54692	1.98	phage infection protein
	fun36133	1.98	MULTISPECIES: DUF1236 domain-containing protein
	fun33496	1.96	MULTISPECIES: beta-(1–>2)glucan export ATP-binding/permease NdvA
	fun36059	1.95	MULTISPECIES: DTW domain-containing protein
	fun47687	1.95	MULTISPECIES: TIGR01620 family protein
	fun34596	1.94	MULTISPECIES: Clp protease ATP-binding protein

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month	gene	$\log_2$Fold Change	function.
	fun40545	1.92	MULTISPECIES: IS630 family transposase
	fun4120	1.91	amidotransferase-related protein
	fun65799	1.90	small secreted protein
	fun42691	1.90	MULTISPECIES: nickel-dependent hydrogenase large subunit
	fun37004	1.88	MULTISPECIES: DUF3592 domain-containing protein
	fun70635	1.88	tRNA-(MS(2)IO(6)A)-hydroxylase-like protein
	fun56958	1.88	polysaccharide deacetylase family protein, partial
	fun43909	1.86	MULTISPECIES: phosoglucose isomerase
	fun63581	1.86	RNA polymerase principal sigma factor hrdD
	fun55656	1.85	phosphonate C-P lyase system protein PhnL
	fun33573	1.85	MULTISPECIES: bicarbonate-binding protein
	fun47398	1.84	MULTISPECIES: taurine catabolism dioxygenase
	fun39214	1.82	MULTISPECIES: glycine reductase
	fun6459	1.82	benzoate 1,2-dioxygenase subunit alpha
	fun3998	1.81	alpha/beta family hydrolase
	fun35309	1.81	MULTISPECIES: cytochrome-c peroxidase
	fun34348	1.81	MULTISPECIES: chaperone protein ClpB
	fun5404	1.79	aspartate transaminase
	fun73788	1.78	YfdX protein
	fun37484	1.78	MULTISPECIES: DUF5076 domain-containing protein
	fun29549	1.77	Methane/Phenol/Toluene Hydroxylase
	fun42750	1.77	MULTISPECIES: nitrate transporter
	fun42659	1.75	MULTISPECIES: NHL repeat containing protein
	fun37521	1.75	MULTISPECIES: DUF680 domain-containing protein
	fun55652	1.75	phosphonate C-P lyase system protein PhnG
	fun66794	1.74	Styrene monooxygenase subunit A
	fun34249	1.74	MULTISPECIES: cell envelope integrity/translocation protein TolA
	fun36423	1.74	MULTISPECIES: DUF2130 domain-containing protein
	fun39698	1.73	MULTISPECIES: heparin-binding hemagglutinin
	fun33484	1.73	MULTISPECIES: benzoylformate decarboxylase
	fun34094	1.72	MULTISPECIES: caspase
	fun41626	1.72	MULTISPECIES: methane monooxygenase
	fun42693	1.71	MULTISPECIES: nickel-responsive transcriptional regulator NikR
	fun72878	1.70	uroporphyrinogen-III decarboxylase
	fun42738	1.70	MULTISPECIES: nitrate ABC transporter, permease protein
	fun49663	1.69	N-acetyl-D-glucosamine ABC transporter substrate-binding protein
	fun55329	1.69	phosphatidylserine decarboxylase, partial
	fun8121	1.68	carbon-phosphorus lyase
	fun31726	1.68	MULTISPECIES: 4'-phosphopantetheinyl transferase
	fun50841	1.67	negative regulator of septation ring formation
	fun52782	1.66	oxalate decarboxylase family bicupin
	fun36532	1.64	MULTISPECIES: DUF2339 domain-containing protein
	fun64279	1.63	SapB/AmfS family lantipeptide
	fun69139	1.63	Toluene-4-monooxygenase system protein A
	fun30184	1.62	MmoB/DmpM family protein
	fun41762	1.62	MULTISPECIES: MexH family multidrug efflux RND transporter periplasmic adaptor subunit
	fun41934	1.62	MULTISPECIES: molybdopterin biosynthesis protein
	fun15621	1.62	DUF3494 domain-containing protein, partial
	fun22334	1.61	GuaB3 family IMP dehydrogenase-related protein, partial
	fun68891	1.60	TIGR03118 family protein, partial

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month	gene	$\log_2$Fold Change	function.
	fun49268	1.59	MULTISPECIES: YecA family protein
	fun30862	1.59	mspA family protein
	fun33131	1.59	MULTISPECIES: aspartate dehydrogenase
	fun40809	1.58	MULTISPECIES: L-idonate 5-dehydrogenase
	fun39932	1.57	MULTISPECIES: hydratase
	fun27144	1.57	Lactonase, 7-bladed beta propeller
	fun63440	1.56	right-handed parallel beta-helix repeat-containing protein, partial
	fun73988	1.55	zinc finger, SWIM-type
	fun16370	1.55	DUF459 domain-containing protein, partial
	fun69889	1.54	transglycosylase-like protein
	fun14260	1.54	DUF1428 domain-containing protein, partial
	fun10312	1.54	conjugal transfer protein TrbG
	fun1545	1.54	5-oxopent-3-ene-1,2,5-tricarboxylate decarboxylase, partial
	fun52404	1.53	organic solvent tolerance protein
	fun65664	1.53	SirA-like protein
	fun39570	1.53	MULTISPECIES: heat-shock protein IbpA
	fun39234	1.52	MULTISPECIES: glycine/sarcosine/betaine reductase complex selenoprotein A
	fun64360	1.51	SCP-like protein
	fun67661	1.50	surfeit 1 protein
	fun32538	1.50	MULTISPECIES: alpha-D-ribose 1-methylphosphonate 5-triphosphate diphosphatase
	fun32471	1.50	MULTISPECIES: alkyl hydroperoxide reductase AhpD
	fun51909	1.50	nucleotide sugar dehydrogenase subfamily
	fun31558	1.50	MULTISPECIES: 3-oxoadipate CoA-transferase subunit B
	fun45803	1.50	MULTISPECIES: ribonuclease activity regulator RraA
	fun63858	1.50	RND efflux system, hypothetical protein CmeC
	fun13445	1.49	DNA repair protein RecN, partial
	fun34688	1.49	MULTISPECIES: cobalt-precorrin-6A reductase
	fun33391	1.49	MULTISPECIES: BA14K family protein
	fun37060	1.48	MULTISPECIES: DUF3830 domain-containing protein
	fun33546	1.47	MULTISPECIES: beta-ketoacyl-ACP synthase II
	fun20254	1.47	gamma-D-glutaminyl-L-lysyl-endopeptidase
	fun37175	1.46	MULTISPECIES: DUF4164 domain-containing protein
	fun36454	1.46	MULTISPECIES: DUF2189 domain-containing protein
	fun44659	1.46	MULTISPECIES: precorrin-6A synthase (deacetylating)
	fun3709	1.46	alkylphosphonate uptake protein
	fun3655	1.45	alkanesulfonate monooxygenase, FMNH(2)-dependent
	fun36515	1.45	MULTISPECIES: DUF2306 domain-containing protein
	fun31849	1.43	MULTISPECIES: 6-carboxyhexanoate–CoA ligase
	fun28289	1.43	LytR family transcriptional regulator, partial
	fun23670	1.43	HupB
	fun26027	1.42	IS6 family transposase, partial
	fun22599	1.42	heat shock protein HspR family protein
	fun37178	1.41	MULTISPECIES: DUF4168 domain-containing protein
	fun23850	1.41	hydrogenase large subunit
	fun45724	1.41	MULTISPECIES: resuscitation-promoting factor RpfB
	fun36403	1.40	MULTISPECIES: DUF2065 domain-containing protein
	fun52563	1.40	outer membrane biogenesis protein BamB
	fun32524	1.40	MULTISPECIES: alpha-2-macroglobulin
	fun23147	1.39	HflK protein, partial
	fun47629	1.39	MULTISPECIES: thiosulfohydrolase SoxB

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month	gene	$\log_2$Fold Change	function.
	fun8125	1.39	carbon-phosphorus lyase complex subunit PhnJ
	fun11667	1.39	cytochrome-c3 hydrogenase
	fun13683	1.39	DNA-directed RNA polymerase subunit beta", partial
	fun38289	1.38	MULTISPECIES: FkbM family methyltransferase
	fun18405	1.38	fe-s cluster assembly nifu-like protein
	fun47116	1.38	MULTISPECIES: SUF system Fe-S cluster assembly protein
	fun36389	1.37	MULTISPECIES: DUF2007 domain-containing protein
	fun34130	1.37	MULTISPECIES: cation transport regulator
	fun24241	1.37	ImpA family type VI secretion-associated protein
	fun35106	1.36	MULTISPECIES: cytochrome
	fun36418	1.36	MULTISPECIES: DUF2093 domain-containing protein
	fun42808	1.36	MULTISPECIES: nitrogen regulatory protein
	fun31625	1.36	MULTISPECIES: 30S ribosomal protein S5 alanine N-acetyltransferase
	fun62822	1.35	resuscitation-promoting factor-like protein
	fun56475	1.35	plasmid partitioning protein RepB
	fun70835	1.35	tungsten ABC transporter permease
	fun41988	1.35	MULTISPECIES: monooxygenase FAD-binding
	fun39564	1.34	MULTISPECIES: heat-shock protein HspB
	fun16650	1.34	DUF680 domain-containing protein
	fun35051	1.34	MULTISPECIES: cys-tRNA(pro)/cys-tRNA(cys) deacylase
	fun23064	1.33	heparin-binding hemagglutinin
	fun49296	1.33	MULTISPECIES: YHS domain-containing protein
	fun36829	1.32	MULTISPECIES: DUF3126 domain-containing protein
	fun2395	1.32	acetone carboxylase subunit beta
	fun48822	1.32	MULTISPECIES: ubiquinone/menaquinone biosynthesis C-methyltransferase UbiE
	fun63202	1.32	Ribose ABC transport system, permease protein RbsC
	fun55654	1.32	phosphonate C-P lyase system protein PhnK
	fun22627	1.32	heat-shock protein C2
	fun41658	1.32	MULTISPECIES: methionine gamma-lyase
	fun10049	1.31	coenzyme F420-reducing hydrogenase subunit beta
	fun10284	1.31	conjugal transfer protein TraI
	fun40780	1.31	MULTISPECIES: L-carnitine dehydratase
	fun13206	1.31	DNA ligase III
	fun67441	1.31	sulfonate ABC transporter substrate-binding protein
	fun28003	1.28	LPS ABC transporter substrate-binding protein LptA
	fun26256	1.28	ISAzo13 family transposase ISMfu1
	fun40928	1.28	MULTISPECIES: lectin
	fun24650	1.28	intracellular septation protein
	fun35077	1.28	MULTISPECIES: cysteine desulfuration protein SufE
	fun32003	1.27	MULTISPECIES: acetoacetate decarboxylase
	fun9049	1.27	Chaperonin Cpn60/TCP-1
	fun20999	1.27	glutamate synthase large subunit, partial
	fun7625	1.27	C4-type zinc finger protein, DksA/TraR family
	fun39963	1.27	MULTISPECIES: hydrogenase expression/formation protein HypE
	fun21616	1.27	glycoside hydrolase family 25
	fun42591	1.26	MULTISPECIES: NADPH–cytochrome P450 reductase
	fun38214	1.26	MULTISPECIES: ferric reductase
	fun56110	1.26	physarolisin II
	fun55663	1.26	phosphonate metabolism protein
	fun25425	1.25	IS256 family transposase, partial

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month	gene	$\log_2$Fold Change	function.
	fun70877	1.25	twin-arginine translocation (Tat)
	fun45123	1.25	MULTISPECIES: PTS sorbitol transporter subunit IIC
	fun37579	1.25	MULTISPECIES: DUF938 domain-containing protein
	fun15493	1.25	DUF3309 domain-containing protein, partial
	fun52781	1.25	oxalate decarboxylase
	fun42272	1.24	MULTISPECIES: N-formylglutamate amidohydrolase
	fun28867	1.24	mannosyl-glycoendo-beta-N-acetylglucosaminidase
	fun47832	1.24	MULTISPECIES: toluene hydroxylase
	fun11903	1.24	D-glucose O-methyltransferase
	fun39884	1.23	MULTISPECIES: Hsp33 family molecular chaperone
	fun27914	1.23	long-chain fatty acid–ACP ligase
	fun47408	1.22	MULTISPECIES: tautomerase
	fun37966	1.22	MULTISPECIES: exosortase system-associated protein, TIGR04073 family
	fun36307	1.21	MULTISPECIES: DUF1775 domain-containing protein
	fun36282	1.21	MULTISPECIES: DUF1674 domain-containing protein
	fun10318	1.21	conjugal transfer protein TrbI
	fun36657	1.20	MULTISPECIES: DUF2778 domain-containing protein
	fun53981	1.19	peptidase S8 and S53
	fun8284	1.19	caspase (peptidase)
	fun36700	1.19	MULTISPECIES: DUF2852 domain-containing protein
	fun34504	1.19	MULTISPECIES: chromosome partitioning protein ParA
	fun2393	1.19	acetone carboxylase subunit alpha
	fun22109	1.18	glyxoylase
	fun15385	1.18	DUF3147 domain-containing protein
	fun54350	1.18	periplasmic dipeptide transport substrate-binding protein
	fun51193	1.18	nitrate/nitrite ABC transporter substrate-binding protein
	fun32622	1.17	MULTISPECIES: amine oxidase
	fun44085	1.16	MULTISPECIES: phosphonate ABC transporter substrate-binding protein
	fun56996	1.16	polysaccharide pyruvyl transferase
	fun23738	1.15	hydantoin utilization protein C
	fun39841	1.15	MULTISPECIES: homospermidine synthase
	fun4305	1.15	aminodeoxychorismate component I
	fun51303	1.15	nitrite transporter NirC
	fun5468	1.15	assimilatory nitrite reductase large subunit
	fun45577	1.15	MULTISPECIES: recombinase family protein
	fun19564	1.15	fluoren-9-ol dehydrogenase
	fun45759	1.15	MULTISPECIES: rhodanese
	fun34494	1.14	MULTISPECIES: chromosomal replication initiator protein DnaA
	fun39560	1.14	MULTISPECIES: heat-shock protein Hsp20
	fun42874	1.14	MULTISPECIES: nodulation protein NodN
	fun8375	1.14	cation transport regulator
	fun66726	1.13	streptomycin biosynthesis protein
	fun23974	1.13	hydrophobic protein
	fun40556	1.13	MULTISPECIES: IS66 family transposase
	fun31234	1.13	MULTISPECIES: 2-dehydro-3-deoxy-phosphogluconate aldolase
	fun14818	1.13	DUF2285 domain-containing protein
	fun16271	1.13	DUF4392 domain-containing protein
	fun9085	1.12	chemoreceptor glutamine deamidase CheD
	fun67896	1.12	taurine catabolism dioxygenase TauD/TfdA
	fun14881	1.12	DUF2383 domain-containing protein

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month	gene	$\log_2$Fold Change	function.
	fun45971	1.12	MULTISPECIES: ring-cleavage extradiol dioxygenase
	fun36435	1.12	MULTISPECIES: DUF2155 domain-containing protein
	fun36229	1.11	MULTISPECIES: DUF1489 domain-containing protein
	fun64896	1.09	septicolysin
	fun51284	1.09	nitrite reductase (NAD(P)H)
	fun42848	1.09	MULTISPECIES: NmrA family transcriptional regulator
	fun22651	1.09	heat-shock protein SP21
	fun71902	1.08	type IV secretion protein DotU
	fun30327	1.08	molecular chaperone GroEL, partial
	fun26388	1.08	ISNCY family transposase ISRjo3
	fun64975	1.08	serine hydroxymethyltransferase, partial
	fun22633	1.07	heat-shock protein Hsp18
	fun66377	1.07	spore protein SP21
	fun65095	1.07	serine/threonine kinase
	fun33233	1.07	MULTISPECIES: ATP-dependent acyl-CoA ligase
	fun47777	1.07	MULTISPECIES: Tim44 domain-containing protein
	fun44616	1.06	MULTISPECIES: potassium-transporting ATPase subunit C
	fun10937	1.06	cycloisomerase
	fun5681	1.06	ATP-dependent acyl-CoA ligase
	fun39727	1.06	MULTISPECIES: HflK protein
	fun31143	1.06	MULTISPECIES: (S)-ureidoglycine aminohydrolase
	fun51618	1.05	Non-motile and phage-resistance protein
	fun59122	1.05	putative 3-carboxymuconate cyclase
	fun65043	1.05	serine protease spb1
	fun68508	1.05	thiol oxidoreductase-like
	fun36517	1.04	MULTISPECIES: DUF2312 domain-containing protein
	fun5963	1.04	ATPase Mrp homolog
	fun34054	1.04	MULTISPECIES: carboxylesterase family protein
	fun51334	1.04	nitrogen fixation protein FixK
	fun21672	1.04	glycoside hydrolase family 9
	fun67987	1.04	Tellurite resistance protein TerB
	fun4500	1.04	ammonium transporter, partial
	fun6004	1.04	atrA protein
	fun8292	1.03	caspase-like domain-containing protein
	fun15060	1.03	DUF2735 domain-containing protein
	fun64082	1.03	S-(hydroxymethyl)glutathione dehydrogenase, partial
	fun48978	1.02	MULTISPECIES: urea ABC transporter substrate-binding protein
	fun60451	1.02	putative naringenin-chalcone synthase
	fun44924	1.02	MULTISPECIES: protein translocase subunit SecA
	fun53438	1.02	penicillin epimerase
	fun39859	1.01	MULTISPECIES: HoxN/HupN/NixA family nickel/cobalt transporter
	fun41862	1.00	MULTISPECIES: modulator protein
	fun48835	1.00	MULTISPECIES: UDP pyrophosphate synthase
	fun20187	1.00	galactose oxidase, partial
	fun72587	1.00	Uma1 protein
	fun45453	0.99	MULTISPECIES: pyruvate carboxyltransferase
	fun4498	0.99	ammonium transporter
	fun68890	0.99	TIGR03118 family protein
	fun47739	0.99	MULTISPECIES: TIGR03118 family protein
	fun32142	0.98	MULTISPECIES: acriflavine resistance protein B

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month	gene	$\log_2$Fold Change	function.
	fun14520	0.98	DUF1842 domain-containing protein
	fun3584	0.98	aliphatic sulfonate ABC transporter ATP-binding protein
	fun36223	0.97	MULTISPECIES: DUF1476 domain-containing protein
	fun44250	0.97	MULTISPECIES: photosystem reaction center subunit H
	fun39386	0.97	MULTISPECIES: glyoxalase/bleomycin resistance/dioxygenase family protein
	fun23604	0.97	HSP20 family protein
	fun40659	0.96	MULTISPECIES: J domain-containing protein
	fun68509	0.96	thiol oxidoreductase-like protein
	fun10323	0.96	conjugal transfer protein TrbL
	fun73031	0.96	vanillate O-demethylase oxygenase
	fun58190	0.96	protein of unknown function DUF140
	fun38927	0.95	MULTISPECIES: globin domain protein
	fun42787	0.95	MULTISPECIES: nitrite reductase large subunit
	fun15406	0.94	DUF3175 domain-containing protein
	fun52266	0.94	oligosaccharide deacetylase
	fun28634	0.94	malonate transporter
	fun67611	0.94	SurA N- domain family protein
	fun33914	0.93	MULTISPECIES: calcium-binding protein
	fun36414	0.93	MULTISPECIES: DUF2089 domain-containing protein
	fun36065	0.93	MULTISPECIES: DUF1007 domain-containing protein
	fun26820	0.93	Ku protein, partial
	fun64597	0.93	secretion protein HylD
	fun30314	0.93	molecular chaperone
	fun65472	0.92	sigma54 specific transcriptional regulator with PAS/PAC sensor, Fis family
	fun38980	0.92	MULTISPECIES: glucose dehydrogenase
	fun2397	0.92	acetone carboxylase subunit gamma
	fun20945	0.92	glutaconate CoA-transferase
	fun23889	0.92	hydrogenase, partial
	fun6524	0.92	beta-(1-6) glucans synthase
	fun50847	0.92	negative transcriptional regulator
	fun32764	0.91	MULTISPECIES: ammonium transporter
	fun48167	0.91	MULTISPECIES: trehalose-6-phosphate synthase
	fun62152	0.91	rare lipoprotein A
	fun33384	0.91	MULTISPECIES: B/F/G family RNA polymerase sigma-70 factor
	fun14143	0.91	DUF1236 domain-containing protein
	fun1604	0.91	50S ribosomal protein L11, partial
	fun34692	0.90	MULTISPECIES: cobaltochelatase subunit CobS
	fun13903	0.90	drug exporter-like protein of the RND superfamily
	fun13803	0.90	dockerin-like protein
	fun27082	0.90	L,D-transpeptidase, partial
	fun72734	0.89	uracil-DNA glycosylase-associated domain / uracil-DNA glycosylase family 6
	fun3999	0.89	alpha/beta fold family hydrolase
	fun36088	0.89	MULTISPECIES: DUF1097 domain-containing protein
	fun39846	0.89	MULTISPECIES: hopanoid biosynthesis associated radical SAM protein HpnH
	fun2923	0.88	acyl-coenzyme A–6-aminopenicillanic-acid-acyltransferase form
	fun22625	0.88	heat-shock protein A
	fun60593	0.88	putative Penicillin-binding protein 1A (PBP-1a)
	fun43638	0.88	MULTISPECIES: peptide-binding protein
	fun69121	0.87	toluene monooxygenase
	fun62529	0.87	repeat-containing regulatory protein

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month	gene	$\log_2$Fold Change	function.
	fun15667	0.87	DUF3568 domain-containing protein
	fun15650	0.87	DUF3551 domain-containing protein
	fun2474	0.87	acetyl-CoA carboxylase, partial
	fun58302	0.86	protein PhaF
	fun37864	0.86	MULTISPECIES: ethanolamine ammonia lyase-activating protein
	fun66840	0.86	substrate-binding protein
	fun31489	0.86	MULTISPECIES: 3-hydroxyacyl-ACP dehydratase
	fun9054	0.86	chaperonin GroL, partial
	fun9774	0.86	co-chaperone GroES
	fun39550	0.86	MULTISPECIES: heat-shock protein
	fun41872	0.85	MULTISPECIES: molecular chaperone GroEL, partial
	fun66544	0.85	ST7 protein
	fun23831	0.85	hydrogenase expression protein HypE
	fun62808	0.85	Resuscitation-promoting factor Rpf2
	fun16584	0.85	DUF5123 domain-containing protein
	fun48785	0.85	MULTISPECIES: UbiD family decarboxylase
	fun32272	0.85	MULTISPECIES: adenosine kinase
	fun9121	0.85	chemotaxis protein CheD
	fun36860	0.84	MULTISPECIES: DUF3185 domain-containing protein
	fun29535	0.84	methane monooxygenase
	fun14098	0.84	DUF1150 domain-containing protein
	fun20209	0.84	galactosylceramidase
	fun34351	0.84	MULTISPECIES: chaperone protein dnaK2
	fun94	0.83	1, 4-beta cellobiohydrolase
	fun9053	0.83	chaperonin GroL
	fun6767	0.83	bicarbonate-binding protein
	fun41212	0.83	MULTISPECIES: lysylphosphatidylglycerol synthetase family protein
	fun34061	0.83	MULTISPECIES: carboxymuconolactone decarboxylase
	fun73592	0.83	XdhC/CoxF family protein
	fun25133	0.83	IS1380 family transposase
	fun6147	0.83	BA14K family protein
	fun9783	0.82	co-factor of molecular chaperone
	fun9051	0.82	chaperonin GroEL
	fun37713	0.82	MULTISPECIES: EmrB/QacA family drug resistance transporter
	fun59597	0.82	putative contains C-terminal CBS domains
	fun70358	0.82	tripartite motif-containing protein 71
	fun37578	0.81	MULTISPECIES: DUF937 domain-containing protein
	fun34361	0.81	MULTISPECIES: chaperonin GroL
	fun30326	0.81	molecular chaperone GroEL
	fun50956	0.80	Ni,Fe-hydrogenase I small subunit
	fun30939	0.80	multi-copper enzyme maturation ABC transporter permease
	fun69120	0.80	toluene hydroxylase
	fun51294	0.80	nitrite reductase large subunit
	fun19421	0.80	flavin-nucleotide-binding protein
	fun74042	0.79	zinc-binding dehydrogenase family oxidoreductase
	fun40183	0.79	MULTISPECIES: integration host factor
	fun24605	0.79	intein-containing tRNA-splicing ligase RtcB
	fun4818	0.79	antigen
	fun20362	0.79	GcrA-like regulator
	fun51371	0.79	nitrogen regulatory protein P-II

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month	gene	$\log_2$Fold Change	function.
	fun66326	0.79	spore coat protein
	fun57165	0.78	potassium-transporting ATPase subunit A
	fun55679	0.78	phosphonate monoester hydrolase
	fun21630	0.78	glycoside hydrolase family 3 protein
	fun27243	0.78	large adhesin
	fun15415	0.78	DUF3185 domain-containing protein
	fun26817	0.77	ku family containing domain-containing protein
	fun46408	0.77	MULTISPECIES: septal ring lytic transglycosylase RlpA family lipoprotein
	fun8675	0.77	cell envelope biogenesis protein AsmA
	fun26901	0.77	L-asparagine permease
	fun14082	0.77	DUF1111 domain-containing protein
	fun63954	0.77	RpoD family RNA polymerase sigma factor
	fun51373	0.77	nitrogen regulatory protein P-II 1
	fun15162	0.77	DUF2865 domain-containing protein
	fun43869	0.77	MULTISPECIES: phenylacetate-CoA oxygenase subunit PaaB
	fun34360	0.77	MULTISPECIES: chaperonin GroEL
	fun48162	0.76	MULTISPECIES: trehalose synthase
	fun31365	0.76	MULTISPECIES: 2,3-bisphosphoglycerate-dependent phosphoglycerate mutase
	fun23611	0.76	Hsp33 family molecular chaperone
	fun22601	0.76	heat shock protein SP21
	fun28370	0.76	M50 family peptidase
	fun69793	0.76	transcriptional regulators, TraR/DksA family protein
	fun27958	0.76	low molecular weight heat shock protein
	fun41100	0.76	MULTISPECIES: low affinity iron permease family protein
	fun1849	0.75	AAA ATPase central domain protein
	fun67205	0.75	sugar phosphorylase
	fun37872	0.75	MULTISPECIES: ethanolamine utilization protein EutA
	fun31013	0.75	multidrug MFS transporter
	fun1186	0.75	30S ribosomal protein S2, partial
	fun21722	0.75	glycosyl hydrolase BNR repeat-containing protein
	fun23252	0.75	histamine oxidase
	fun65805	0.75	small-conductance mechanosensitive channel
	fun4491	0.75	ammonia channel protein
	fun26517	0.74	isoquinoline 1-oxidoreductase subunit alpha
	fun34991	0.74	MULTISPECIES: CusA/CzcA family heavy metal efflux RND transporter
	fun32785	0.74	MULTISPECIES: amylo-alpha-1,6-glucosidase
	fun6133	0.74	B/F/G family RNA polymerase sigma-70 factor
	fun13693	0.74	DNA-directed RNA polymerase subunit omega, partial
	fun2457	0.74	acetyl-CoA carboxylase carboxyltransferase subunit alpha/beta
	fun68818	0.74	TIGR01906 family membrane protein
	fun73382	0.74	WD-40 repeat protein, beta transducin-like protein
	fun3867	0.73	alpha-D-ribose 1-methylphosphonate 5-triphosphate diphosphatase
	fun51472	0.73	NmrA family transcriptional regulator
	fun23366	0.73	histone H1-like protein
	fun330	0.73	2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate hydrolase
	fun34614	0.73	MULTISPECIES: co-chaperone GroES
	fun22634	0.73	heat-shock protein Hsp20
	fun39359	0.73	MULTISPECIES: glycosyltransferase family 4 protein
	fun29015	0.73	MCP methyltransferase/methylesterase
	fun25373	0.72	IS21 family transposase, partial

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month	gene	$\log_2$Fold Change	function.
	fun28686	0.72	maltose ABC transporter substrate-binding protein MalE
	fun22646	0.72	heat-shock protein HspR
	fun37940	0.72	MULTISPECIES: exopolysaccharide biosynthesis protein
	fun62134	0.72	Raf kinase inhibitor-like protein, YbhB/YbcL family
	fun40214	0.72	MULTISPECIES: invasion associated locus B family protein
	fun61883	0.72	pyruvate kinase, partial
	fun42084	0.72	MULTISPECIES: multidrug MFS transporter
	fun42651	0.72	MULTISPECIES: neutral zinc metallopeptidase
	fun21490	0.72	glycine/sarcosine/betaine reductase complex selenoprotein A
	fun66418	0.72	sporulation protein SsgA
	fun30328	0.71	molecular chaperone GroES
	fun40643	0.71	MULTISPECIES: isoprenylcysteine carboxylmethyltransferase family protein
	fun44163	0.71	MULTISPECIES: phosphoribulokinase
	fun61602	0.71	pyridoxal kinase
	fun53612	0.71	peptidase C14, caspase catalytic subunit p20
	fun38949	0.71	MULTISPECIES: glucan export ABC transporter ATP-binding protein
	fun41871	0.71	MULTISPECIES: molecular chaperone GroEL
	fun4164	0.71	amino acid ABC transporter substrate-binding protein, partial
	fun46912	0.71	MULTISPECIES: squalene–hopene cyclase
	fun28991	0.70	MCD, Malonyl-CoA decarboxylase MCD
	fun23817	0.70	hydrogenase assembly protein HupF
	fun19443	0.70	flavodoxin protein
	fun8029	0.70	carbohydrate porin
	fun40328	0.70	MULTISPECIES: IS110 family transposase
	fun48403	0.70	MULTISPECIES: two-component response regulator
	fun32295	0.70	MULTISPECIES: adenylate/guanylate cyclase domain-containing protein
	fun400	0.70	2-keto-gluconate dehydrogenase
	fun8507	0.69	CDP-6-deoxy-delta-3,4-glucoseen reductase
	fun68880	0.69	TIGR03066 family protein
	fun38227	0.69	MULTISPECIES: ferritin-like domain-containing protein
	fun45017	0.69	MULTISPECIES: PrsW family intramembrane metalloprotease
	fun50955	0.69	Ni,Fe-hydrogenase I large subunit
	fun73530	0.68	xanthine dehydrogenase molybdenum-binding subunit XdhA
	fun47706	0.68	MULTISPECIES: TIGR02300 family protein
	fun44604	0.68	MULTISPECIES: potassium transporter Kup
	fun3487	0.68	aldehyde oxidase and xanthine dehydrogenase molybdopterin binding
	fun73614	0.68	XRE family transcriptional regulator, partial
	fun45616	0.68	MULTISPECIES: reductive dehalogenase
	fun61336	0.68	putative tRNA-dihydrouridine synthase
	fun5602	0.67	ATP synthase subunit alpha, partial
	fun227	0.67	2-acyl-glycerophospho-ethanolamine acyltransferase
	fun17465	0.67	ErfK/YbiS/YcfS/YnhG
	fun63292	0.67	ribosomal small subunit Rsm22
	fun38608	0.67	MULTISPECIES: formylglycine-generating enzyme family protein
	fun53208	0.67	PAS
	fun39873	0.67	MULTISPECIES: HrcA family transcriptional regulator
	fun46203	0.67	MULTISPECIES: S-adenosylmethionine synthase
	fun37144	0.67	MULTISPECIES: DUF4112 domain-containing protein
	fun7749	0.67	calmodulin
	fun53012	0.67	P-II family nitrogen regulator

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month	gene	$\log_2$Fold Change	function.
	fun15960	0.67	DUF4118 domain-containing protein
	fun10859	0.66	cyclic 2,3-phosphoglycerate synthetase
	fun41084	0.66	MULTISPECIES: Lon protease
	fun29546	0.66	methane/phenol/toluene hydroxylase
	fun14718	0.66	DUF2171 domain-containing protein
	fun62344	0.66	reductive dehalogenase
	fun17007	0.66	electron transfer flavoprotein
	fun23607	0.66	Hsp20/alpha crystallin family protein
	fun37599	0.66	MULTISPECIES: DUF992 domain-containing protein
	fun49400	0.66	MULTISPECIES: zinc-binding dehydrogenase
	fun57788	0.66	protein archease
	fun3430	0.66	Alcohol dehydrogenase zinc-binding domain protein
	fun72222	0.66	UBA/THIF-type NAD/FAD-binding fold protein
	fun28443	0.66	maf protein
	fun73774	0.66	YebC/PmpR family DNA-binding transcriptional regulator, partial
	fun17497	0.65	erythromycin esterase-like enzyme
	fun12828	0.65	dinucleotide-binding protein
	fun37231	0.65	MULTISPECIES: DUF4242 domain-containing protein
	fun25375	0.65	IS256 family transposase
	fun59052	0.65	PUR-alpha/beta/gamma DNA/RNA-binding
	fun43249	0.65	MULTISPECIES: oxalate/formate MFS antiporter
	fun37654	0.65	MULTISPECIES: efflux transporter periplasmic adaptor subunit
	fun36140	0.64	MULTISPECIES: DUF1259 domain-containing protein
	fun62923	0.64	rhodanese
	fun42931	0.64	MULTISPECIES: nuclear transport factor 2 family protein
	fun33090	0.64	MULTISPECIES: arylsulfatase
	fun63527	0.64	RNA degradosome polyphosphate kinase, partial
	fun36445	0.64	MULTISPECIES: DUF2171 domain-containing protein
	fun71197	0.64	type I 3-dehydroquinate dehydratase
	fun8003	0.64	carbohydrate binding:glycosyltransferase 36:glycosyltransferase 36 associated protein
	fun51143	0.63	nitrate ABC transporter, permease protein
	fun8174	0.63	carboxylesterase family protein
	fun47301	0.63	MULTISPECIES: superoxide dismutase family protein
	fun57257	0.63	PRC-barrel domain-containing protein
	fun25058	0.63	IS110 family transposase, partial
	fun46355	0.63	MULTISPECIES: selenium-binding protein
	fun3313	0.63	AfsR/SARP family transcriptional regulator, partial
	fun30322	0.63	molecular chaperone DnaK
	fun41619	0.63	MULTISPECIES: metalloprotease
	fun26682	0.63	Kelch repeat type 1-containing protein
	fun65614	0.63	single-strand-binding protein
	fun9610	0.63	class D sortase
	fun24938	0.63	iron-sulfur containing oxygenase
	fun17573	0.62	ESX secretion-associated protein EspG
	fun3848	0.62	alpha-amylase, partial
	fun14655	0.62	DUF2076 domain-containing protein
	fun73824	0.62	YHS domain-containing protein
	fun30308	0.62	MoeZ/MoeB
	fun51023	0.62	nicotinamidase-like amidase
	fun18646	0.62	ferric reductase

Table D.4 continued from previous page

month	gene	$\log_2$Fold Change	function.
	fun23588	0.62	Hrp-dependent type III effector protein
	fun18425	0.62	Fe-S containing oxidoreductase
	fun73449	0.62	X-Pro dipeptidase
	fun50754	0.62	naringenin-chalcone synthase
	fun34641	0.62	MULTISPECIES: cob(I)yrinic acid a,c-diamide adenosyltransferase
	fun62392	0.62	regulator protein
	fun62807	0.62	resuscitation-promoting factor
	fun14362	0.62	DUF1579 domain-containing protein
	fun2895	0.62	acyl-CoA thioester hydrolase
	fun65918	0.62	SnoaL-like polyketide cyclase
	fun67976	0.61	tellerium resistance protein
	fun11012	0.61	cysteine biosynthesis protein CysZ
	fun8772	0.61	cell wall biosynthesis glycosyltransferase
	fun38039	0.61	MULTISPECIES: FAD-binding protein
	fun4229	0.61	amino acid regulated cytosolic protein
	fun37256	0.61	MULTISPECIES: DUF427 domain-containing protein
	fun18234	0.61	family 3 glycosyl hydrolase
	fun184	0.61	16S rRNA (guanine(527)-N(7))-methyltransferase
	fun68999	0.61	TlpA-like protein
	fun68881	0.60	TIGR03067 domain-containing protein
	fun4708	0.60	anti-anti-sigma regulatory factor
	fun36471	0.60	MULTISPECIES: DUF2231 domain-containing protein
	fun39955	0.60	MULTISPECIES: hydrogenase expression protein HypE
	fun10249	0.60	condensation domain protein
	fun23581	0.60	HrcA family transcriptional regulator
	fun11646	0.60	cytochrome P460
	fun42730	0.60	MULTISPECIES: NIPSNAP family protein
	fun20493	0.60	general stress protein CsbD
	fun37756	0.60	MULTISPECIES: endopeptidase La
	fun30652	0.60	monogalactosyldiacylglycerol synthase
	fun66669	0.60	sterol carrier protein
	fun3919	0.59	alpha-ketoglutarate decarboxylase, partial
	fun40303	0.59	MULTISPECIES: iron-sulfur cluster assembly scaffold protein NifU
	fun20659	0.59	gliding motility-associated lipoprotein GldK
	fun57161	0.59	potassium-transporting ATPase A chain
	fun48383	0.59	MULTISPECIES: twin-arginine translocase TatA/TatE family subunit
	fun40490	0.59	MULTISPECIES: IS481 family transposase
	fun60744	0.59	putative PhoU
	fun16275	0.59	DUF4394 domain-containing protein
	fun5262	0.59	aryl-sulfate sulfohydrolase
	fun29734	0.59	methylamine utilization protein MauG
	fun5931	0.59	ATPase AAA, partial
	fun15656	0.59	DUF3558 domain-containing protein
	fun27951	0.59	low affinity iron permease family protein
	fun34480	0.59	MULTISPECIES: CHRD domain-containing protein
	fun4933	0.59	apple domain-containing protein
	fun14208	0.59	DUF1328 domain-containing protein
	fun63756	0.58	RNA polymerase, sigma 70 subunit, RpoD subfamily
	fun30337	0.58	molecular chaperone Hsp20
	fun56109	0.58	Phyllosphere-induced regulator PhyR

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month	gene	$\log_2$Fold Change	function.
	fun1283	0.58	4-diphosphocytidyl-2C-methyl-D-erythritol kinase
	fun11723	0.58	cytosolic protein
	fun1636	0.58	50S ribosomal protein L25, partial
	fun37902	0.58	MULTISPECIES: exo-alpha-sialidase
	fun14762	0.58	DUF2231 domain-containing protein
	fun10760	0.58	cupin, partial
	fun34163	0.58	MULTISPECIES: CBS domain-containing protein
	fun23522	0.58	hopanoid biosynthesis-associated RND transporter HpnN
	fun2159	0.57	ABC-type dipeptide transport system, periplasmic component
	fun49771	0.57	N-acetyltransferase GCN5
	fun37562	0.57	MULTISPECIES: DUF899 domain-containing protein
	fun17358	0.57	enoyl-CoA hydratase/isomerase
	fun36901	0.57	MULTISPECIES: DUF3309 domain-containing protein
	fun70891	0.56	Twin-arginine translocation pathway signal sequence domain-containing protein
	fun59892	0.56	putative FMN-dependent luciferase-like monooxygenase
	fun61620	0.56	pyridoxal-5-phosphate-dependent protein subunit beta
	fun30227	0.56	MoaD family protein
	fun58486	0.56	protein tyrosine/serine phosphatase
	fun109	0.56	1,3-1,4-beta-glycanase
	fun46787	0.56	MULTISPECIES: SOS response-associated peptidase
	fun14266	0.56	DUF1440 domain-containing protein
	fun26626	0.56	KaiC 1, partial
	fun71405	0.56	type IA DNA topoisomerase
	fun15084	0.55	DUF2778 domain-containing protein
	fun70386	0.55	tripeptidyl aminopeptidase
	fun20734	0.55	glucan export ABC transporter ATP-binding protein
	fun28551	0.55	major pilin protein FimA
	fun18417	0.55	Fe-S cluster assembly scaffold protein NifU
	fun26033	0.55	IS630 family transposase
	fun44155	0.55	MULTISPECIES: phosphoribosylformylglycinamidine synthase subunit PurS
	fun51614	0.55	non-homologous end joining protein Ku
	fun51894	0.55	nucleotide exchange factor GrpE
	fun59036	0.55	PUCC protein
	fun41986	0.54	MULTISPECIES: monooxygenase
	fun4835	0.54	antirepressor regulating drug resistance protein
	fun61660	0.54	pyridoxamine 5'-phosphate oxidase FMN–binding protein
	fun71025	0.54	two-component system response regulator protein-glutamate methylesterase
	fun14616	0.54	DUF2000 domain-containing protein
	fun34244	0.54	MULTISPECIES: cell envelope biogenesis protein OmpA
	fun58941	0.54	PTS N-acetyl-D-glucosamine transporter
	fun18145	0.53	FAD-binding molybdopterin dehydrogenase
	fun37778	0.53	MULTISPECIES: enolase
	fun8468	0.53	CBS domain-containing protein
	fun40850	0.53	MULTISPECIES: L,D-transpeptidase
	fun15492	0.53	DUF3309 domain-containing protein
	fun15263	0.53	DUF3008 domain-containing protein
	fun44162	0.53	MULTISPECIES: phosphoribosyltransferase
	fun55753	0.53	phosphoribosyl transferase
	fun17978	0.53	ExsB family transcriptional regulator
	fun1679	0.53	50S ribosomal protein L7/L12, partial

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month	gene	$\log_2$Fold Change	function.
	fun6503	0.53	beta and gamma crystallin
	fun69105	0.53	TolQ protein
	fun41867	0.53	MULTISPECIES: molecular chaperone DnaJ
	fun21447	0.53	glycine reductase complex selenoprotein B
	fun43538	0.52	MULTISPECIES: peptidase M48 Ste24p
	fun51451	0.52	NLP/P60 protein
	fun66765	0.52	stress-induced bacterial acidophilic repeat motif family protein
	fun21790	0.52	glycosyl hydrolase family 57
	fun74039	0.52	zinc-binding dehydrogenase
	fun15232	0.51	DUF2959 domain-containing protein
	fun26695	0.51	ketoacyl reductase
	fun53963	0.51	peptidase S53
	fun22649	0.51	heat-shock protein IbpA
	fun10801	0.51	cutinase family protein
	fun34536	0.51	MULTISPECIES: citrate synthase/methylcitrate synthase
	fun10857	0.51	cyclase/dehydrase
	fun69776	0.51	transcriptional regulator, TraR/DksA family
	fun21759	0.51	glycosyl hydrolase family 3
	fun56526	0.51	plastocyanin
	fun4538	0.51	amylo-alpha-1,6-glucosidase
	fun72322	0.51	ubiquitin–protein ligase
	fun39881	0.51	MULTISPECIES: Hsp20/alpha crystallin family protein
	fun63708	0.51	RNA polymerase sigma-H factor
	fun21107	0.50	glutamine synthetase type III
	fun17708	0.50	exo-alpha-sialidase
	fun53318	0.50	PBP family phospholipid-binding protein
	fun55594	0.50	phospholipid biosynthesis protein
	fun38485	0.50	MULTISPECIES: flavin-dependent oxidoreductase
	fun46014	0.50	MULTISPECIES: RNA polymerase sigma factor
	fun55438	0.50	phosphoesterase ICC
	fun5686	0.50	ATP-dependent chaperone ClpB
	fun7374	0.50	BON domain-containing protein
	fun7926	0.49	carbamoyl phosphate synthase
	fun53831	0.49	peptidase M52
	fun16806	0.49	DUF982 domain-containing protein
	fun40752	0.49	MULTISPECIES: Ku protein
	fun55613	0.49	phospholipid/glycerol acyltransferase
	fun30187	0.49	MMPL domain protein
	fun41873	0.49	MULTISPECIES: molecular chaperone GroES
	fun41868	0.49	MULTISPECIES: molecular chaperone DnaK
	fun66608	0.49	stage V sporulation protein S
	fun18691	0.49	ferritin-like domain-containing protein
	fun43639	0.49	MULTISPECIES: peptide-methionine (R)-S-oxide reductase
	fun33162	0.49	MULTISPECIES: ATP F0F1 synthase subunit C
	fun5704	0.49	ATP-dependent Clp protease ATP-binding protein
	fun15746	0.49	DUF3754 domain-containing protein
	fun55310	0.48	phosphatidylinositol alpha-mannosyltransferase
	fun52375	0.48	organic hydroperoxide resistance protein
	fun21679	0.48	glycoside hydrolase family protein
	fun14155	0.48	DUF1259 domain-containing protein

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month	gene	$\log_2$Fold Change	function.
	fun62142	0.48	raiA ribosome-associated inhibitor A
	fun8143	0.48	carboxy-terminal-processing protease
	fun68873	0.48	TIGR02996 domain-containing protein
	fun42739	0.48	MULTISPECIES: nitrate reductase
	fun51824	0.48	nucleoside 2-deoxyribosyltransferase
	fun9820	0.48	CoA-substrate-specific enzyme activase
	fun73594	0.48	XdhC/CoxI family protein
	fun33235	0.48	MULTISPECIES: ATP-dependent chaperone ClpB
	fun52928	0.48	oxidoreductase, partial
	fun41836	0.48	MULTISPECIES: MMPL domain-containing protein
	fun24487	0.48	integral membrane protein TerC
	fun38491	0.48	MULTISPECIES: flavodoxin
	fun16825	0.48	dynamin
	fun17014	0.47	Electron transfer flavoprotein alpha/beta-subunit
	fun64080	0.47	S-(hydroxymethyl)glutathione dehydrogenase
	fun14950	0.47	DUF2516 domain-containing protein
	fun64883	0.47	septal ring lytic transglycosylase RlpA family lipoprotein
	fun9706	0.47	Clp protease ATP-binding protein
	fun5263	0.47	aryl-sulfate sulfotransferase
	fun61764	0.47	pyrroloquinoline quinone biosynthesis protein B
	fun2670	0.47	ACP S-malonyltransferase
	fun12928	0.47	dipeptidyl-peptidase III
	fun56066	0.47	phthalate 4,5-dioxygenase
	fun8612	0.47	cell division protein FtsH, partial
	fun33104	0.47	MULTISPECIES: Asp-tRNA(Asn)/Glu-tRNA(Gln) amidotransferase GatCAB subunit C
	fun881	0.47	3-hydroxyacyl-ACP dehydratase
	fun24388	0.47	inosine-5-monophosphate dehydrogenase
	fun67554	0.47	superfamily I DNA/RNA helicase
	fun4729	0.47	anti-sigma B factor RsbW
	fun61768	0.47	pyrroloquinoline quinone biosynthesis protein PqqB
	fun64928	0.47	Ser-tRNA(Ala) deacylase AlaX
	fun53845	0.47	peptidase M6 immune inhibitor A
	fun30948	0.47	multi-sensor hybrid histidine kinase
	fun56053	0.47	photosystem reaction center subunit H
	fun16315	0.47	DUF4430 domain-containing protein
	fun24686	0.46	invasion-associated locus B family protein
	fun67623	0.46	surface antigen
	fun21092	0.46	glutamine synthetase
	fun23057	0.46	heparan N-sulfatase
	fun23410	0.46	HlyD family secretion protein
	fun16022	0.46	DUF4169 domain-containing protein
	fun30188	0.46	MMPL domain-containing protein
	fun47547	0.46	MULTISPECIES: thiamine-phosphate kinase
	fun58211	0.46	protein of unknown function DUF1745
	fun33637	0.46	MULTISPECIES: bifunctional folylpolyglutamate synthase/dihydrofolate synthase
	fun27338	0.46	lectin
	fun33122	0.46	MULTISPECIES: aspartate aminotransferase
	fun14146	0.45	DUF1244 domain-containing protein
	fun36103	0.45	MULTISPECIES: DUF1150 domain-containing protein
	fun2118	0.45	ABC-1 domain protein

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month	gene	$\log_2$Fold Change	function.
	fun8525	0.45	CDP-diacylglycerol–inositol 3-phosphatidyltransferase
	fun63537	0.45	RNA ligase RtcB family protein
	fun15186	0.45	DUF2892 domain-containing protein
	fun10812	0.45	cyanase
	fun62845	0.45	reverse transcriptase
	fun9897	0.45	cobalamin biosynthesis protein CobW
	fun9704	0.45	Clp protease
	fun25739	0.44	IS4 family transposase, partial
	fun14677	0.44	DUF2125 domain-containing protein
	fun39490	0.44	MULTISPECIES: GYD domain-containing protein
	fun39378	0.44	MULTISPECIES: glyoxalase
	fun18877	0.44	fimbrial assembly protein PilM
	fun12265	0.44	delta fatty acid desaturase
	fun50304	0.44	NADH (or F420H2) dehydrogenase subunit C
	fun68874	0.44	TIGR03000 domain-containing protein
	fun26819	0.44	Ku protein
	fun30346	0.44	molecular chaperone HtpG
	fun36169	0.44	MULTISPECIES: DUF1328 domain-containing protein
	fun42978	0.44	MULTISPECIES: nucleotide exchange factor GrpE
	fun30486	0.44	molybdopterin binding aldehyde oxidase and xanthine dehydrogenase
	fun52340	0.44	OpgC domain-containing protein
	fun16674	0.44	DUF748 domain-containing protein
	fun12120	0.44	deacylase
	fun10334	0.43	conjugative relaxase
	fun17495	0.43	erythromycin esterase
	fun57661	0.43	propionyl-CoA carboxylase subunit beta
	fun18011	0.43	extracellular solute-binding protein
	fun15586	0.43	DUF3455 domain-containing protein
	fun34031	0.43	MULTISPECIES: carbon-monoxide dehydrogenase large subunit
	fun51762	0.43	nuclear transport factor 2 family protein
	fun63578	0.43	RNA polymerase major sigma-43 factor (sigma-A)
	fun16813	0.43	DUF992 domain-containing protein
	fun67888	0.42	taurine catabolism dioxygenase
	fun36203	0.42	MULTISPECIES: DUF1428 domain-containing protein
	fun26519	0.42	isoquinoline 1-oxidoreductase subunit beta
	fun10715	0.42	CTP synthetase, partial
	fun19724	0.42	formate dehydrogenase-N subunit alpha
	fun21737	0.42	glycosyl hydrolase family 15
	fun73267	0.42	VOC family protein
	fun28973	0.42	MBL fold metallo-hydrolase, partial
	fun23836	0.42	hydrogenase expression/formation protein HypE
	fun6239	0.42	bacteriocin
	fun60334	0.42	putative methyltransferase
	fun48061	0.42	MULTISPECIES: transglycosylase
	fun22055	0.42	glycosyltransferase/methyltransferase
	fun30320	0.41	molecular chaperone DnaJ
	fun14830	0.41	DUF2304 domain-containing protein
	fun16242	0.41	DUF4365 domain-containing protein
	fun14623	0.41	DUF2017 domain-containing protein
	fun52787	0.41	oxalate/formate MFS antiporter

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month	gene	$\log_2$Fold Change	function.
	fun63362	0.41	ribulose 1,5-bisphosphate carboxylase
	fun70241	0.41	trehalose synthase
	fun2944	0.41	acylaldehyde oxidase
	fun6486	0.41	benzoylformate decarboxylase
	fun48539	0.41	MULTISPECIES: type II citrate synthase
	fun57008	0.41	polysaccharide transporter
	fun53484	0.41	penicillin-binding protein, partial
	fun16326	0.41	DUF4437 domain-containing protein
	fun21877	0.41	glycosyl transferase family 36
	fun14484	0.41	DUF1802 domain-containing protein
	fun30321	0.41	molecular chaperone DnaJ, partial
	fun22002	0.41	glycosyltransferase family 4 protein
	fun13676	0.41	DNA-directed RNA polymerase subunit alpha, partial
	fun5687	0.40	ATP-dependent chaperone ClpB, partial
	fun71207	0.40	type I glutamate–ammonia ligase
	fun56512	0.40	plasmid stabilization protein
	fun641	0.40	2,4-dichlorophenoxyacetate dioxygenase
	fun55154	0.40	phosoglucose isomerase
	fun18154	0.40	FAD-binding oxidoreductase, partial
	fun2809	0.40	acyl-CoA dehydrogenase domain-containing protein
	fun8108	0.40	carbon-monoxide dehydrogenase large subunit
	fun16283	0.40	DUF4398 domain-containing protein
	fun41105	0.39	MULTISPECIES: low specificity L-threonine aldolase
	fun30297	0.39	modulator protein
	fun68780	0.39	TIGR00296 family protein
	fun1417	0.39	4-phytase
	fun15221	0.39	DUF2948 domain-containing protein
	fun21834	0.39	glycosyl hydrolase, partial
	fun26555	0.39	J domain-containing protein
	fun63571	0.39	RNA polymerase factor sigma-32
	fun16369	0.39	DUF459 domain-containing protein
	fun16561	0.39	DUF5069 domain-containing protein
	fun64085	0.39	S-(hydroxymethyl)glutathione synthase
	fun14259	0.39	DUF1428 domain-containing protein
	fun57172	0.38	potassium-transporting ATPase subunit KdpA
	fun30987	0.38	multidrug efflux protein
	fun26619	0.38	KaiB 1
	fun5871	0.38	ATP-dependent zinc metalloprotease FtsH
	fun41761	0.38	MULTISPECIES: MexE family multidrug efflux RND transporter periplasmic adaptor subunit
	fun17588	0.38	ethanolamine ammonia lyase-activating protein
	fun65188	0.38	SGNH hydrolase
	fun68840	0.38	TIGR02302 family protein
	fun3493	0.38	aldehyde oxidase and xanthine dehydrogenase molybdopterin-binding protein
	fun7725	0.38	calcium-binding protein
	fun6763	0.38	bi-functional transferase/deacetylase
	fun27959	0.38	low molecular weight phosphatase family protein
	fun15513	0.38	DUF3341 domain-containing protein
	fun38645	0.38	MULTISPECIES: fructose-bisphosphatase class II
	fun19457	0.38	flavohemoprotein
	fun4516	0.38	AMP-dependent acyl-CoA synthetase

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month	gene	$\log_2$Fold Change	function.
	fun49116	0.38	MULTISPECIES: VOC family protein
	fun51919	0.38	nucleotide-binding protein
	fun57167	0.38	potassium-transporting ATPase subunit B
	fun73029	0.38	vanillate O-demethylase oxidoreductase VanB
	fun29932	0.38	MexE family multidrug efflux RND transporter periplasmic adaptor subunit
	fun18155	0.38	FAD-binding protein
	fun14732	0.37	DUF2188 domain-containing protein
	fun16744	0.37	DUF899 domain-containing protein
	fun9988	0.37	cobaltochelatase subunit CobS
	fun18875	0.37	fimbrial assembly protein FimA
	fun19985	0.37	fumarate hydrolyase
	fun18960	0.37	FKBP-type peptidyl-prolyl cis-trans isomerase
	fun2283	0.37	acetamidase/formamidase family protein
	fun64108	0.37	S-adenosylmethionine decarboxylase proenzyme
	fun13484	0.37	DNA starvation/stationary phase protection protein Dps
	fun18231	0.37	family 3 adenylate cyclase
	fun787	0.36	3-alpha-hydroxysteroid dehydrogenase
	fun54987	0.36	phenoxybenzoate dioxygenase
	fun65479	0.36	signal peptidase
	fun73541	0.36	xanthine dehydrogenase subunit D
	fun2346	0.36	acetoin:2,6-dichlorophenolindophenol oxidoreductase subunit alpha
	fun5281	0.36	arylsulfatase
	fun15091	0.36	DUF2784 domain-containing protein
	fun21651	0.36	glycoside hydrolase family 5
	fun32683	0.36	MULTISPECIES: aminoacylase
	fun14120	0.36	DUF1194 domain-containing protein
	fun10865	0.36	cyclic beta-1,2-glucan ABC transporter
	fun52200	0.36	oligoendopeptidase
	fun44175	0.36	MULTISPECIES: phosphoserine transaminase
	fun1705	0.36	6-carboxyhexanoate–CoA ligase
	fun32411	0.36	MULTISPECIES: aldehyde-activating protein
	fun18956	0.35	FkbM family methyltransferase
	fun10852	0.35	cyclase
	fun9376	0.35	CHRD domain-containing protein
	fun14346	0.35	DUF1559 domain-containing protein
	fun26760	0.35	kinase inhibitor
	fun1023	0.35	3-oxoacid CoA-transferase
	fun65126	0.35	serine/threonine protein kinase-related protein
	fun55185	0.35	phosphate ABC transporter substrate-binding protein PstS
	fun18036	0.35	extradiol ring-cleavage dioxygenase
	fun15304	0.35	DUF3048 domain-containing protein
	fun5683	0.35	ATP-dependent carboxylate-amine ligase
	fun24657	0.35	intradiol ring-cleavage dioxygenase
	fun55825	0.35	phosphoribosyltransferase
	fun19574	0.35	FmdB family transcriptional regulator
	fun59415	0.35	putative ATPase
	fun12736	0.35	dihydrouridine synthase
	fun18243	0.34	family 5 extracellular solute-binding protein
	fun23843	0.34	hydrogenase formation protein HypD
	fun14526	0.34	DUF1849 domain-containing protein

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month	gene	$\log_2$Fold Change	function.
	fun69943	0.34	translation initiation factor IF-2, partial
	fun33386	0.34	MULTISPECIES: B12-binding domain-containing radical SAM protein
	fun22072	0.34	glyoxalase
	fun13196	0.34	DNA ligase D
	fun28666	0.34	malto-oligosyltrehalose trehalohydrolase
	fun28295	0.34	LytTR family transcriptional regulator
	fun13501	0.34	DNA topoisomerase I
	fun31767	0.34	MULTISPECIES: 5-oxoprolinase
	fun55520	0.34	phosphoglycolate phosphatase
	fun24062	0.33	hydroxyquinol 1,2-dioxygenase
	fun62059	0.33	quinonprotein alcohol dehydrogenase
	fun14192	0.33	DUF1304 domain-containing protein
	fun26728	0.33	ketosteroid isomerase
	fun53268	0.33	PAS/PAC sensor signal transduction histidine kinase
	fun18174	0.33	FAD-dependent oxidoreductase, partial
	fun53661	0.33	peptidase E

Table D.5: Variation in the expression of soil microbial genes between years (2017 vs. 2016), based on RefSeq eukaryotic functional annotation (DESeq2, adjusted $P < 0.05$).

year	gene	$\log_2$Fold Change	function.
	fun16215	3.58	hypothetical protein AOL_s00007g9
	fun14532	2.90	hevamine-A
	fun10934	2.81	dienelactone hydrolase family
	fun27163	2.62	hypothetical protein CRE_05421
	fun18838	2.45	hypothetical protein AOL_s00080g432
	fun33691	2.29	hypothetical protein LOAG_16048, partial
	fun51914	2.14	UcrQ
	fun10398	1.62	cytochrome b-c1 complex subunit 2
	fun785	1.62	acid phosphatase 1 precursor
	fun26957	1.61	hypothetical protein CRE_04457
	fun45644	1.53	protein RARE-COLD-INDUCIBLE 2A-like
	fun1445	1.49	amino acid permease 1
	fun14919	1.31	Hsp90 binding co-chaperone Sba1
	fun45898	1.25	proton myo-inositol cotransporter
	fun18659	1.18	hypothetical protein AOL_s00080g127
	fun6337	1.14	CHAP domain-containing protein
	fun29005	1.10	hypothetical protein CRE_17186
	fun5468	1.08	C6 transcription factor RosA-like
	fun34195	1.07	hypothetical protein LOAG_18340
	fun786	1.06	acid phosphatase 1-like precursor
	fun4	1.06	(2R)-phospho-3-sulfolactate synthase, ComA
	fun2636	1.06	BolA-like protein 3
	fun9240	1.04	CRE-RPS-15 protein
	fun39193	1.03	hypothetical protein VFPFJ_10816

Table D.5 continued from previous page

year	gene	$\log_2$Fold Change	function.
	fun14077	0.96	GrpE protein homolog, mitochondrial precursor
	fun53277	0.96	universal stress family protein
	fun10946	0.96	dihydrodipicolinate synthetase family protein
	fun10413	0.96	cytochrome b5 reductase
	fun24292	0.95	Hypothetical protein CBG12121
	fun15174	0.92	hypothetical protein AOL_s00004g240
	fun49905	0.90	sugar transporter protein 12
	fun42435	0.89	NIPSNAP family protein
	fun1275	0.87	alpha beta hydrolase fold protein
	fun21338	0.87	hypothetical protein AOL_s00215g103
	fun33374	0.86	hypothetical protein LOAG_12436
	fun1520	0.85	ammonium transporter 2
	fun29093	0.84	hypothetical protein CRE_17801
	fun42862	0.84	O-methyltransferase, family 3
	fun10966	0.84	dihydroorotate dehydrogenase 2 domain protein
	fun575	0.83	7alpha-cephem-methoxylase P8 chain related protein
	fun31255	0.83	hypothetical protein CRE_30972
	fun21575	0.81	hypothetical protein AOL_s00215g4
	fun16598	0.79	hypothetical protein AOL_s00043g68
	fun22154	0.77	hypothetical protein Bm1_25520
	fun13371	0.76	generic methyltransferase
	fun16206	0.75	hypothetical protein AOL_s00007g6
	fun26776	0.74	hypothetical protein CRE_03593
	fun33170	0.73	hypothetical protein LOAG_10620
	fun52658	0.72	Uncharacterized protein CELE_R05D8.7
	fun47110	0.72	pyruvate synthase
	fun42470	0.72	NmrA family protein
	fun27205	0.70	hypothetical protein CRE_05701
	fun32838	0.70	hypothetical protein LOAG_08353
	fun30026	0.70	hypothetical protein CRE_24044
	fun43271	0.69	PDZ/DHR/GLGF domain protein
	fun27872	0.69	hypothetical protein CRE_10272
	fun2067	0.69	ATP synthase protein 9 precursor (Lipid-binding protein)
	fun41371	0.68	mitochondrial cytochrome
	fun1758	0.68	aquaporin-2
	fun23320	0.67	Hypothetical protein CBG05718
	fun51176	0.67	trehalose synthase (Ccg-9)
	fun14359	0.67	heat shock protein
	fun41626	0.66	Molybdopterin synthase catalytic subunit
	fun16627	0.66	hypothetical protein AOL_s00043g715
	fun12131	0.66	epoxide hydrolase 1
	fun43257	0.65	PDZ domain and Leucine-rich repeat and Leucine-rich repeat, typical subtype and Leucine rich repeat 4-containing protein
	fun43670	0.65	phenylalanine ammonia-lyase
	fun5786	0.65	carbonic anhydrase
	fun39743	0.65	Isochorismatase-like domain-containing protein
	fun20276	0.65	hypothetical protein AOL_s00112g95
	fun2976	0.64	C. briggsae CBR-ARS-1 protein
	fun44056	0.64	pirin domain-containingprotein
	fun16425	0.64	hypothetical protein AOL_s00043g467

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year	gene	$\log_2$Fold Change	function.
	fun2234	0.63	ATPase ASNA1
	fun12551	0.63	FAD monooxygenase
	fun9596	0.63	CRE-TAG-320 protein
	fun19042	0.62	hypothetical protein AOL_s00081g339
	fun33564	0.62	hypothetical protein LOAG_14827
	fun16936	0.61	hypothetical protein AOL_s00054g390
	fun6705	0.60	CO dehydrogenase flavoprotein, partial
	fun12943	0.60	formamidase
	fun20828	0.59	hypothetical protein AOL_s00188g131
	fun14090	0.58	GTP cyclohydrolase-2
	fun16441	0.58	hypothetical protein AOL_s00043g487
	fun42158	0.58	NADPH:adrenodoxin oxidoreductase
	fun45477	0.58	Protein mac-1
	fun20016	0.58	hypothetical protein AOL_s00109g89
	fun13085	0.57	G-patch domain-containingprotein
	fun1684	0.57	antiviral helicase SKI2
	fun9491	0.57	CRE-STO-2 protein
	fun43840	0.56	Phosphoenolypyruvate CarboxyKinase
	fun37833	0.56	hypothetical protein NECAME_19255, partial
	fun31287	0.56	hypothetical protein CRE_31139
	fun14256	0.56	GYD domain protein
	fun21846	0.55	hypothetical protein AOL_s00215g783
	fun45007	0.55	programmed cell death 8
	fun16001	0.55	hypothetical protein AOL_s00007g292
	fun14074	0.55	GrpE nucleotide exchange factor
	fun28662	0.55	hypothetical protein CRE_14925
	fun11547	0.54	drug resistance protein
	fun12944	0.54	formate dehydrogenase
	fun1389	0.54	alpha/beta hydrolase family domain-containing protein
	fun9479	0.54	CRE-SRT-41 protein
	fun20073	0.54	hypothetical protein AOL_s00110g160
	fun27417	0.54	hypothetical protein CRE_07190
	fun2070	0.53	ATP synthase subunit 5
	fun28697	0.53	hypothetical protein CRE_15156
	fun17691	0.53	hypothetical protein AOL_s00076g334
	fun18164	0.53	hypothetical protein AOL_s00078g353
	fun47066	0.53	pyridoxine biosynthesis protein
	fun53650	0.53	VOC domain-containing protein
	fun9697	0.53	CRE-TRX-2 protein
	fun199	0.53	3-demethylubiquinone-9 3-methyltransferase
	fun23838	0.53	Hypothetical protein CBG09060
	fun21883	0.53	hypothetical protein AOL_s00215g829
	fun42493	0.52	non-heme chloroperoxidase
	fun43677	0.52	Phenylalanine-4-hydroxylase
	fun29188	0.52	hypothetical protein CRE_18396
	fun29639	0.52	hypothetical protein CRE_21238
	fun22807	0.52	Hypothetical protein CBG02613
	fun16871	0.52	hypothetical protein AOL_s00054g322
	fun13692	0.52	glycerone kinase
	fun231	0.51	3-methyl-2-oxobutanoate hydroxymethyltransferase

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year	gene	$\log_2$Fold Change	function.
	fun54250	0.51	zinc transporter ZitB
	fun24564	0.51	Hypothetical protein CBG13739
	fun41638	0.51	monothiol glutaredoxin-4
	fun28543	0.51	hypothetical protein CRE_14174
	fun8027	0.50	CRE-GLRX-5 protein
	fun47073	0.50	pyrroline-5-carboxylate reductase
	fun3806	0.50	C. briggsae CBR-HPD-1 protein
	fun14262	0.50	H(+)-ATPase 4
	fun20344	0.50	hypothetical protein AOL_s00140g4
	fun40740	0.50	malic enzyme, NAD binding domain protein, partial
	fun41167	0.50	MFS toxin efflux pump
	fun931	0.50	acyl-CoA synthetase
	fun46215	0.49	putative deoxyribose-phosphate aldolase
	fun8775	0.49	CRE-NUO-2 protein
	fun22776	0.49	Hypothetical protein CBG02437, partial
	fun22986	0.49	Hypothetical protein CBG03613
	fun705	0.49	acetate–CoA ligase
	fun24383	0.49	Hypothetical protein CBG12633
	fun17744	0.48	hypothetical protein AOL_s00076g411
	fun717	0.48	Acetyl-CoA acetyltransferase homolog, mitochondrial
	fun13906	0.48	Glyoxalase domain-containing protein 5
	fun1158	0.48	AIF-like mitochondrial oxidoreductase (Nfrl)
	fun17136	0.48	hypothetical protein AOL_s00054g648
	fun12554	0.48	FAD_binding_2 domain-containing protein
	fun48029	0.48	Rieske domain-containing protein
	fun49883	0.47	Succinyl-CoA synthetase, beta subunit
	fun39290	0.47	Hypothetical UPF0055 protein YPL059w
	fun7446	0.47	CRE-COQ-8 protein
	fun11940	0.47	elongation factor Tu GTP binding domain-containingprotein
	fun15379	0.47	hypothetical protein AOL_s00004g510
	fun11895	0.47	electron-transfer-flavoprotein, alpha polypeptide
	fun730	0.47	acetyl-Coenzyme A synthetase 2
	fun49943	0.47	Sulfurtransferase
	fun40873	0.47	Mechanosensory protein 2, partial
	fun4244	0.47	C. briggsae CBR-NFT-1 protein
	fun52706	0.46	Uncharacterized protein CELE_T02G5.7
	fun1177	0.46	alcohol dehydrogenase
	fun39722	0.46	Iron-sulfur cluster assembly 1 homolog, mitochondrial
	fun20699	0.46	hypothetical protein AOL_s00173g369
	fun40050	0.46	L-xylulose reductase
	fun18482	0.46	hypothetical protein AOL_s00079g291
	fun708	0.46	acetoacetate-CoA ligase
	fun31523	0.46	hypothetical protein LOAG_00527
	fun43660	0.46	phenazine biosynthesis protein
	fun10993	0.45	dihydroxyacetone kinase (DakA)
	fun39739	0.45	isochorismatase family protein
	fun42863	0.45	O-methyltransferase, family 3-containing protein
	fun19778	0.44	hypothetical protein AOL_s00097g549
	fun27540	0.44	hypothetical protein CRE_07978
	fun43836	0.44	Phosphoenolpyruvate carboxykinase, cytosolic

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year	gene	$\log_2$Fold Change	function.
	fun8318	0.44	CRE-LACT-2 protein
	fun1755	0.44	aquaglyceroporin
	fun33208	0.43	hypothetical protein LOAG_10884
	fun46216	0.43	Putative deoxyribose-phosphate aldolase
	fun14086	0.43	GTP cyclohydrolase 1
	fun12699	0.43	ferredoxin–NADP reductase
	fun47065	0.43	pyridoxine
	fun37253	0.43	hypothetical protein NECAME_16228
	fun19012	0.42	hypothetical protein AOL_s00081g301
	fun26481	0.42	hypothetical protein CRE_01860
	fun43420	0.42	peptidase, S1C family
	fun41622	0.42	molybdopterin cofactor biosynthetic protein
	fun13812	0.42	glycosyl hydrolase
	fun10289	0.42	cyclophilin-type peptidyl-prolyl cis-trans isomerase-15
	fun49036	0.42	short chain oxidoreductase protein
	fun28393	0.42	hypothetical protein CRE_13377
	fun29843	0.42	hypothetical protein CRE_22770
	fun28975	0.42	hypothetical protein CRE_16920
	fun8552	0.42	CRE-MMCM-1 protein
	fun20233	0.41	hypothetical protein AOL_s00112g39
	fun46450	0.41	putative LAO/AO transport system ATPase
	fun12994	0.41	Fructose-bisphosphate aldolase 1
	fun18263	0.41	hypothetical protein AOL_s00078g523
	fun16577	0.41	hypothetical protein AOL_s00043g656
	fun19748	0.41	hypothetical protein AOL_s00097g509
	fun42419	0.41	NIF system FeS cluster assembly, NifU, C-terminal domain and NIF system FeS cluster assembly, NifU-like scaffold, N-terminal domain and HIRA-interacting protein 5 family-containing protein
	fun28813	0.41	hypothetical protein CRE_15840, partial
	fun41372	0.41	mitochondrial cytochrome b2
	fun43641	0.41	PHB domain-containing protein
	fun47082	0.41	pyruvate decarboxylase
	fun2371	0.41	Band 7 protein family and Stomatin family-containing protein
	fun43831	0.40	phosphoadenosine phosphosulfate reductase
	fun21900	0.40	hypothetical protein AOL_s00215g851
	fun15657	0.40	hypothetical protein AOL_s00006g28
	fun718	0.40	Acetyl-CoA acetyltransferase, mitochondrial
	fun706	0.40	acetate-CoA ligase
	fun40749	0.40	maltase 1
	fun10650	0.40	DAK1 domain containing protein
	fun40763	0.40	manganese/iron superoxide dismutase, binding site
	fun232	0.40	3-methyl-2-oxobutanoate hydroxymethyltransferase PanB
	fun28479	0.40	hypothetical protein CRE_13873, partial
	fun29615	0.40	hypothetical protein CRE_21100, partial
	fun475	0.40	6-phosphogluconolactonase family protein
	fun44055	0.39	pirin
	fun7598	0.39	CRE-DAO-3 protein
	fun13033	0.39	fungal specific transcription factor domain-containing protein
	fun20416	0.39	hypothetical protein AOL_s00169g233
	fun701	0.39	acetamidase/formamidase
	fun26636	0.39	hypothetical protein CRE_02795, partial

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year	gene	$\log_2$Fold Change	function.
	fun21005	0.39	hypothetical protein AOL_s00188g90
	fun2045	0.39	ATP synthase F0 subunit 9 (mitochondrion)
	fun10943	0.39	dihydrodipicolinate synthase
	fun13899	0.38	glycyl-tRNA synthetase 1
	fun12995	0.38	fructose-bisphosphate aldolase 2
	fun16118	0.38	hypothetical protein AOL_s00007g474
	fun14507	0.38	HesB/YadR/YfhF family protein
	fun93	0.38	2-oxoisovalerate dehydrogenase subunit alpha
	fun51180	0.38	trehalose-phosphatase
	fun1863	0.38	Arp2/3 complex subunit Arc16
	fun17464	0.38	hypothetical protein AOL_s00075g226
	fun11880	0.38	electron transfer flavoprotein alpha-subunit
	fun11894	0.38	electron-transfer-flavoprotein
	fun12902	0.38	FMN-dependent dehydrogenase domain and Alpha-hydroxy acid dehydrogenase, FMN-dependent family and Aldolase-type TIM barrel domain-containing protein
	fun49772	0.38	steroid monooxygenase (CpmA)
	fun10600	0.38	Cytosolic Fe-S cluster assembly factor NUBP1
	fun49571	0.38	SPFH/Band 7 domain protein
	fun40425	0.37	lipoyl synthase, mitochondrial
	fun40418	0.37	lipoic acid synthetase
	fun20776	0.37	hypothetical protein AOL_s00176g4
	fun26955	0.37	hypothetical protein CRE_04449, partial
	fun729	0.37	Acetyl-coenzyme A synthetase
	fun50635	0.37	toxin-antitoxin system, toxin component, PIN family
	fun22766	0.37	Hypothetical protein CBG02379
	fun27934	0.36	hypothetical protein CRE_10734
	fun40428	0.36	lipoyl(octanoyl) transferase
	fun49570	0.36	SPFH domain/band 7 family domain protein
	fun18400	0.36	hypothetical protein AOL_s00079g163
	fun41623	0.36	molybdopterin converting factor, subunit 2
	fun29500	0.36	hypothetical protein CRE_20422
	fun42875	0.36	Octanoyltransferase family and Peptidase M14, carboxypeptidase A domain and Biotin/lipoate A/B protein ligase domain and Ribosomal protein S32, mitochondrial family-containing protein
	fun1404	0.36	alpha/beta-Hydrolase
	fun22444	0.36	Hypothetical protein CBG00466, partial
	fun6638	0.36	cleavage and polyadenylation specificity factor subunit 2
	fun31142	0.36	hypothetical protein CRE_30358
	fun9432	0.36	CRE-SOD-3 protein
	fun5801	0.36	Carboxyl transferase domain and Acetyl-coenzyme A carboxyltransferase, N-terminal domain and Acetyl-coenzyme A carboxyltransferase, C-terminal domain-containing protein
	fun17217	0.36	hypothetical protein AOL_s00054g762
	fun731	0.36	Acetyl-coenzyme A synthetase 2-like, mitochondrial
	fun5591	0.35	calcium-transporting ATPase 1
	fun41608	0.35	molybdenum cofactor biosynthesis protein A
	fun32734	0.35	hypothetical protein LOAG_07714
	fun14444	0.35	helix-turn-helix, AraC type, DNA binding protein
	fun40426	0.35	Lipoyl synthase, mitochondrial
	fun10800	0.35	delta-1-pyrroline-5-carboxylate dehydrogenase, mitochondrial precursor
	fun27795	0.35	hypothetical protein CRE_09948, partial
	fun50458	0.35	Thioredoxin, mitochondrial
	fun30198	0.35	hypothetical protein CRE_25164

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year	gene	$\log_2$Fold Change	function.
	fun4484	0.35	C. briggsae CBR-PNG-1 protein
	fun26006	0.35	Hypothetical protein CBG23896
	fun73	0.35	2-methyl branched-chain enoyl CoA reductase isoform I
	fun340	0.34	4-hydroxyphenylpyruvate dioxygenase
	fun18384	0.34	hypothetical protein AOL_s00079g139
	fun23713	0.34	Hypothetical protein CBG08279
	fun18178	0.34	hypothetical protein AOL_s00078g38
	fun5781	0.34	carbon monoxide dehydrogenase, small subunit
	fun6962	0.34	COX1 gene product (mitochondrion)
	fun40872	0.34	mechanosensory protein 2
	fun43542	0.34	peroxidase/catalase 2
	fun50870	0.34	transcriptional regulator, AraC family
	fun26971	0.34	hypothetical protein CRE_04493
	fun18380	0.34	hypothetical protein AOL_s00079g131
	fun18094	0.34	hypothetical protein AOL_s00078g256
	fun49959	0.34	superoxide dismutase, partial
	fun10595	0.34	cytosolic Fe-S cluster assembling factor cfd1
	fun16593	0.33	hypothetical protein AOL_s00043g674
	fun53283	0.33	unnamed protein product
	fun30149	0.33	hypothetical protein CRE_24910
	fun26401	0.33	hypothetical protein CRE_01517, partial
	fun19628	0.33	hypothetical protein AOL_s00097g329
	fun92	0.33	2-oxoisovalerate dehydrogenase complex, E1 component, alpha subunit
	fun21261	0.33	hypothetical protein AOL_s00210g336
	fun46488	0.33	putative methylmalonyl-CoA mutase large subunit
	fun6917	0.33	copper transporting ATPase
	fun28943	0.33	hypothetical protein CRE_16773
	fun44818	0.33	Probable methylmalonyl-CoA mutase, mitochondrial
	fun2921	0.33	C. briggsae CBR-ALH-3 protein
	fun34726	0.33	hypothetical protein NECAME_01825
	fun474	0.33	6-phosphogluconolactonase
	fun16737	0.33	hypothetical protein AOL_s00054g13
	fun24877	0.33	Hypothetical protein CBG16084
	fun49929	0.33	sulfite oxidase
	fun89	0.32	2-oxoisovalerate dehydrogenase alpha subunit
	fun46010	0.32	putative acyl-CoA dehydrogenase
	fun10778	0.32	dehydrogenase, FMN-dependent
	fun12897	0.32	FMN dependent dehydrogenase
	fun16283	0.32	hypothetical protein AOL_s00043g22
	fun20767	0.32	hypothetical protein AOL_s00176g25
	fun41109	0.32	methylmalonyl-CoA mutase
	fun27653	0.32	hypothetical protein CRE_08633
	fun49796	0.32	Stomatin-like protein 2, mitochondrial
	fun12665	0.32	Fe-containing alcohol dehydrogenase
	fun1433	0.32	Amidophosphoribosyltransferase
	fun29519	0.32	hypothetical protein CRE_20534
	fun40748	0.32	maltase
	fun30825	0.32	hypothetical protein CRE_28484
	fun19835	0.32	hypothetical protein AOL_s00097g629
	fun6085	0.32	cbs-prov protein, variant

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year	gene	$\log_2$Fold Change	function.
	fun13929	0.32	GMP synthase domain protein
	fun467	0.32	6-phosphofructokinase alpha subunit
	fun44838	0.31	Probable NADH-ubiquinone oxidoreductase 20 kDa subunit, mitochondrialprecursor
	fun1208	0.31	aldehyde oxidase and xanthine dehydrogenase, molybdopterin binding domain protein, partial
	fun50917	0.31	transketolase
	fun18088	0.31	hypothetical protein AOL_s00078g245
	fun14400	0.31	heavy metal translocating P-type ATPase
	fun803	0.31	aconitase family protein
	fun10325	0.31	Cysteine desulfurase, mitochondrial precursor
	fun10805	0.31	delta-aminolevulinic acid dehydratase
	fun2232	0.31	ATPase
	fun31140	0.31	hypothetical protein CRE_30355
	fun49853	0.31	succinate dehydrogenase
	fun21034	0.31	hypothetical protein AOL_s00193g126
	fun29839	0.31	hypothetical protein CRE_22764
	fun43863	0.31	phosphoglycerate dehydrogenase
	fun2093	0.31	ATP-binding cassette sub-family A member 3, partial
	fun29926	0.31	hypothetical protein CRE_23552
	fun28815	0.31	hypothetical protein CRE_15855
	fun45513	0.31	protein NBP35
	fun50311	0.31	tetrapyrrole biosynthesis, hydroxymethylbilane synthase
	fun15289	0.31	hypothetical protein AOL_s00004g395
	fun11955	0.31	Elp3 domain-containing protein
	fun27286	0.30	hypothetical protein CRE_06271
	fun2091	0.30	ATP-binding cassette sub-family A member 2
	fun10322	0.30	cysteine desulfurase
	fun41639	0.30	monothiol glutaredoxin-5
	fun16287	0.30	hypothetical protein AOL_s00043g230
	fun26909	0.30	hypothetical protein CRE_04116
	fun43134	0.30	pantoate-beta-alanine ligase
	fun13591	0.30	glutamyl-tRNA(Gln) amidotransferase subunit A
	fun48370	0.30	S-(hydroxymethyl)glutathione dehydrogenase
	fun32354	0.30	hypothetical protein LOAG_05301
	fun13486	0.30	glucose-6-phosphate 1-dehydrogenase
	fun5155	0.30	C. briggsae CBR-TRX-2 protein
	fun6923	0.30	copper-exporting ATPase
	fun29378	0.30	hypothetical protein CRE_19634, partial
	fun53673	0.29	voltage-gated potassium channel beta-1 subunit
	fun90	0.29	2-oxoisovalerate dehydrogenase alpha subunit, mitochondrial precursor
	fun28477	0.29	hypothetical protein CRE_13870, partial
	fun17526	0.29	hypothetical protein AOL_s00076g108
	fun41616	0.29	molybdenum cofactor synthesis domain protein
	fun553	0.29	60S ribosomal protein L40
	fun1258	0.29	allantoinase
	fun13681	0.29	glycerol kinase 2
	fun49859	0.29	succinate dehydrogenase flavoprotein subunit, mitochondrial precursor
	fun6927	0.29	copper-transporting ATPase 2
	fun30169	0.29	hypothetical protein CRE_25044
	fun919	0.29	Acyl-CoA dehydrogenase, C-terminal domain containing protein
	fun13488	0.29	glucose-6-phosphate dehydrogenase

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year	gene	$\log_2$Fold Change	function.
	fun30944	0.29	hypothetical protein CRE_29393
	fun10802	0.29	Delta-1-pyrroline-5-carboxylate dehydrogenase,mitochondrial
	fun28463	0.29	hypothetical protein CRE_13818
	fun10348	0.29	cysteine synthase
	fun49572	0.29	SPFH/Band 7/PHB domain protein
	fun42006	0.29	NAD dehydrogenase
	fun18874	0.29	hypothetical protein AOL_s00080g8
	fun41449	0.29	mitochondrial NADH-ubiquinone oxidoreductase subunit
	fun41740	0.29	multidrug resistance protein fnx1
	fun18939	0.28	hypothetical protein AOL_s00081g180
	fun2153	0.28	ATP-dependent metalloprotease FtsH family protein
	fun50863	0.28	transcriptional regulator AraC protein
	fun17622	0.28	hypothetical protein AOL_s00076g255
	fun53963	0.28	xylulose-5-phosphate phosphoketolase
	fun51359	0.28	Tryptophan 2,3-dioxygenase
	fun1500	0.28	Aminotran_5 domain-containing protein
	fun52057	0.28	Uncharacterized protein CELE_B0495.5
	fun27903	0.28	hypothetical protein CRE_10408
	fun39724	0.28	Iron-sulfur cluster assembly accessory protein
	fun44581	0.28	prevent-host-death family protein
	fun43023	0.28	oxoglutarate dehydrogenase (succinyl-transferring), E1 component
	fun17646	0.28	hypothetical protein AOL_s00076g282
	fun30477	0.28	hypothetical protein CRE_26453
	fun7005	0.28	CRE-AARS-2 protein
	fun14087	0.28	GTP cyclohydrolase I
	fun13600	0.28	Glutaredoxin
	fun204	0.28	3-hydroxyacyl-CoA dehydrogenase protein
	fun723	0.28	acetyl-CoA C-acetyltransferase
	fun10068	0.28	CU (copper) ATPase
	fun2533	0.28	beta-lactamase
	fun17157	0.27	hypothetical protein AOL_s00054g678
	fun43436	0.27	peptide methionine sulfoxide reductase msrB
	fun21010	0.27	hypothetical protein AOL_s00188g96
	fun20410	0.27	hypothetical protein AOL_s00169g227
	fun20489	0.27	hypothetical protein AOL_s00170g104
	fun37262	0.27	hypothetical protein NECAME_16242
	fun41110	0.27	Methylmalonyl-CoA mutase, mitochondrial
	fun13702	0.27	Glycine cleavage system H protein, mitochondrial
	fun720	0.27	acetyl-CoA acyltransferase
	fun42355	0.27	Nfs1p
	fun26956	0.26	hypothetical protein CRE_04450
	fun20038	0.26	hypothetical protein AOL_s00110g113
	fun14321	0.26	HD domain protein
	fun13688	0.26	glycerol-3-phosphate dehydrogenase, mitochondrial precursor
	fun50445	0.26	thioredoxin-1
	fun49054	0.26	Short-chain dehydrogenase/reductase SDR
	fun49164	0.26	siroheme synthase
	fun53924	0.26	Xanthine DeHydrogenase homolog
	fun13487	0.26	Glucose-6-phosphate 1-dehydrogenase
	fun47061	0.26	pyridoxal-phosphate dependent protein, partial

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year	gene	$\log_2$Fold Change	function.
	fun26050	0.26	Hypothetical protein CBG24278
	fun40714	0.26	malate dehydrogenase
	fun40419	0.26	lipoic acid synthetase, mitochondrial precursor
	fun46883	0.26	putative transcriptional activator protein IrlR
	fun28046	0.26	hypothetical protein CRE_11384
	fun43384	0.26	peptidase S14, ClpP
	fun1196	0.26	Aldedh domain-containing protein
	fun27292	0.26	hypothetical protein CRE_06284
	fun39723	0.26	iron-sulfur cluster assembly accessory protein
	fun50433	0.26	thioredoxin peroxidase, identical
	fun42420	0.25	NifU_N domain-containing protein
	fun15184	0.25	hypothetical protein AOL_s00004g254
	fun21091	0.25	hypothetical protein AOL_s00193g4
	fun27	0.25	1-pyrroline-5-carboxylate dehydrogenase
	fun42354	0.25	NFS1 protein
	fun5805	0.25	carboxyl transferase domain protein, partial
	fun21520	0.25	hypothetical protein AOL_s00215g326
	fun17858	0.25	hypothetical protein AOL_s00076g618
	fun930	0.24	Acyl-CoA oxidase/dehydrogenase, central domain and Acyl-CoA dehydrogenase/oxidase C-terminal domain and Acyl-CoA dehydrogenase/oxidase, N-terminal and middle domain and Acyl-CoA dehydrogenase/oxidase, N-terminal domain-containing protein
	fun42053	0.24	NADH dehydrogenase I, B subunit
	fun10777	0.24	Dehydrogenase, E1 component domain and Transketolase-like, pyrimidine-binding domain and 2-oxoglutarate dehydrogenase, E1 component family-containing protein
	fun21046	0.24	hypothetical protein AOL_s00193g143
	fun15100	0.24	hypothetical protein AOL_s00004g120
	fun44721	0.24	Probable cytoplasmic aconitate hydratase
	fun23798	0.24	Hypothetical protein CBG08829, partial
	fun14881	0.24	homoserine O-acetyltransferase
	fun2481	0.24	Beta_elim_lyase domain-containing protein
	fun28078	0.24	hypothetical protein CRE_11637
	fun44839	0.24	Probable NADH-ubiquinone oxidoreductase 49 kDa subunit, mitochondrialprecursor
	fun49877	0.24	succinyl-CoA ligase alpha-chain
	fun16011	0.23	hypothetical protein AOL_s00007g305
	fun20237	0.23	hypothetical protein AOL_s00112g43
	fun10555	0.23	Cytoplasmic aconitate hydratase
	fun47080	0.23	Pyruvate carboxylase 1
	fun29621	0.23	hypothetical protein CRE_21113, partial
	fun10372	0.23	cysteinyl-tRNA synthetase, cytoplasmic
	fun16455	0.23	hypothetical protein AOL_s00043g502
	fun10955	0.23	dihydrolipoamide succinyltransferase
	fun11888	0.23	electron transfer flavoprotein-ubiquinone oxidoreductase, mitochondrial precursor
	fun2585	0.23	bifunctional purine biosynthesis protein ADE16
	fun12070	0.23	enoyl-CoA hydratase
	fun53563	0.23	vanillin dehydrogenase
	fun19844	0.23	hypothetical protein AOL_s00097g64
	fun70	0.23	2-isopropylmalate synthase
	fun46337	0.23	Putative glutamate synthase
	fun41457	0.23	mitochondrial peroxiredoxin PRX1
	fun12541	0.22	FAD binding domain-containingprotein
	fun2092	0.22	ATP-binding cassette sub-family A member 3

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year	gene	$\log_2$Fold Change	function.
	fun18024	0.22	hypothetical protein AOL_s00078g17
	fun39748	0.22	isocitrate dehydrogenase subunit 2, mitochondrial precursor
	fun16493	0.22	hypothetical protein AOL_s00043g556
	fun18444	0.22	hypothetical protein AOL_s00079g226
	fun29698	0.22	hypothetical protein CRE_21713, partial
	fun40047	0.22	L-threonine 3-dehydrogenase
	fun50919	0.22	transketolase 1
	fun51675	0.22	ubiE/COQ5 methyltransferase
	fun32617	0.22	hypothetical protein LOAG_06870
	fun41606	0.22	Molybdenum cofactor biosynthesis protein 1
	fun18035	0.22	hypothetical protein AOL_s00078g182
	fun18530	0.22	hypothetical protein AOL_s00079g361
	fun19111	0.22	hypothetical protein AOL_s00083g125
	fun44072	0.21	PKS_ER domain-containing protein
	fun1411	0.21	Amidase
	fun42421	0.21	NifU-like protein
	fun27146	0.21	hypothetical protein CRE_05366
	fun29180	0.21	hypothetical protein CRE_18371
	fun20755	0.21	hypothetical protein AOL_s00173g96
	fun46998	0.21	putative xanthine dehydrogenase accessory factor
	fun13549	0.21	glutamate–tRNA ligase
	fun47559	0.21	retinal dehydrogenase 2
	fun10324	0.21	Cysteine desulfurase, mitochondrial
	fun26773	0.21	hypothetical protein CRE_03582
	fun2876	0.21	C. briggsae CBR-ACO-1 protein
	fun49055	0.21	Short-chain dehydrogenase/reductase SDR family and Glucose/ribitol dehydrogenase family and NAD(P)-binding domain-containing protein
	fun48018	0.21	ribulose-phosphate 3-epimerase
	fun24448	0.21	Hypothetical protein CBG13036, partial
	fun28081	0.21	hypothetical protein CRE_11651
	fun43434	0.21	peptide methionine sulfoxide reductase
	fun50310	0.21	tetrahydroxynaphthalene reductase-like protein
	fun49864	0.20	succinate dehydrogenase, flavoprotein subunit
	fun13015	0.20	fumarate hydratase, class II
	fun53674	0.20	voltage-gated potassium channel subunit beta-1 channel subunit beta-1
	fun22797	0.20	Hypothetical protein CBG02570
	fun225	0.20	3-ketoacyl-CoA thiolase, mitochondrial
	fun41611	0.20	molybdenum cofactor biosynthetic protein (CnxF)
	fun13673	0.20	Glyceraldehyde-3-phosphate dehydrogenase 4
	fun230	0.20	3-methyl-2-oxobutanoate dehydrogenase
	fun10314	0.20	cystathionine gamma-lyase
	fun904	0.20	Acyl CoA DeHydrogenase
	fun45078	0.20	Propionyl Coenzyme A Carboxylase Beta subunit
	fun42038	0.20	NAD(P) transhydrogenase, mitochondrial precursor
	fun17138	0.20	hypothetical protein AOL_s00054g65
	fun27291	0.19	hypothetical protein CRE_06283
	fun635	0.19	ABC transporter ced-7
	fun649	0.19	ABC transporter, ATP-binding protein, partial
	fun21053	0.19	hypothetical protein AOL_s00193g153
	fun43294	0.19	PEPCK_GTP domain-containing protein

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year	gene	$\log_2$Fold Change	function.
	fun12075	0.19	enoyl-CoA hydratase/isomerase
	fun13700	0.19	glycine cleavage system H protein
	fun19356	0.19	hypothetical protein AOL_s00083g45
	fun7055	0.19	CRE-ACO-1 protein
	fun25117	0.19	Hypothetical protein CBG18013
	fun43835	0.18	phosphoenolpyruvate carboxykinase
	fun47560	0.18	Retinal dehydrogenase 2
	fun31132	0.18	hypothetical protein CRE_30344
	fun41607	0.18	molybdenum cofactor biosynthesis protein 1 B
	fun721	0.18	ACetyl-CoA Acyltransferase 2 homolog
	fun41619	0.18	molybdenum cofactor synthesis protein 3
	fun27942	0.18	hypothetical protein CRE_10797
	fun44759	0.18	Probable fumarate hydratase, mitochondrial
	fun46350	0.18	putative glycine dehydrogenase
	fun10306	0.17	cystathionine beta-lyase
	fun16571	0.17	hypothetical protein AOL_s00043g647
	fun50916	0.17	Transket_pyr domain-containing protein
	fun630	0.17	ABC transporter
	fun1541	0.16	AMP-binding enzyme
	fun13669	0.16	glyceraldehyde-3-phosphate dehydrogenase
	fun709	0.16	acetoacetyl-CoA reductase
	fun15489	0.16	hypothetical protein AOL_s00004g654
	fun18661	0.16	hypothetical protein AOL_s00080g129
	fun47060	0.16	pyridoxal-phosphate dependent protein
	fun27654	0.15	hypothetical protein CRE_08636, partial
	fun15466	0.15	hypothetical protein AOL_s00004g626
	fun29088	0.15	hypothetical protein CRE_17788
	fun27278	0.15	hypothetical protein CRE_06254
	fun45080	0.15	propionyl-CoA carboxylase, beta subunit
	fun1202	0.15	aldehyde dehydrogenase family protein
	fun43828	0.14	phospho-2-dehydro-3-deoxyheptonate aldolase
	fun637	0.14	ABC Transporter family
	fun10634	0.14	D-ribulose-5-phosphate-3-epimerase
	fun10309	0.14	cystathionine beta-synthase
	fun2798	0.13	C-1-tetrahydrofolate synthase, mitochondrial precursor
	fun51279	0.13	tRNA synthetase class II
	fun48678	0.11	serine hydroxymethyltransferase 4
	fun28222	-0.12	hypothetical protein CRE_12456
	fun50488	-0.15	threonyl-tRNA synthetase, cytoplasmic
	fun13326	-0.15	GDP-mannose 4,6-dehydratase
	fun46362	-0.17	putative GTP-binding protein LepA
	fun16901	-0.19	hypothetical protein AOL_s00054g354
	fun293	-0.19	37S ribosomal protein S12
	fun1020	-0.19	Adenosylhomocysteinase
	fun13525	-0.19	Glutamate dehydrogenase, mitochondrial
	fun17390	-0.19	hypothetical protein AOL_s00075g138
	fun20323	-0.19	hypothetical protein AOL_s00117g8
	fun45998	-0.20	putative acetyl-CoA carboxylase, biotin carboxylase subunit
	fun1019	-0.20	adenosylhomocysteinase
	fun15541	-0.21	hypothetical protein AOL_s00006g123

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year	gene	$\log_2$Fold Change	function.
	fun17404	-0.21	hypothetical protein AOL_s00075g156
	fun2061	-0.22	ATP synthase gamma chain, mitochondrial precursor
	fun51936	-0.22	UDP-glucose 6-dehydrogenase
	fun46045	-0.22	putative angiotensin-converting enzyme, somatic isoform
	fun46091	-0.22	putative bacteriochlorophyll 4-vinyl reductase
	fun48179	-0.22	RNA polymerase Rpb1, domain 1 family protein
	fun25142	-0.22	Hypothetical protein CBG18137
	fun50487	-0.23	Threonyl-tRNA synthetase
	fun26452	-0.23	hypothetical protein CRE_01727
	fun11349	-0.23	DNA-directed RNA polymerase III subunit RPC1
	fun13323	-0.23	GDP-mannose 4,6 dehydratase
	fun45812	-0.24	protein ZK524.3
	fun12007	-0.24	endopeptidase La, partial
	fun33542	-0.24	hypothetical protein LOAG_14564
	fun17135	-0.24	hypothetical protein AOL_s00054g647
	fun18239	-0.24	hypothetical protein AOL_s00078g477
	fun11943	-0.24	elongation factor Tu, mitochondrial precursor
	fun11942	-0.24	Elongation factor Tu, mitochondrial
	fun6258	-0.24	Centromere/kinetochore Zw10 family protein
	fun47437	-0.24	RecQ family ATP-dependent DNA helicase
	fun45079	-0.24	Propionyl-CoA carboxylase alpha chain, mitochondrial
	fun47884	-0.24	ribosomal protein S12
	fun49657	-0.24	Spo0B-associated GTP-binding protein
	fun29980	-0.25	hypothetical protein CRE_23866
	fun647	-0.25	ABC transporter-like domain and AAA+ ATPase domain and P-loop containing nucleoside triphosphate hydrolase domain-containing protein
	fun27409	-0.25	hypothetical protein CRE_07173
	fun46629	-0.25	putative polyribonucleotide nucleotidyltransferase
	fun30552	-0.25	hypothetical protein CRE_26933
	fun25334	-0.25	Hypothetical protein CBG19373
	fun31184	-0.25	hypothetical protein CRE_30647
	fun17084	-0.25	hypothetical protein AOL_s00054g582
	fun11337	-0.26	DNA-directed RNA polymerase II subunit RPB1
	fun651	-0.26	ABC transporter, class F
	fun278	-0.26	30S ribosomal protein S12
	fun42871	-0.26	OBG-type G domain-containing protein
	fun15551	-0.27	hypothetical protein AOL_s00006g137
	fun21154	-0.27	hypothetical protein AOL_s00210g131
	fun44266	-0.27	Polyribonucleotide nucleotidyltransferase 1, mitochondrial
	fun13729	-0.27	glycogen phosphorylase
	fun50959	-0.27	Translation initiation factor IF-2, mitochondrial
	fun11934	-0.27	elongation factor Tu domain 2
	fun7096	-0.28	CRE-AHCY-1 protein
	fun41330	-0.28	mitochondrial 37S ribosomal protein MRPS9
	fun50658	-0.28	Tr-type G domain-containing protein
	fun42062	-0.28	NADH dehydrogenase subunit 4 (mitochondrion)
	fun32105	-0.28	hypothetical protein LOAG_03897
	fun11335	-0.29	DNA-directed RNA polymerase II polypeptide
	fun50932	-0.29	translation elongation factor Tu, partial
	fun16970	-0.29	hypothetical protein AOL_s00054g433

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year	gene	$\log_2$Fold Change	function.
	fun29086	-0.30	hypothetical protein CRE_17786
	fun2194	-0.30	ATP-dependent RNA helicase DHX8
	fun26772	-0.30	hypothetical protein CRE_03575, partial
	fun29542	-0.30	hypothetical protein CRE_20665
	fun48375	-0.30	S-adenosyl-L-homocysteine hydrolase, NAD binding domain protein
	fun29767	-0.30	hypothetical protein CRE_22285
	fun50929	-0.30	translation elongation factor G
	fun5590	-0.30	Calcium-transporting ATPase
	fun11930	-0.30	elongation factor TS family protein
	fun16820	-0.30	hypothetical protein AOL_s00054g251
	fun11928	-0.30	Elongation factor G C-terminus family protein
	fun51111	-0.31	transport energizing protein, ExbD/TolR family
	fun24739	-0.32	Hypothetical protein CBG15142
	fun43680	-0.32	phenylalanyl-tRNA synthetase alpha chain
	fun18600	-0.32	hypothetical protein AOL_s00079g496
	fun47882	-0.32	ribosomal protein S11
	fun6931	-0.32	coproporphyrinogen III oxidase
	fun17215	-0.32	hypothetical protein AOL_s00054g760
	fun44636	-0.33	Probable 39S ribosomal protein L11, mitochondrial
	fun370	-0.33	40s ribosomal protein s20 protein
	fun18625	-0.33	hypothetical protein AOL_s00079g79
	fun41331	-0.33	mitochondrial 37S ribosomal protein SWS2
	fun52999	-0.33	Uncharacterized protein CELE_Y69A2AR.18
	fun20184	-0.33	hypothetical protein AOL_s00110g92
	fun12841	-0.34	FKBP-type peptidyl-prolyl cis-trans isomerase-59, BmFKBP59
	fun15169	-0.34	hypothetical protein AOL_s00004g229
	fun48394	-0.34	S1 motif domain-containing protein
	fun46257	-0.34	putative elongation factor Tu GTP binding domain protein
	fun11926	-0.34	elongation factor G
	fun3532	-0.34	C. briggsae CBR-ERS-1 protein
	fun41041	-0.34	metalloprotease 1 protein
	fun19830	-0.34	hypothetical protein AOL_s00097g624
	fun253	-0.35	3' exoribonuclease family, domain 2 containing protein
	fun10300	-0.35	cyclopropane-fatty-acyl-phospholipid synthase
	fun10941	-0.35	diguanylate cyclase domain protein
	fun16136	-0.35	hypothetical protein AOL_s00007g497
	fun26449	-0.35	hypothetical protein CRE_01704
	fun18648	-0.36	hypothetical protein AOL_s00080g109
	fun4667	-0.36	C. briggsae CBR-RPC-1 protein
	fun20488	-0.36	hypothetical protein AOL_s00170g103
	fun48214	-0.36	RNA recognition motif domain-containingprotein
	fun50936	-0.36	Translation factor waclaw, mitochondrial
	fun37286	-0.36	hypothetical protein NECAME_16334
	fun50489	-0.36	Threonyl-tRNA synthetase, cytoplasmic family protein
	fun30790	-0.36	hypothetical protein CRE_28335
	fun21106	-0.37	hypothetical protein AOL_s00193g6
	fun51123	-0.37	transporter protein smf2
	fun6766	-0.37	cohesin complex subunit (Psm1)
	fun11927	-0.37	elongation factor G 1, mitochondrial precursor
	fun2103	-0.37	ATP-binding cassette sub-family F member 2

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year	gene	$\log_2$Fold Change	function.
	fun44206	-0.37	polyadenylate-binding protein
	fun31385	-0.38	hypothetical protein EMIHUDRAFT_226409
	fun366	-0.38	40S ribosomal protein S2
	fun29783	-0.38	hypothetical protein CRE_22391
	fun49330	-0.38	SNF2 family N-terminal domain containing protein
	fun50687	-0.38	transcription accessory protein
	fun16199	-0.39	hypothetical protein AOL_s00007g573
	fun48311	-0.39	rRNA biogenesis protein RRP5
	fun51255	-0.39	tRNA ligase class II, partial
	fun2104	-0.39	ATP-binding cassette sub-family F member 3
	fun47811	-0.40	ribosomal protein L11
	fun28082	-0.40	hypothetical protein CRE_11653
	fun29601	-0.40	hypothetical protein CRE_20935
	fun17782	-0.40	hypothetical protein AOL_s00076g495
	fun22831	-0.40	Hypothetical protein CBG02783
	fun48127	-0.41	RNA helicase HRH1
	fun254	-0.41	3' exoribonuclease family, variant
	fun10201	-0.41	cyclic diguanylate phosphodiesterase domain protein
	fun19722	-0.41	hypothetical protein AOL_s00097g477
	fun14416	-0.41	Helicase conserved C-terminal domain containing protein
	fun11931	-0.41	Elongation factor TS family protein
	fun40816	-0.41	MAP/microtubule affinity-regulating kinase 3
	fun19459	-0.41	hypothetical protein AOL_s00091g68
	fun207	-0.42	3-hydroxyacyl-CoA dehydrogenase, C-terminal domain and 3-hydroxyacyl-CoA dehydrogenase, NAD binding domain and 6-phosphogluconate dehydrogenase, C-terminal-like domain and Dehydrogenase, multihelical domain and NAD(P)-binding domain-containing protein
	fun459	-0.42	50S ribosome-binding GTPase domain-containing protein
	fun11925	-0.42	elongation factor 3
	fun32703	-0.42	hypothetical protein LOAG_07439
	fun48206	-0.42	RNA recognition motif containing protein
	fun441	-0.42	50S ribosomal protein L14
	fun15359	-0.42	hypothetical protein AOL_s00004g484
	fun46083	-0.42	putative ATP-dependent RNA helicase Pl10
	fun11929	-0.42	Elongation factor G, mitochondrial
	fun17279	-0.42	hypothetical protein AOL_s00054g848
	fun34453	-0.42	hypothetical protein NECAME_00436
	fun46376	-0.42	putative heat shock protein 90
	fun17647	-0.43	hypothetical protein AOL_s00076g283
	fun276	-0.44	30S ribosomal protein S10
	fun19581	-0.44	hypothetical protein AOL_s00097g258
	fun923	-0.44	acyl-CoA desaturase
	fun48374	-0.44	S-adenosyl-L-homocysteine hydrolase
	fun10697	-0.44	Ddx49-A-prov protein
	fun17520	-0.45	hypothetical protein AOL_s00075g95
	fun17605	-0.45	hypothetical protein AOL_s00076g230
	fun49774	-0.45	sterol 24-C-methyltransferase
	fun15878	-0.45	hypothetical protein AOL_s00006g8
	fun2531	-0.45	Beta-ketoacyl synthase, N-terminal domain containing protein
	fun44877	-0.46	Probable pre-mRNA-splicing factor ATP-dependent RNA helicase mog-5
	fun26781	-0.46	hypothetical protein CRE_03606

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year	gene	$\log_2$Fold Change	function.
	fun53430	-0.46	vacuolar ATP synthase catalytic subunit A
	fun52120	-0.46	Uncharacterized protein CELE_C14F11.6
	fun16431	-0.46	hypothetical protein AOL_s00043g473
	fun17597	-0.46	hypothetical protein AOL_s00076g218
	fun41510	-0.46	Mitogen-activated protein kinase
	fun18698	-0.47	hypothetical protein AOL_s00080g19
	fun4020	-0.47	C. briggsae CBR-LIN-28 protein, partial
	fun41081	-0.47	methionyl-tRNA synthetase, cytoplasmic
	fun41759	-0.47	multiple RNA-binding domain-containing protein 1
	fun6278	-0.47	CG10333-PA
	fun19756	-0.47	hypothetical protein AOL_s00097g519
	fun45987	-0.47	Putative 39S ribosomal protein L3, mitochondrial
	fun39983	-0.47	KP-43 peptidase, serine peptidase, MEROPS family S08A
	fun15178	-0.47	hypothetical protein AOL_s00004g247
	fun11484	-0.47	domain protein, SNF2 family
	fun35726	-0.48	hypothetical protein NECAME_07561, partial
	fun48228	-0.48	RNA-binding protein 24
	fun6646	-0.48	cleavage stimulation factor
	fun28445	-0.48	hypothetical protein CRE_13733
	fun48308	-0.48	RRM domain-containing protein
	fun20148	-0.48	hypothetical protein AOL_s00110g340
	fun25226	-0.48	Hypothetical protein CBG18692
	fun2191	-0.48	ATP-dependent RNA helicase DHH1
	fun15576	-0.48	hypothetical protein AOL_s00006g166
	fun42425	-0.48	NimA-like kinase
	fun41339	-0.49	mitochondrial 54S ribosomal protein YmL7/YmL5
	fun11056	-0.49	Dis3, partial
	fun45375	-0.49	protein kinase (Gcn2)
	fun27083	-0.49	hypothetical protein CRE_04940
	fun2159	-0.49	ATP-dependent RNA helicase
	fun517	-0.50	60S ribosomal protein L19 precursor
	fun18764	-0.50	hypothetical protein AOL_s00080g3
	fun1070	-0.50	ADP-ribose pyrophosphatase
	fun25222	-0.50	Hypothetical protein CBG18684
	fun48250	-0.51	RNA-binding S4 domain and Pseudouridine synthase, RsuA/RluB/C/D/E/F domain and Pseudouridine synthase, RluC/RluD family and Pseudouridine synthase, catalytic domain-containing protein
	fun16435	-0.51	hypothetical protein AOL_s00043g479
	fun20911	-0.51	hypothetical protein AOL_s00188g29
	fun51260	-0.52	tRNA modification GTPase GTPBP3, mitochondrial
	fun21124	-0.52	hypothetical protein AOL_s00193g80
	fun10726	-0.52	DEAD/DEAH box RNA helicase
	fun28907	-0.52	hypothetical protein CRE_16594
	fun1674	-0.53	antibiotic synthetase
	fun17320	-0.53	hypothetical protein AOL_s00054g921
	fun44269	-0.53	polysaccharide export inner-membrane protein, BexC/CtrB/KpsE family
	fun49740	-0.53	STE/STE11/SSK protein kinase
	fun44265	-0.53	polyribonucleotide nucleotidyltransferase
	fun519	-0.53	60S ribosomal protein L2
	fun47943	-0.53	ribosomal protein, L4/L1 family
	fun51767	-0.53	ubiquitin family protein

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year	gene	$\log_2$Fold Change	function.
	fun15706	-0.54	hypothetical protein AOL_s00006g340
	fun12238	-0.54	eukaryotic initiation factor 4A-III
	fun499	-0.54	60S ribosomal protein L11
	fun16561	-0.54	hypothetical protein AOL_s00043g635
	fun42731	-0.54	nucleolar GTP-binding protein 1
	fun5673	-0.54	calreticulin family protein
	fun41340	-0.54	mitochondrial 54S ribosomal protein YmL9
	fun46085	-0.54	Putative ATP-dependent RNA helicase T26G10.1
	fun16003	-0.55	hypothetical protein AOL_s00007g295
	fun21784	-0.55	hypothetical protein AOL_s00215g679
	fun41027	-0.56	metallopeptidase family M24 containing protein
	fun41338	-0.56	mitochondrial 54S ribosomal protein YmL6
	fun1082	-0.56	ADP-ribosylation factor family protein
	fun563	-0.56	60S ribosomal protein L8
	fun7857	-0.57	CRE-EXO-3 protein
	fun524	-0.57	60S ribosomal protein L23
	fun12256	-0.57	eukaryotic translation initiation factor 1A
	fun16961	-0.58	hypothetical protein AOL_s00054g421
	fun12235	-0.58	Eukaryotic initiation factor 4A
	fun44213	-0.58	Polyadenylate-binding protein 4
	fun46855	-0.58	putative tetratricopeptide repeat-containing domain protein
	fun48761	-0.58	serine/threonine-protein kinase 24
	fun10704	-0.58	dead box ATP-dependent rna helicase
	fun52532	-0.58	Uncharacterized protein CELE_F55F8.2
	fun29177	-0.58	hypothetical protein CRE_18366
	fun47883	-0.59	ribosomal protein S11 containing protein
	fun25223	-0.59	Hypothetical protein CBG18685
	fun33987	-0.59	hypothetical protein LOAG_17498
	fun75	-0.59	2-methylcitrate synthase
	fun1939	-0.59	Aspartic peptidase family and Aspartic peptidase domain-containing protein
	fun44676	-0.60	Probable ATP-dependent RNA helicase DDX41
	fun7592	-0.60	CRE-DAF-21 protein
	fun16074	-0.60	hypothetical protein AOL_s00007g412
	fun2175	-0.60	ATP-dependent RNA helicase DBP8
	fun19342	-0.60	hypothetical protein AOL_s00083g423
	fun44205	-0.60	polyadenylate binding protein
	fun51388	-0.60	tubulin A
	fun17591	-0.61	hypothetical protein AOL_s00076g205
	fun4148	-0.61	C. briggsae CBR-MOG-5 protein
	fun19877	-0.61	hypothetical protein AOL_s00109g109
	fun15525	-0.61	hypothetical protein AOL_s00006g105
	fun48282	-0.61	RNB-like protein
	fun25224	-0.61	Hypothetical protein CBG18686
	fun20318	-0.62	hypothetical protein AOL_s00117g6
	fun14712	-0.62	histone H3, embryonic
	fun1031	-0.62	adenylate kinase isoenzyme
	fun11340	-0.62	DNA-directed RNA polymerase II subunit RPB2
	fun17903	-0.62	hypothetical protein AOL_s00076g675
	fun21860	-0.62	hypothetical protein AOL_s00215g802
	fun9589	-0.62	CRE-TAG-300 protein

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year	gene	$\log_2$Fold Change	function.
	fun18849	-0.62	hypothetical protein AOL_s00080g47
	fun14407	-0.63	HECT domain-containing protein
	fun20386	-0.63	hypothetical protein AOL_s00169g134
	fun48696	-0.63	serine proteinase inhibitor
	fun7344	-0.63	CRE-CEY-1 protein
	fun30791	-0.63	hypothetical protein CRE_28336
	fun19277	-0.63	hypothetical protein AOL_s00083g337
	fun53432	-0.63	vacuolar ATP synthase catalytic subunit A, partial
	fun48775	-0.63	Serine/threonine-protein kinase Chk1
	fun30230	-0.63	hypothetical protein CRE_25313
	fun14418	-0.64	helicase protein
	fun12390	-0.64	exodeoxyribonuclease III family protein
	fun24405	-0.65	Hypothetical protein CBG12746
	fun23842	-0.65	Hypothetical protein CBG09074
	fun277	-0.65	30S ribosomal protein S11
	fun44679	-0.65	Probable ATP-dependent RNA helicase DDX49
	fun12236	-0.65	Eukaryotic initiation factor 4A-I
	fun10041	-0.66	CSD_1 domain-containing protein
	fun25150	-0.66	Hypothetical protein CBG18195
	fun30847	-0.67	hypothetical protein CRE_28621
	fun44407	-0.68	Pre-mRNA-splicing factor 8 homolog
	fun16512	-0.68	hypothetical protein AOL_s00043g579
	fun5913	-0.68	cathepsin B
	fun11055	-0.68	DIS3-like exonuclease 2
	fun44370	-0.68	pre-mRNA processing RNA-helicase
	fun27280	-0.69	hypothetical protein CRE_06259
	fun15967	-0.69	hypothetical protein AOL_s00007g228
	fun13563	-0.69	glutaminase DH11.1
	fun20984	-0.69	hypothetical protein AOL_s00188g65
	fun52964	-0.70	Uncharacterized protein CELE_Y54G11A.3
	fun5871	-0.70	Casein kinase I
	fun6822	-0.70	common central of tyrosinase domain-containing protein
	fun51403	-0.70	Tubulin beta chain, partial
	fun12795	-0.70	Fic protein family protein
	fun11336	-0.70	DNA-directed RNA polymerase II second largest subunit
	fun23767	-0.70	Hypothetical protein CBG08614, partial
	fun28709	-0.70	hypothetical protein CRE_15249
	fun13837	-0.70	glycosyl hydrolase, family 31, partial
	fun27269	-0.70	hypothetical protein CRE_06212
	fun44554	-0.71	pregnancy-associated plasma protein-E
	fun29766	-0.71	hypothetical protein CRE_22283
	fun2217	-0.71	ATP-dependent rRNA helicase RRP3
	fun48208	-0.71	RNA recognition motif domain and Nucleotide-binding, alpha-beta plait domain-containing protein
	fun44388	-0.71	Pre-mRNA-processing ATP-dependent RNA helicase PRP5, partial
	fun1973	-0.72	Astacin-like metalloendopeptidase
	fun14521	-0.72	Heterogeneous nuclear ribonucleoprotein A1
	fun34459	-0.72	hypothetical protein NECAME_00456
	fun47814	-0.72	Ribosomal protein L11, N-terminal domain containing protein
	fun18471	-0.72	hypothetical protein AOL_s00079g271
	fun9562	-0.72	CRE-TAG-194 protein

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year	gene	$\log_2$Fold Change	function.
	fun6036	-0.72	CBR-MOG-5 protein
	fun16037	-0.72	hypothetical protein AOL_s00007g357
	fun48205	-0.73	RNA recognition motif
	fun10723	-0.73	DEAD/DEAH box helicase family protein
	fun44207	-0.73	Polyadenylate-binding protein
	fun1110	-0.73	ADP/ATP translocase 4
	fun7854	-0.73	CRE-EXC-7 protein
	fun47763	-0.73	ribonucleoside-diphosphate reductase large subunit
	fun314	-0.73	39S ribosomal protein L3, mitochondrial
	fun634	-0.73	ABC transporter CDR4
	fun2169	-0.73	ATP-dependent RNA helicase Dbp2
	fun44678	-0.73	Probable ATP-dependent RNA helicase DDX47
	fun47480	-0.74	Regulator of nonsense transcripts 1 homolog
	fun52236	-0.74	Uncharacterized protein CELE_C46F11.4
	fun50857	-0.74	transcriptional enhancer factor TEF-4
	fun27034	-0.74	hypothetical protein CRE_04763
	fun24170	-0.74	Hypothetical protein CBG11408
	fun12411	-0.74	exosome complex exonuclease RRP44
	fun47764	-0.74	Ribonucleoside-diphosphate reductase large subunit
	fun2183	-0.75	ATP-dependent RNA helicase DDX3Y
	fun20905	-0.75	hypothetical protein AOL_s00188g279
	fun47765	-0.75	ribonucleoside-diphosphate reductase large subunit, partial
	fun9197	-0.75	CRE-RPL-2 protein
	fun17527	-0.75	hypothetical protein AOL_s00076g109
	fun12237	-0.76	Eukaryotic initiation factor 4A-II
	fun41515	-0.76	mitogen-activated protein kinase 15
	fun8561	-0.76	CRE-MOG-5 protein
	fun1077	-0.76	ADP-ribosylation factor 1-like 2
	fun518	-0.77	60S ribosomal protein L19, partial
	fun21535	-0.77	hypothetical protein AOL_s00215g344
	fun2187	-0.77	ATP-dependent RNA helicase DDX54
	fun44690	-0.78	Probable ATP-dependent RNA helicase pitchoune
	fun8814	-0.78	CRE-PAB-2 protein
	fun9608	-0.78	CRE-TAG-61 protein
	fun27199	-0.79	hypothetical protein CRE_05666
	fun2200	-0.79	ATP-dependent RNA helicase glh-3
	fun18955	-0.79	hypothetical protein AOL_s00081g211
	fun996	-0.80	Adenine Nucleotide Translocator
	fun10444	-0.80	cytochrome C oxidase assembly protein CtaG family protein
	fun28204	-0.80	hypothetical protein CRE_12342
	fun41084	-0.80	methyl-accepting chemotaxis protein signaling domain protein, partial
	fun32108	-0.80	hypothetical protein LOAG_03914
	fun17018	-0.81	hypothetical protein AOL_s00054g491
	fun36076	-0.81	hypothetical protein NECAME_09621
	fun50156	-0.81	T-complex protein 1, zeta subunit
	fun47766	-0.81	ribonucleoside-diphosphate reductase small chain
	fun39898	-0.81	KIAA0801 protein
	fun21715	-0.81	hypothetical protein AOL_s00215g590
	fun47762	-0.81	ribonucleoside-diphosphate reductase large chain
	fun2197	-0.81	ATP-dependent RNA helicase DRS1

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year	gene	$\log_2$Fold Change	function.
	fun44675	-0.81	Probable ATP-dependent RNA helicase DDX27
	fun50148	-0.82	T-complex protein 1, alpha subunit
	fun44677	-0.82	Probable ATP-dependent RNA helicase DDX46
	fun15156	-0.83	hypothetical protein AOL_s00004g208
	fun10705	-0.83	DEAD box ATP-dependent RNA helicase
	fun2216	-0.83	ATP-dependent rRNA helicase Rrp3
	fun20059	-0.83	hypothetical protein AOL_s00110g137
	fun16844	-0.83	hypothetical protein AOL_s00054g289
	fun41516	-0.84	Mitogen-activated protein kinase 15
	fun2202	-0.84	ATP-dependent RNA helicase in chromosome III
	fun2010	-0.84	Ataxin-2 binding protein 1
	fun11863	-0.85	EH domain-containing protein
	fun53511	-0.85	Vacuolar protein sorting-associated protein 53 homolog
	fun17323	-0.85	hypothetical protein AOL_s00054g927
	fun12749	-0.85	FI14826p
	fun1107	-0.86	ADP/ATP translocase
	fun2163	-0.86	ATP-dependent RNA helicase An3
	fun2189	-0.86	ATP-dependent RNA helicase ded1
	fun13198	-0.86	Gal_mutarotas_2 domain-containing protein
	fun44758	-0.86	Probable exosome complex exonuclease RRP44
	fun10725	-0.86	DEAD/DEAH box helicase, partial
	fun14917	-0.87	hsp70-like protein, variant 1
	fun10719	-0.87	DEAD-box ATP-dependent RNA helicase rde-12
	fun10817	-0.87	Delta(9)-fatty-acid desaturase fat-7
	fun24190	-0.87	Hypothetical protein CBG11510
	fun30207	-0.87	hypothetical protein CRE_25199
	fun4650	-0.88	C. briggsae CBR-RNR-1 protein
	fun32781	-0.88	hypothetical protein LOAG_07994
	fun44683	-0.88	Probable ATP-dependent RNA helicase DDX6
	fun2203	-0.88	ATP-dependent RNA helicase laf-1
	fun12623	-0.88	fatty acid desaturase
	fun10087	-0.88	cullin family protein
	fun2178	-0.89	ATP-dependent RNA helicase DDX18
	fun33086	-0.89	hypothetical protein LOAG_10109
	fun40977	-0.89	Membrane-bound transcription factor site-1 protease precursor, putative
	fun7450	-0.89	CRE-CPF-2 protein
	fun2208	-0.89	ATP-dependent RNA helicase RhlE
	fun2166	-0.89	ATP-dependent RNA helicase cgh-1
	fun34537	-0.89	hypothetical protein NECAME_00800
	fun17211	-0.89	hypothetical protein AOL_s00054g751
	fun1000	-0.90	adenosine 3'-phospho 5'-phosphosulfate transporter 1
	fun10721	-0.90	DEAD/DEAH box helicase
	fun47772	-0.91	ribonucleotide reductase R2 subunit
	fun15787	-0.91	hypothetical protein AOL_s00006g461
	fun17913	-0.91	hypothetical protein AOL_s00076g686
	fun13841	-0.91	Glycosyl hydrolases family 31 protein
	fun3434	-0.92	C. briggsae CBR-DRR-2 protein
	fun18451	-0.92	hypothetical protein AOL_s00079g234
	fun49962	-0.92	Suppressor of ACY-4 sterility
	fun24024	-0.92	Hypothetical protein CBG10097

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year	gene	$\log_2$Fold Change	function.
	fun44112	-0.93	PLaSTin (actin bundling protein) homolog
	fun14525	-0.93	heterogeneous nuclear ribonucleoprotein HRP1
	fun26999	-0.93	hypothetical protein CRE_04620
	fun41518	-0.94	Mitogen-activated protein kinase ERK-A
	fun2207	-0.94	ATP-dependent RNA helicase P62
	fun48112	-0.94	RNA binding protein, identical
	fun7624	-0.94	CRE-DDX-23 protein
	fun8663	-0.94	CRE-NDX-1 protein
	fun21289	-0.94	hypothetical protein AOL_s00210g38
	fun2718	-0.94	Bruno-3
	fun39755	-0.95	isoflavone 2'-hydroxylase-like
	fun48834	-0.95	serine/threonine-protein kinase polo
	fun10818	-0.95	Delta5 fatty acid desaturase, partial
	fun48982	-0.95	SF3b1 domain-containing protein
	fun9918	-0.95	CRE-VBH-1 protein
	fun47992	-0.95	ribosome biogenesis protein NSA2-like protein, partial
	fun39090	-0.95	hypothetical protein VFPFJ_09348
	fun10706	-0.95	DEAD boX helicase homolog
	fun13472	-0.96	Gluconokinase
	fun32662	-0.96	hypothetical protein LOAG_07126
	fun40167	-0.96	LD15481p
	fun26856	-0.96	hypothetical protein CRE_03900
	fun38479	-0.97	hypothetical protein VFPFJ_01453
	fun2214	-0.97	ATP-dependent RNA helicase vasa, partial
	fun22996	-0.97	Hypothetical protein CBG03667
	fun14425	-0.97	Helicase, C-terminal domain and DNA/RNA helicase, DEAD/DEAH box type, N-terminal domain and Helicase, superfamily 1/2, ATP-binding domain and RNA helicase, DEAD-box type, Q motif domain and P-loop containing nucleoside triphosphate hydrolase domain-containing protein
	fun19166	-0.97	hypothetical protein AOL_s00083g198
	fun35111	-0.98	hypothetical protein NECAME_03935
	fun15157	-0.98	hypothetical protein AOL_s00004g209
	fun49811	-0.98	Stress-associated endoplasmic reticulum protein family-containing protein
	fun40471	-0.98	Lon protease proteolytic domain protein, partial
	fun17628	-0.98	hypothetical protein AOL_s00076g264
	fun20256	-0.98	hypothetical protein AOL_s00112g73
	fun41509	-0.98	mitogen-activated protein kinase
	fun45843	-0.98	Protein-tyrosine phosphatase containing protein
	fun4688	-0.99	C. briggsae CBR-RPL-3 protein
	fun48251	-0.99	RNA-dependent helicase
	fun19533	-0.99	hypothetical protein AOL_s00097g196
	fun15138	-1.00	hypothetical protein AOL_s00004g18
	fun48125	-1.00	RNA helicase
	fun31360	-1.01	hypothetical protein CRE_31571
	fun527	-1.01	60S ribosomal protein L23a 2
	fun9162	-1.01	CRE-RNR-1 protein
	fun53400	-1.02	V-type proton ATPase subunit a
	fun6647	-1.02	Cleavage stimulation factor 64 kilodalton subunit
	fun11819	-1.02	EF hand family protein
	fun12899	-1.03	FMN-binding split barrel
	fun46206	-1.03	putative DEAD/DEAH box helicase

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year	gene	$\log_2$Fold Change	function.
	fun2168	-1.03	ATP-dependent RNA helicase DBP10
	fun11677	-1.03	Dynein light chain 1, cytoplasmic
	fun10710	-1.03	DEAD box polypeptide 17, variant 3
	fun7577	-1.04	CRE-CYP-42A1 protein
	fun15722	-1.04	hypothetical protein AOL_s00006g364
	fun16233	-1.05	hypothetical protein AOL_s00043g11
	fun1091	-1.07	ADP-ribosylation factor-like protein 3
	fun18361	-1.08	hypothetical protein AOL_s00079g109
	fun16565	-1.08	hypothetical protein AOL_s00043g641
	fun7884	-1.08	CRE-FAT-4 protein
	fun12520	-1.09	FA_desaturase domain-containing protein
	fun25555	-1.09	Hypothetical protein CBG20666
	fun2521	-1.09	beta-glucuronidase
	fun2171	-1.10	ATP-dependent RNA helicase DBP4
	fun48829	-1.10	serine/threonine-protein kinase plk-2
	fun41830	-1.10	Myosin heavy chain 95F
	fun24350	-1.10	Hypothetical protein CBG12465
	fun43246	-1.10	PCS-1 protein
	fun13830	-1.11	glycosyl hydrolase family 88
	fun32949	-1.11	hypothetical protein LOAG_09017
	fun42291	-1.11	Neuroendocrine convertase 2
	fun42300	-1.11	neurogenic locus notch homolog protein 1-like
	fun50155	-1.12	T-complex protein 1, variant
	fun52579	-1.12	Uncharacterized protein CELE_H20J04.4
	fun44674	-1.12	Probable ATP-dependent RNA helicase DDX23
	fun37285	-1.13	hypothetical protein NECAME_16332
	fun3832	-1.13	C. briggsae CBR-HUM-1 protein
	fun10711	-1.13	DEAD box polypeptide 27
	fun21635	-1.14	hypothetical protein AOL_s00215g49
	fun2185	-1.14	ATP-dependent RNA helicase DDX42
	fun48286	-1.14	RNP (RRM RNA binding domain) containing
	fun17949	-1.14	hypothetical protein AOL_s00076g87
	fun2212	-1.15	ATP-dependent RNA helicase T26G10.1 in chromosome III
	fun24598	-1.15	Hypothetical protein CBG13978
	fun13821	-1.16	glycosyl hydrolase family 31 protein
	fun27157	-1.17	hypothetical protein CRE_05393
	fun44844	-1.17	Probable nucleolar GTP-binding protein 1
	fun2786	-1.18	bZIP transcription factor (AtfA)
	fun53642	-1.18	Vitellogenin-1
	fun29477	-1.18	hypothetical protein CRE_20298
	fun10717	-1.19	DEAD-box ATP-dependent RNA helicase 52C, partial
	fun17957	-1.19	hypothetical protein AOL_s00076g97
	fun2344	-1.19	b-Raf proto-oncogene serine/threonine-protein kinase
	fun21916	-1.20	hypothetical protein AOL_s00215g873
	fun5877	-1.20	Casein kinase I isoform delta
	fun5332	-1.20	C. briggsae CBR-VBH-1 protein
	fun25212	-1.20	Hypothetical protein CBG18556
	fun33751	-1.20	hypothetical protein LOAG_16456
	fun2182	-1.21	ATP-dependent RNA helicase DDX3X family protein
	fun48126	-1.21	RNA helicase (Dbp)

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year	gene	$\log_2$Fold Change	function.
	fun37930	-1.21	hypothetical protein PpBr36_09066
	fun23051	-1.22	Hypothetical protein CBG03992
	fun10724	-1.22	DEAD/DEAH box helicase family protein, partial
	fun40355	-1.22	limpet
	fun46080	-1.22	putative ATP-dependent RNA helicase DDX56
	fun16730	-1.23	hypothetical protein AOL_s00054g117
	fun16555	-1.23	hypothetical protein AOL_s00043g626
	fun23701	-1.24	Hypothetical protein CBG08162
	fun11989	-1.24	endoglucanase-5-like
	fun18068	-1.25	hypothetical protein AOL_s00078g222
	fun49209	-1.25	sluggish A-PC
	fun15124	-1.25	hypothetical protein AOL_s00004g152
	fun12826	-1.26	fizzy-related protein
	fun36122	-1.26	hypothetical protein NECAME_09823
	fun50685	-1.27	transbilayer amphipath transporter protein 2
	fun31883	-1.27	hypothetical protein LOAG_02689
	fun9796	-1.28	CRE-UBQ-1 protein
	fun33381	-1.28	hypothetical protein LOAG_12553
	fun7442	-1.29	CRE-COQ-3 protein
	fun51637	-1.29	U5 small nuclear ribonucleoprotein 200 kDa helicase
	fun11619	-1.31	DUF5110 domain-containing protein
	fun21121	-1.31	hypothetical protein AOL_s00193g78
	fun29063	-1.32	hypothetical protein CRE_17716
	fun39636	-1.32	intermediate filament tail domain protein
	fun12306	-1.32	Eukaryotic translation initiation factor 3 subunit G
	fun52578	-1.33	Uncharacterized protein CELE_H19M22.2
	fun12513	-1.34	F-box/WD repeat-containing protein 1A
	fun40009	-1.34	Kunitz/Bovine pancreatic trypsin inhibitor domain protein
	fun2177	-1.35	ATP-dependent RNA helicase DDX1
	fun14362	-1.35	Heat shock protein 4
	fun3662	-1.35	C. briggsae CBR-GLH-1 protein
	fun48918	-1.35	Serine/threonine-protein phosphatase PP1-beta
	fun45452	-1.36	Protein lin-28
	fun49242	-1.36	Small GTPase superfamily and Ran GTPase family and Small GTPase superfamily, Rho type and Small GTPase superfamily, Rab type and Small GTP-binding protein domain and Small GTPase superfamily, Ras type and P-loop containing nucleoside triphosphate hydrolase domain-containing protein
	fun2188	-1.36	ATP-dependent RNA helicase DDX55
	fun9206	-1.37	CRE-RPL-27 protein
	fun39945	-1.37	kinesin-II subunit
	fun2164	-1.37	ATP-dependent RNA helicase An3, partial
	fun11279	-1.37	DNA topoisomerase 2-beta
	fun2816	-1.39	C-type LECtin
	fun43280	-1.41	pectin methylesterase 3
	fun33300	-1.42	hypothetical protein LOAG_11660
	fun29122	-1.43	hypothetical protein CRE_18002
	fun47364	-1.44	RE32166p
	fun42952	-1.45	Osmotic avoidance abnormal protein 3
	fun39948	-1.46	Kinesin-like protein at 64D
	fun3557	-1.46	C. briggsae CBR-FAT-4 protein
	fun10560	-1.47	cytoplasmic dynein 2 heavy chain 1, variant

Table D.5 continued from previous page

year	gene	$\log_2$Fold Change	function.
	fun34952	-1.48	hypothetical protein NECAME_03096
	fun1090	-1.49	ADP-ribosylation factor-like protein 2
	fun37875	-1.49	hypothetical protein NECAME_19424, partial
	fun4413	-1.50	C. briggsae CBR-PEK-1 protein
	fun39838	-1.50	kap beta 3 protein
	fun42537	-1.50	notch-like transmembrane receptor
	fun13157	-1.51	G-type lectin S-receptor-like serine/threonine-protein kinase RLK1 isoform X1
	fun33655	-1.51	hypothetical protein LOAG_15614, partial
	fun50526	-1.52	TIA-1/TIAL RNA binding protein homolog
	fun46856	-1.53	putative tetratricopeptide repeat-containing domain protein, partial
	fun39929	-1.53	Kinesin heavy chain
	fun11300	-1.54	DNA-binding protein A
	fun28086	-1.54	hypothetical protein CRE_11658, partial
	fun40986	-1.55	mesencephalic astrocyte-derived neurotrophic factor-like family protein
	fun39947	-1.56	Kinesin-like protein
	fun23971	-1.57	Hypothetical protein CBG09816
	fun39962	-1.57	Kinesin-like protein Klp68D
	fun24583	-1.58	Hypothetical protein CBG13909, partial
	fun46449	-1.58	putative laminin G domain protein
	fun27938	-1.58	hypothetical protein CRE_10783
	fun7809	-1.59	CRE-EIF-3.G protein
	fun53572	-1.59	Vasa-and Belle-like Helicase
	fun18328	-1.60	hypothetical protein AOL_s00078g71
	fun46249	-1.60	putative dynamin GTPase effector domain protein
	fun5700	-1.62	CAMK/CAMKL/MELK protein kinase
	fun6998	-1.63	CRE-AAGR-4 protein
	fun3811	-1.66	C. briggsae CBR-HRP-1 protein, partial
	fun14254	-1.70	Gut on exterior protein 2
	fun51659	-1.71	UBA_e1_C domain-containing protein
	fun28076	-1.72	hypothetical protein CRE_11630
	fun41150	-1.74	MFS hexose transporter
	fun1658	-1.75	Annexin family and Annexin, type III family and Annexin repeat-containing protein
	fun47632	-1.76	RH66426p, partial
	fun12284	-1.78	eukaryotic translation initiation factor 3 subunit 4
	fun51570	-1.81	Tyrosine-protein phosphatase cdc-14
	fun2650	-1.81	brahma associated protein
	fun10051	-1.82	CTD small phosphatase-like protein 2
	fun31127	-1.83	hypothetical protein CRE_30323
	fun13213	-1.83	galactoside-binding lectin
	fun3720	-1.88	C. briggsae CBR-GPD-3.1 protein
	fun1992	-1.95	AT09395p
	fun51425	-1.96	Tubulin polyglutamylase TTLL5
	fun17419	-1.97	hypothetical protein AOL_s00075g173
	fun12305	-1.97	eukaryotic translation initiation factor 3 subunit G
	fun28906	-1.97	hypothetical protein CRE_16583
	fun15356	-1.97	hypothetical protein AOL_s00004g481
	fun2989	-2.00	C. briggsae CBR-ASD-1 protein
	fun28395	-2.02	hypothetical protein CRE_13393
	fun44646	-2.13	Probable 40S ribosomal protein S14, mitochondrial
	fun12570	-2.17	Failed axon connections homolog

Table D.5 continued from previous page

year	gene	$\log_2$Fold Change	function.
	fun7883	-2.18	CRE-FAT-3 protein
	fun22557	-2.19	Hypothetical protein CBG01119
	fun40987	-2.33	Mesocentin
	fun30473	-2.35	hypothetical protein CRE_26440
	fun32427	-2.38	hypothetical protein LOAG_05721
	fun51423	-2.38	Tubulin polyglutamylase TTLL11
	fun33008	-2.51	hypothetical protein LOAG_09513
	fun42246	-3.27	nematode resistance protein-like HSPRO2
	fun40004	-4.30	Kunitz trypsin inhibitor precursor

Table D.6: Variation in the expression of soil microbial genes between months (September vs. June), based on RefSeq eukaryotic functional annotation (DESeq2, adjusted $P < 0.05$).

month	gene	log_2Fold Change	function.
	fun40004	4.33	Kunitz trypsin inhibitor precursor
	fun50292	2.75	tensin
	fun3057	2.68	C. briggsae CBR-CBP-1 protein, partial
	fun18838	2.48	hypothetical protein AOL_s00080g432
	fun19357	2.33	hypothetical protein AOL_s00083g450
	fun12281	2.20	eukaryotic translation initiation factor 3 subunit 10
	fun14730	2.14	histone-arginine methyltransferase CARM1, partial
	fun51138	2.14	transportin-SR
	fun10102	2.02	Cullin-5
	fun21909	1.87	hypothetical protein AOL_s00215g867
	fun46638	1.85	putative protease inhibitor
	fun3390	1.66	C. briggsae CBR-DNJ-18 protein
	fun30494	1.66	hypothetical protein CRE_26521
	fun1949	1.65	aspartic protease BmAsp-2, identical
	fun21102	1.64	hypothetical protein AOL_s00193g54
	fun42983	1.63	oxalate decarboxylase oxdC
	fun1521	1.61	ammonium transporter 3
	fun7127	1.60	CRE-AMT-3 protein
	fun26642	1.55	hypothetical protein CRE_02815
	fun27938	1.51	hypothetical protein CRE_10783
	fun5292	1.47	C. briggsae CBR-UNC-33 protein
	fun16335	1.38	hypothetical protein AOL_s00043g327
	fun46036	1.35	Putative ammonium transporter 1
	fun8547	1.30	CRE-MLT-7 protein
	fun46316	1.28	Putative galactocerebrosidase
	fun13621	1.26	Glutathione peroxidase 3
	fun7700	1.24	CRE-DNJ-8 protein
	fun1605	1.22	animal heme peroxidase
	fun8683	1.21	CRE-NHL-1 protein

Table D.6 continued from previous page

month	gene	log_2Fold Change	function.
	fun10016	1.20	CRE-ZYG-8 protein
	fun46038	1.19	Putative ammonium transporter 3
	fun9501	1.17	CRE-SUL-3 protein
	fun24213	1.16	Hypothetical protein CBG11702
	fun242	1.15	3-oxoacyl-(acyl-carrier-protein) reductase protein
	fun24999	1.12	Hypothetical protein CBG17045
	fun15776	1.11	hypothetical protein AOL_s00006g437
	fun46865	1.11	putative thrombospondin type 1 domain protein, partial
	fun42451	1.11	nitrite reductase
	fun18612	1.06	hypothetical protein AOL_s00079g62
	fun1522	1.05	Ammonium transporter 3, partial
	fun18992	1.04	hypothetical protein AOL_s00081g272
	fun29765	1.03	hypothetical protein CRE_22269
	fun8011	1.01	CRE-GLN-2 protein
	fun208	1.01	3-hydroxyacyl-CoA dehydrogenase, NAD binding domain
	fun27228	1.01	hypothetical protein CRE_05879
	fun31010	1.01	hypothetical protein CRE_29740
	fun52875	1.00	Uncharacterized protein CELE_Y22D7AL.10
	fun14075	1.00	GrpE protein homolog 2, mitochondrial
	fun18813	0.99	hypothetical protein AOL_s00080g393
	fun15305	0.98	hypothetical protein AOL_s00004g415
	fun27277	0.98	hypothetical protein CRE_06245
	fun1523	0.98	Ammonium transporter family and Ammonium transporter AmtB-like domain-containing protein
	fun43533	0.96	periplasmic nitrate reductase
	fun51174	0.95	trehalose 6-phosphate synthase 1
	fun6352	0.95	chaperonin GroS
	fun36701	0.93	hypothetical protein NECAME_13010
	fun20434	0.93	hypothetical protein AOL_s00169g269
	fun14312	0.92	HATPase_c domain-containing protein
	fun5380	0.92	C. briggsae CBR-WRN-1 protein, partial
	fun25270	0.92	Hypothetical protein CBG18990
	fun3665	0.92	C. briggsae CBR-GLN-1 protein, partial
	fun4956	0.92	C. briggsae CBR-SUL-1 protein, partial
	fun20276	0.92	hypothetical protein AOL_s00112g95
	fun21288	0.86	hypothetical protein AOL_s00210g376
	fun1520	0.86	ammonium transporter 2
	fun1526	0.86	ammonium transporter MEP3
	fun1518	0.86	ammonium transporter
	fun3826	0.85	C. briggsae CBR-HSP-6 protein
	fun15615	0.84	hypothetical protein AOL_s00006g212
	fun6706	0.84	co-chaperone GrpE
	fun46148	0.84	putative chaperonin GroL
	fun12903	0.84	FMN-dependent oxidoreductase, nitrilotriacetate monooxygenase family, partial
	fun3827	0.84	C. briggsae CBR-HSP-60 protein
	fun3190	0.83	C. briggsae CBR-COQ-5 protein
	fun13543	0.83	glutamate–ammonia ligase, catalytic domain protein
	fun6345	0.83	Chaperone protein dnaJ, partial
	fun16265	0.83	hypothetical protein AOL_s00043g163
	fun29384	0.83	hypothetical protein CRE_19644
	fun48874	0.81	Serine/threonine-protein kinase zyg-8

Table D.6 continued from previous page

month	gene	log_2Fold Change	function.
	fun31239	0.80	hypothetical protein CRE_30886
	fun6354	0.80	chaperonin homolog HSP60, mitochondrial precursor, partial
	fun21485	0.80	hypothetical protein AOL_s00215g289
	fun14337	0.79	Heat shock 60kD protein 1
	fun6353	0.79	Chaperonin homolog Hsp-60, mitochondrial
	fun1525	0.79	ammonium transporter MEP2
	fun13572	0.78	glutamine synthetase, beta-grasp domain protein
	fun14359	0.78	heat shock protein
	fun8195	0.77	CRE-HSP-60 protein
	fun21173	0.77	hypothetical protein AOL_s00210g162
	fun17034	0.76	hypothetical protein AOL_s00054g511
	fun15122	0.76	hypothetical protein AOL_s00004g150
	fun6358	0.76	chaperonine protein HSP60, partial
	fun13451	0.76	Gln-synt_C domain-containing protein
	fun20159	0.75	hypothetical protein AOL_s00110g48
	fun25354	0.75	Hypothetical protein CBG19479
	fun14363	0.75	heat shock protein 60, mitochondrial precursor
	fun39	0.74	10 kDa heat shock protein, mitochondrial
	fun6355	0.74	chaperonin Hsp-60
	fun6339	0.74	chaperone DnaK
	fun6350	0.73	chaperonin
	fun11428	0.73	dnaK protein
	fun21878	0.73	hypothetical protein AOL_s00215g823
	fun10291	0.73	cyclophilin-type peptidyl-prolyl cis-trans isomerase-15, Bmcyp-5
	fun14346	0.73	heat shock 70 kDa protein C precursor
	fun17222	0.72	hypothetical protein AOL_s00054g77
	fun10413	0.71	cytochrome b5 reductase
	fun14968	0.71	hydrolase, alpha/beta fold family protein
	fun17792	0.70	hypothetical protein AOL_s00076g511
	fun45345	0.70	protein GrpE
	fun12130	0.69	Epoxide hydrolase
	fun6346	0.69	chaperone protein DnaK
	fun16676	0.69	hypothetical protein AOL_s00043g789
	fun16089	0.69	hypothetical protein AOL_s00007g434
	fun6351	0.68	chaperonin GroL
	fun51693	0.68	Ubiquinone biosynthesis methyltransferase ZK652.9
	fun14877	0.68	homoserine acetyltransferase
	fun25261	0.68	Hypothetical protein CBG18950
	fun199	0.67	3-demethylubiquinone-9 3-methyltransferase
	fun12132	0.66	Epoxide hydrolase 1
	fun3667	0.66	C. briggsae CBR-GLN-3 protein
	fun11416	0.65	DnaJ protein subfamily B member 11
	fun14386	0.63	heat shock protein SSC1, mitochondrial precursor
	fun14348	0.63	Heat shock 70 kDa protein F, mitochondrial
	fun14390	0.63	heat shockprotein C
	fun44772	0.63	Probable glutamine synthetase
	fun12028	0.63	endoplasmin
	fun12076	0.63	enoyl-coA hydratase/isomerase family protein
	fun6356	0.62	chaperonin-10 kDa
	fun18416	0.62	hypothetical protein AOL_s00079g18

Table D.6 continued from previous page

month	gene	log_2Fold Change	function.
	fun14349	0.62	Heat shock 70 kDa protein F, mitochondrial precursor
	fun16696	0.62	hypothetical protein AOL_s00043g815
	fun42493	0.61	non-heme chloroperoxidase
	fun11401	0.61	DnaJ homolog subfamily B member 11
	fun8194	0.61	CRE-HSP-6 protein
	fun15860	0.61	hypothetical protein AOL_s00006g586
	fun14366	0.61	heat shock protein 70 (chloroplast)
	fun20653	0.61	hypothetical protein AOL_s00173g269
	fun19179	0.60	hypothetical protein AOL_s00083g212
	fun20535	0.60	hypothetical protein AOL_s00170g71
	fun13911	0.60	glyoxylate reductase protein
	fun45456	0.59	Protein lin-41
	fun14352	0.59	heat shock cognate protein 1
	fun18164	0.59	hypothetical protein AOL_s00078g353
	fun11418	0.59	DnaJ protein subfamily B member 6-B
	fun33700	0.58	hypothetical protein LOAG_16099, partial
	fun13495	0.58	glucose-regulated protein precursor
	fun38667	0.58	hypothetical protein VFPFJ_03985
	fun21262	0.57	hypothetical protein AOL_s00210g337
	fun14962	0.57	hydrolase
	fun13571	0.57	Glutamine synthetase 2 cytoplasmic
	fun17728	0.57	hypothetical protein AOL_s00076g39
	fun11388	0.57	DnaJ domain containing protein
	fun14915	0.57	hsp70-like protein
	fun7031	0.57	CRE-ACDH-10 protein
	fun20185	0.56	hypothetical protein AOL_s00110g94
	fun14074	0.56	GrpE nucleotide exchange factor
	fun6086	0.56	CBS/PB1 domain-containing protein
	fun12129	0.56	epoxide hydrolase
	fun22466	0.55	Hypothetical protein CBG00585, partial
	fun11393	0.55	DnaJ domain-containing protein
	fun51692	0.55	ubiquinone biosynthesis methyltransferase
	fun5812	0.54	Carboxylesterase family protein
	fun27288	0.54	hypothetical protein CRE_06278, partial
	fun1374	0.54	alpha-N-arabinofuranosidase precursor
	fun14343	0.54	Heat shock 70 kDa protein 1A/1B
	fun14340	0.54	Heat shock 70 kDa protein 13
	fun43435	0.53	Peptide methionine sulfoxide reductase family protein
	fun37539	0.53	hypothetical protein NECAME_17731
	fun52927	0.53	Uncharacterized protein CELE_Y47G6A.22
	fun14370	0.53	Heat shock protein 83
	fun1413	0.53	amidase family protein
	fun8192	0.53	CRE-HSP-4 protein
	fun11383	0.53	DnaJ carboxy terminal region family protein, partial
	fun14350	0.53	Heat shock cognate 71 kDa protein
	fun13758	0.53	Glycoprotein 93
	fun46146	0.52	putative chaperone protein DnaK
	fun37537	0.51	hypothetical protein NECAME_17729, partial
	fun6343	0.51	chaperone protein dnaJ 2
	fun6342	0.51	Chaperone protein dnaJ

Table D.6 continued from previous page

month	gene	log_2Fold Change	function.
	fun42344	0.51	Neutral and basic amino acid transport protein rBAT
	fun52475	0.51	Uncharacterized protein CELE_F44E5.5
	fun16376	0.50	hypothetical protein AOL_s00043g399
	fun31850	0.50	hypothetical protein LOAG_02490
	fun1389	0.50	alpha/beta hydrolase family domain-containing protein
	fun11397	0.50	DnaJ homolog dnj-20
	fun39786	0.50	J domain-containing protein
	fun3393	0.50	C. briggsae CBR-DNJ-20 protein
	fun51987	0.50	UMP-CMP kinase family protein
	fun24414	0.50	Hypothetical protein CBG12802
	fun17783	0.49	hypothetical protein AOL_s00076g498
	fun37833	0.49	hypothetical protein NECAME_19255, partial
	fun11547	0.49	drug resistance protein
	fun3388	0.49	C. briggsae CBR-DNJ-16 protein
	fun14365	0.49	heat shock protein 70
	fun4655	0.49	C. briggsae CBR-ROP-1 protein
	fun42449	0.48	nitrile hydratase, beta subunit
	fun37790	0.48	hypothetical protein NECAME_19092, partial
	fun14345	0.47	Heat shock 70 kDa protein C
	fun20692	0.47	hypothetical protein AOL_s00173g361
	fun18549	0.47	hypothetical protein AOL_s00079g4
	fun43454	0.47	Peptidyl-glycine alpha-amidating monooxygenase
	fun31176	0.47	hypothetical protein CRE_30616
	fun46396	0.47	putative Hsp90 protein, partial
	fun13569	0.47	glutamine synthetase
	fun3823	0.47	C. briggsae CBR-HSP-3 protein
	fun30247	0.46	hypothetical protein CRE_25393
	fun6357	0.46	chaperonin, 10 kDa
	fun29485	0.46	hypothetical protein CRE_20338
	fun30547	0.46	hypothetical protein CRE_26901
	fun12031	0.46	Endoplasmin precursor
	fun11414	0.45	DnaJ protein
	fun13	0.44	1-Cys peroxiredoxin, variant
	fun29077	0.44	hypothetical protein CRE_17774
	fun47132	0.44	r10h10-like protein TO42
	fun1403	0.44	alpha/beta-hydrolase
	fun46920	0.43	putative TROVE domain protein
	fun12013	0.43	Endoplasmic reticulum chaperone BiP homolog
	fun12029	0.43	Endoplasmin
	fun16919	0.43	hypothetical protein AOL_s00054g373
	fun51920	0.43	UDP-galactopyranose mutase
	fun14338	0.43	heat shock 70 kDa protein
	fun14256	0.42	GYD domain protein
	fun14922	0.42	Hsp90 protein
	fun6601	0.42	Class II glutamine amidotransferase domain and Glutamine amidotransferase type 2 domain-containing protein
	fun37627	0.42	hypothetical protein NECAME_18231
	fun11384	0.42	DnaJ chaperonine
	fun12	0.42	1-Cys peroxiredoxin
	fun11386	0.42	DnaJ domain and Chaperone DnaJ, C-terminal domain and HSP40/DnaJ peptide-binding domain-containing protein

Table D.6 continued from previous page

month	gene	log_2Fold Change	function.
	fun7691	0.41	CRE-DNJ-20 protein
	fun14389	0.41	heat shockprotein A
	fun11399	0.41	DnaJ homolog subfamily A member 3, mitochondrial
	fun8191	0.41	CRE-HSP-3 protein
	fun29364	0.41	hypothetical protein CRE_19549
	fun11403	0.41	DnaJ homolog subfamily B member 4
	fun46886	0.40	putative transketolase, thiamine diphosphate binding domain protein
	fun19574	0.40	hypothetical protein AOL_s00097g249
	fun19296	0.40	hypothetical protein AOL_s00083g363
	fun20581	0.39	hypothetical protein AOL_s00173g14
	fun18631	0.39	hypothetical protein AOL_s00079g87
	fun18265	0.39	hypothetical protein AOL_s00078g527
	fun35582	0.39	hypothetical protein NECAME_06632
	fun49915	0.39	Sulfatase domain-containing protein
	fun12032	0.38	endoplasmin protein
	fun41469	0.38	mitochondrial protein import protein MAS5
	fun43870	0.38	phosphoglycerate mutase family protein
	fun11390	0.38	DnaJ domain protein
	fun11424	0.38	DnaJ-like-2
	fun16577	0.38	hypothetical protein AOL_s00043g656
	fun11932	0.37	Elongation factor Ts, mitochondrial
	fun24371	0.37	Hypothetical protein CBG12562
	fun46260	0.37	putative endopeptidase La, partial
	fun25236	0.37	Hypothetical protein CBG18761
	fun11404	0.37	DnaJ homolog subfamily B member 9
	fun26006	0.36	Hypothetical protein CBG23896
	fun51708	0.36	ubiquinone/menaquinone biosynthesis methyltransferase
	fun39174	0.36	hypothetical protein VFPFJ_10516
	fun10980	0.35	Dihydropyrimidinase 1
	fun50918	0.34	Transketolase
	fun343	0.34	4-phosphoerythronate dehydrogenase
	fun50917	0.34	transketolase
	fun48396	0.34	S1 RNA binding domain protein
	fun15732	0.34	hypothetical protein AOL_s00006g376
	fun13256	0.33	Gamma-glutamyltranspeptidase family-containing protein
	fun19748	0.33	hypothetical protein AOL_s00097g509
	fun40917	0.33	Medium-chain specific acyl-CoA dehydrogenase, mitochondrial
	fun30954	0.32	hypothetical protein CRE_29422, partial
	fun11381	0.31	DnaJ and TPR domain-containing protein
	fun20544	0.31	hypothetical protein AOL_s00170g93
	fun46077	0.31	putative ATP-dependent protease La
	fun13800	0.31	Glycoside hydrolase, family 13, N-terminal domain and Glycosyl hydrolase, family 13, catalytic domain and Alpha-amylase, C-terminal all beta domain and 1,4-alpha-glucan-branching enzyme, GlgB family and Glycosyl hydrolase, family 13, subfamily, catalytic domain and Glycosyl hydrolase, family 13, all-beta domain and Glycoside hydrolase, catalytic domain and Immunoglobulin-like fold domain and Immunoglobulin E-set domain and Glycoside hydrolase, superfamily domain-containing protein
	fun40074	0.31	lactate dehydrogenase
	fun41077	0.31	methionine-R-sulfoxide reductase
	fun1385	0.31	alpha,alpha-trehalose-phosphate synthase 1
	fun46010	0.30	putative acyl-CoA dehydrogenase
	fun14368	0.30	heat shock protein 78, mitochondrial precursor

Table D.6 continued from previous page

month	gene	log_2Fold Change	function.
	fun2248	0.30	ATPase, AAA family
	fun41639	0.30	monothiol glutaredoxin-5
	fun41379	0.30	mitochondrial DnaJ chaperone (Mdj1)
	fun44812	0.30	Probable medium-chain specific acyl-CoA dehydrogenase 10, mitochondrial
	fun19696	0.29	hypothetical protein AOL_s00097g448
	fun41336	0.29	mitochondrial 54S ribosomal protein YmL23
	fun7108	0.29	CRE-ALH-1 protein
	fun213	0.29	3-hydroxyisobutyrate dehydrogenase
	fun19514	0.29	hypothetical protein AOL_s00097g171
	fun15766	0.29	hypothetical protein AOL_s00006g422
	fun43134	0.28	pantoate-beta-alanine ligase
	fun25891	0.28	Hypothetical protein CBG23035
	fun13844	0.28	glycosyl transferase
	fun1800	0.28	argininosuccinate lyase
	fun5853	0.27	carnitinyl-CoA dehydratase
	fun14966	0.27	hydrolase, alpha/beta domain protein
	fun46256	0.26	putative elongation factor TS
	fun21653	0.26	hypothetical protein AOL_s00215g514
	fun12829	0.26	FK506-binding protein 2
	fun21352	0.26	hypothetical protein AOL_s00215g119
	fun42445	0.26	Nitrilase homolog
	fun12006	0.26	endopeptidase Clp
	fun41481	0.26	Mitochondrial Ribosomal Protein, Large
	fun40468	0.26	Lon protease homolog 2, peroxisomal
	fun12007	0.26	endopeptidase La, partial
	fun7401	0.25	CRE-CLPP-1 protein
	fun53963	0.25	xylulose-5-phosphate phosphoketolase
	fun20561	0.25	hypothetical protein AOL_s00173g116
	fun17873	0.25	hypothetical protein AOL_s00076g641
	fun2157	0.25	ATP-dependent protease La
	fun12635	0.24	fatty acid oxidation complex, alpha subunit, partial
	fun15464	0.23	hypothetical protein AOL_s00004g624
	fun50433	0.23	thioredoxin peroxidase, identical
	fun46999	0.22	putative xanthine dehydrogenase, small subunit
	fun17604	0.21	hypothetical protein AOL_s00076g229
	fun2158	0.21	ATP-dependent protease La 2
	fun49055	0.20	Short-chain dehydrogenase/reductase SDR family and Glucose/ribitol dehydrogenase family and NAD(P)-binding domain-containing protein
	fun43558	0.20	Peroxiredoxin-6
	fun2127	0.19	ATP-dependent Clp protease proteolytic subunit 1, mitochondrial
	fun29083	0.18	hypothetical protein CRE_17781, partial
	fun43433	0.18	peptide methionine sulfoxide
	fun10609	0.17	D-3-phosphoglycerate dehydrogenase
	fun2152	0.17	ATP-dependent metallopeptidase HflB
	fun14937	0.17	Human spg
	fun53561	0.16	valyl-tRNA synthetase, mitochondrial precursor
	fun41080	0.15	methionyl-tRNA synthetase
	fun16890	0.14	hypothetical protein AOL_s00054g342
	fun42049	-0.13	NADH dehydrogenase
	fun19558	-0.15	hypothetical protein AOL_s00097g226

Table D.6 continued from previous page

month	gene	log_2Fold Change	function.
	fun42066	-0.19	NADH dehydrogenase subunit 5 (mitochondrion)
	fun27145	-0.20	hypothetical protein CRE_05365, partial
	fun20032	-0.22	hypothetical protein AOL_s00110g104
	fun29695	-0.23	hypothetical protein CRE_21708
	fun42062	-0.23	NADH dehydrogenase subunit 4 (mitochondrion)
	fun11886	-0.23	Electron transfer flavoprotein subunit beta
	fun1443	-0.23	amino acid adenylation domain-containing protein
	fun42506	-0.25	nonribosomal peptide synthase
	fun6794	-0.26	cold-shock DNA-binding domain-containing protein
	fun20014	-0.27	hypothetical protein AOL_s00109g87
	fun2249	-0.27	ATPase, AAA family protein
	fun10965	-0.27	Dihydroorotate dehydrogenase (quinone), mitochondrial
	fun44857	-0.30	Probable peroxiredoxin prdx-3
	fun21480	-0.30	hypothetical protein AOL_s00215g283
	fun20862	-0.30	hypothetical protein AOL_s00188g194
	fun48206	-0.31	RNA recognition motif containing protein
	fun6710	-0.32	CoA binding domain protein
	fun5790	-0.32	carbonic anhydrase 2
	fun807	-0.32	aconitate hydratase, mitochondrial precursor
	fun45509	-0.32	Protein MTO1 homolog, mitochondrial
	fun11140	-0.33	DNA mismatch repair protein Mlh1
	fun6211	-0.33	Cell division protein ftsZ
	fun606	-0.34	AAA domain-containing protein
	fun29091	-0.34	hypothetical protein CRE_17796, partial
	fun13772	-0.35	glycoside hydrolase family 2 protein
	fun18183	-0.36	hypothetical protein AOL_s00078g389
	fun16599	-0.36	hypothetical protein AOL_s00043g680
	fun50430	-0.37	thioredoxin peroxidase 1
	fun44615	-0.37	Probable 26S proteasome regulatory subunit 6B
	fun19505	-0.38	hypothetical protein AOL_s00097g160
	fun37287	-0.38	hypothetical protein NECAME_16335
	fun37519	-0.38	hypothetical protein NECAME_17644
	fun19269	-0.39	hypothetical protein AOL_s00083g329
	fun6205	-0.40	cell division cycle protein 48
	fun18648	-0.40	hypothetical protein AOL_s00080g109
	fun41041	-0.40	metalloprotease 1 protein
	fun16082	-0.40	hypothetical protein AOL_s00007g424
	fun8248	-0.41	CRE-INF-1 protein
	fun43971	-0.41	phosphoribosylformylglycinamidine synthase
	fun10201	-0.41	cyclic diguanylate phosphodiesterase domain protein
	fun24418	-0.41	Hypothetical protein CBG12821
	fun13353	-0.42	general secretion pathway protein G
	fun1926	-0.43	Aspartate aminotransferase
	fun14090	-0.44	GTP cyclohydrolase-2
	fun49896	-0.44	sugar isomerase (SIS)
	fun19284	-0.44	hypothetical protein AOL_s00083g347
	fun74	-0.44	2-methylcitrate dehydratase
	fun45897	-0.45	Protoheme IX farnesyltransferase, mitochondrial
	fun14708	-0.45	Histone H3
	fun6581	-0.46	citrate synthase, mitochondrial precursor

Table D.6 continued from previous page

month	gene	log_2Fold Change	function.
	fun31130	-0.46	hypothetical protein CRE_30338
	fun43839	-0.48	phosphoenolpyruvate synthase
	fun39549	-0.48	inositol-3-phosphate synthase
	fun42087	-0.48	NADH-quinone oxidoreductase, E subunit
	fun16407	-0.48	hypothetical protein AOL_s00043g445
	fun5588	-0.49	calcium-translocating P-type ATPase, PMCA-type
	fun39722	-0.49	Iron-sulfur cluster assembly 1 homolog, mitochondrial
	fun17840	-0.49	hypothetical protein AOL_s00076g589
	fun47887	-0.49	ribosomal protein S14, S11 family
	fun50150	-0.49	T-complex protein 1, delta subunit
	fun21105	-0.49	hypothetical protein AOL_s00193g59
	fun43612	-0.50	PEX6 protein
	fun26526	-0.50	hypothetical protein CRE_02046
	fun39550	-0.50	Inositol-3-phosphate synthase 1
	fun19021	-0.50	hypothetical protein AOL_s00081g313
	fun17314	-0.51	hypothetical protein AOL_s00054g907
	fun29379	-0.52	hypothetical protein CRE_19635
	fun417	-0.52	5-methyltetrahydropteroyltriglutamate-homocysteine S-methyltransferase
	fun42943	-0.52	Orotate phosphoribosyltransferase
	fun2926	-0.52	C. briggsae CBR-ALH-9 protein
	fun29842	-0.53	hypothetical protein CRE_22769
	fun346	-0.53	40S ribosomal protein
	fun19647	-0.53	hypothetical protein AOL_s00097g365
	fun17635	-0.55	hypothetical protein AOL_s00076g270
	fun47826	-0.56	ribosomal protein L19
	fun47858	-0.56	ribosomal protein L37e
	fun28907	-0.56	hypothetical protein CRE_16594
	fun29064	-0.57	hypothetical protein CRE_17717, partial
	fun13642	-0.57	glutathione S-transferase protein
	fun17035	-0.57	hypothetical protein AOL_s00054g512
	fun53430	-0.57	vacuolar ATP synthase catalytic subunit A
	fun19339	-0.58	hypothetical protein AOL_s00083g420
	fun31139	-0.58	hypothetical protein CRE_30353
	fun20676	-0.58	hypothetical protein AOL_s00173g337
	fun16245	-0.58	hypothetical protein AOL_s00043g133
	fun18692	-0.58	hypothetical protein AOL_s00080g174
	fun43718	-0.59	Phosphate carrier protein, mitochondrial
	fun15382	-0.59	hypothetical protein AOL_s00004g515
	fun4020	-0.59	C. briggsae CBR-LIN-28 protein, partial
	fun51687	-0.60	ubiquinol-cytochrome c reductase, iron-sulfur subunit family protein
	fun47764	-0.60	Ribonucleoside-diphosphate reductase large subunit
	fun47846	-0.61	ribosomal protein L3
	fun16181	-0.61	hypothetical protein AOL_s00007g551
	fun812	-0.61	actin
	fun501	-0.61	60S ribosomal protein L11-2
	fun51212	-0.62	tripeptidyl peptidase SED3
	fun47847	-0.63	ribosomal protein L30p/L7e
	fun13633	-0.64	glutathione S-transferase domain-containing protein
	fun23546	-0.64	Hypothetical protein CBG07031
	fun50215	-0.64	TATA-box binding protein

Table D.6 continued from previous page

month	gene	log_2Fold Change	function.
	fun47819	-0.64	ribosomal protein L14b/L23e
	fun18932	-0.64	hypothetical protein AOL_s00081g168
	fun17794	-0.65	hypothetical protein AOL_s00076g519
	fun10497	-0.66	cytochrome c1, heme protein, mitochondrial precursor
	fun40103	-0.66	laminin receptor 1
	fun43590	-0.67	peroxisome biogenesis factor 1
	fun47925	-0.67	ribosomal protein S6e
	fun12237	-0.67	Eukaryotic initiation factor 4A-II
	fun19911	-0.67	hypothetical protein AOL_s00109g150
	fun16830	-0.68	hypothetical protein AOL_s00054g268
	fun19036	-0.68	hypothetical protein AOL_s00081g332
	fun43465	-0.68	Peptidyl-prolyl cis-trans isomerase 3
	fun12876	-0.68	flavohemoglobin
	fun50041	-0.69	SWIB/MDM2 domain-containingprotein
	fun51160	-0.69	TRAP transporter solute receptor, TAXI family, partial
	fun14699	-0.69	histone H2B
	fun6764	-0.69	cofilin/tropomyosin-type actin-binding protein
	fun18373	-0.69	hypothetical protein AOL_s00079g121
	fun25493	-0.69	Hypothetical protein CBG20269
	fun47931	-0.69	ribosomal protein S8
	fun14716	-0.70	Histone H3.3
	fun7170	-0.70	CRE-ARX-1 protein
	fun20445	-0.72	hypothetical protein AOL_s00169g4
	fun46279	-0.72	Putative exosome complex exonuclease RRP41
	fun26246	-0.72	hypothetical protein CRE_00653
	fun17553	-0.73	hypothetical protein AOL_s00076g149
	fun5539	-0.74	calcium ATPase
	fun540	-0.74	60S ribosomal protein L33
	fun15436	-0.74	hypothetical protein AOL_s00004g585
	fun44656	-0.74	Probable actin-related protein 2/3 complex subunit 4
	fun15757	-0.74	hypothetical protein AOL_s00006g41
	fun16300	-0.74	hypothetical protein AOL_s00043g26
	fun815	-0.74	actin 1
	fun50857	-0.74	transcriptional enhancer factor TEF-4
	fun284	-0.75	30S ribosomal protein S9
	fun26838	-0.75	hypothetical protein CRE_03839
	fun47900	-0.76	ribosomal protein S22, S8 family
	fun41358	-0.76	mitochondrial carrier protein
	fun365	-0.76	40S ribosomal protein S19S, variant
	fun43670	-0.77	phenylalanine ammonia-lyase
	fun47259	-0.77	Ras protein let-60
	fun14155	-0.77	GTPase activating protein Sar1
	fun44089	-0.77	plasma membrane ATPase 2
	fun12343	-0.78	eukaryotic translation initiation factor eIF-1A
	fun15347	-0.79	hypothetical protein AOL_s00004g468
	fun20753	-0.79	hypothetical protein AOL_s00173g93
	fun12814	-0.80	fimbrin
	fun15752	-0.80	hypothetical protein AOL_s00006g40
	fun11833	-0.81	Ef1alpha-like factor
	fun49249	-0.81	small GTPase superfamily, Rho type

Table D.6 continued from previous page

month	gene	log_2Fold Change	function.
	fun46267	-0.81	putative epimerase/reductase
	fun813	-0.81	Actin
	fun20451	-0.82	hypothetical protein AOL_s00169g5
	fun14706	-0.82	histone H2B, partial
	fun41852	-0.82	myosin type II heavy chain
	fun21798	-0.83	hypothetical protein AOL_s00215g7
	fun18806	-0.83	hypothetical protein AOL_s00080g371
	fun26790	-0.83	hypothetical protein CRE_03641
	fun53533	-0.83	Vacuolar proton pump subunit B
	fun12489	-0.83	F-actin-capping protein subunit beta
	fun3460	-0.84	C. briggsae CBR-ECH-4 protein
	fun49008	-0.84	SH3 domain protein
	fun47810	-0.85	ribosomal protein L10.e
	fun41858	-0.85	myosin-1
	fun814	-0.86	ACTin
	fun47277	-0.86	Ras-like protein 3
	fun40481	-0.86	long-chain fatty acid transporter
	fun816	-0.86	actin 2
	fun47791	-0.87	ribosomal L18ae protein
	fun16506	-0.87	hypothetical protein AOL_s00043g572
	fun831	-0.88	actin-binding protein
	fun40665	-0.89	maintenance of ploidy protein mob1
	fun44794	-0.90	Probable histone H2B 3
	fun18324	-0.90	hypothetical protein AOL_s00078g68
	fun5428	-0.91	C2 domain-containing protein
	fun48857	-0.92	serine/threonine-protein kinase srk1
	fun47825	-0.92	ribosomal protein L17
	fun45138	-0.92	proteasome subunit alpha type-5
	fun867	-0.93	Actin, alpha cardiac muscle 1
	fun27551	-0.94	hypothetical protein CRE_08027
	fun20291	-0.94	hypothetical protein AOL_s00117g2
	fun41166	-0.94	MFS sugar transporter
	fun1329	-0.94	alpha-actinin, sarcomeric (f-actin cross linking protein)
	fun1857	-0.95	ARP2/3 complex 21 kDa subunit
	fun40639	-0.95	Macrophage-capping protein
	fun13371	-0.95	generic methyltransferase
	fun18711	-0.95	hypothetical protein AOL_s00080g218
	fun8995	-0.96	CRE-PRDX-3 protein
	fun21856	-0.96	hypothetical protein AOL_s00215g8
	fun14725	-0.97	Histone H4 family and Histone core domain and Histone-fold domain-containing protein
	fun14689	-0.98	Histone H2A domain and Histone core domain and Histone-fold domain-containing protein
	fun13342	-0.98	gelsolin repeat protein
	fun44795	-0.98	Probable histone H2B 4
	fun7072	-0.98	CRE-ACT-5 protein
	fun50039	-0.99	SWIB/MDM2 domain protein
	fun3066	-1.00	C. briggsae CBR-CCT-6 protein
	fun48498	-1.00	SCP-like protein
	fun6018	-1.00	CBR-HUM-5 protein
	fun41836	-1.00	myosin heavy chain, nonmuscle type 1
	fun6195	-1.03	cell division control protein 42-like protein

Table D.6 continued from previous page

month	gene	log_2Fold Change	function.
	fun47326	-1.03	Ras-related protein Rap-1
	fun7295	-1.03	CRE-CCT-6 protein
	fun49548	-1.03	spectrin beta chain
	fun6922	-1.04	copper-containing nitrite reductase
	fun38726	-1.05	hypothetical protein VFPFJ_04663
	fun47327	-1.05	Ras-related protein Rap-1A
	fun1861	-1.05	ARP2/3 complex subunit
	fun1406	-1.05	Alternative oxidase
	fun26322	-1.05	hypothetical protein CRE_01043
	fun32599	-1.06	hypothetical protein LOAG_06763
	fun45486	-1.07	protein mannosyltransferase 1
	fun1	-1.07	Unannotated
	fun17079	-1.07	hypothetical protein AOL_s00054g577
	fun1854	-1.07	ARP2/3 actin-organizing complex subunit Sop2
	fun29063	-1.08	hypothetical protein CRE_17716
	fun20232	-1.08	hypothetical protein AOL_s00112g37
	fun485	-1.09	60s Acidic ribosomal protein
	fun16598	-1.09	hypothetical protein AOL_s00043g68
	fun1797	-1.10	arginine/ornithine antiporter domain protein, partial
	fun14914	-1.11	Hsp70 family protein
	fun18768	-1.12	hypothetical protein AOL_s00080g303
	fun45452	-1.13	Protein lin-28
	fun43299	-1.13	Peptidase A1 domain-containing protein
	fun25689	-1.13	Hypothetical protein CBG21460
	fun47254	-1.14	ras GTPase activator
	fun1757	-1.16	aquaporin PIP2-6
	fun7172	-1.17	CRE-ARX-3 protein
	fun26782	-1.18	hypothetical protein CRE_03607
	fun8153	-1.19	CRE-HIS-51 protein
	fun50178	-1.21	Talin 1, partial
	fun16371	-1.22	hypothetical protein AOL_s00043g392
	fun17606	-1.23	hypothetical protein AOL_s00076g231
	fun6953	-1.23	Coronin-like protein
	fun43215	-1.23	patched sphingolipid transporter (Ncr1)
	fun42883	-1.23	oligopeptide transporter
	fun859	-1.26	Actin-related protein 2/3 complex subunit 4
	fun828	-1.27	Actin-4
	fun38575	-1.28	hypothetical protein VFPFJ_02788
	fun40329	-1.29	LIM and SH3 domain protein 1
	fun44994	-1.30	Profilin
	fun32010	-1.31	hypothetical protein LOAG_03476
	fun51150	-1.31	Transthyretin-like family protein
	fun42907	-1.32	OPT oligopeptide transporter family
	fun45001	-1.32	profilin-like protein
	fun46291	-1.36	putative Fe-S protein assembly co-chaperone HscB
	fun3110	-1.36	C. briggsae CBR-CGP-1 protein, partial
	fun5484	-1.36	cache domain protein, partial
	fun7171	-1.38	CRE-ARX-2 protein
	fun38479	-1.38	hypothetical protein VFPFJ_01453
	fun44113	-1.38	plastin-2

Table D.6 continued from previous page

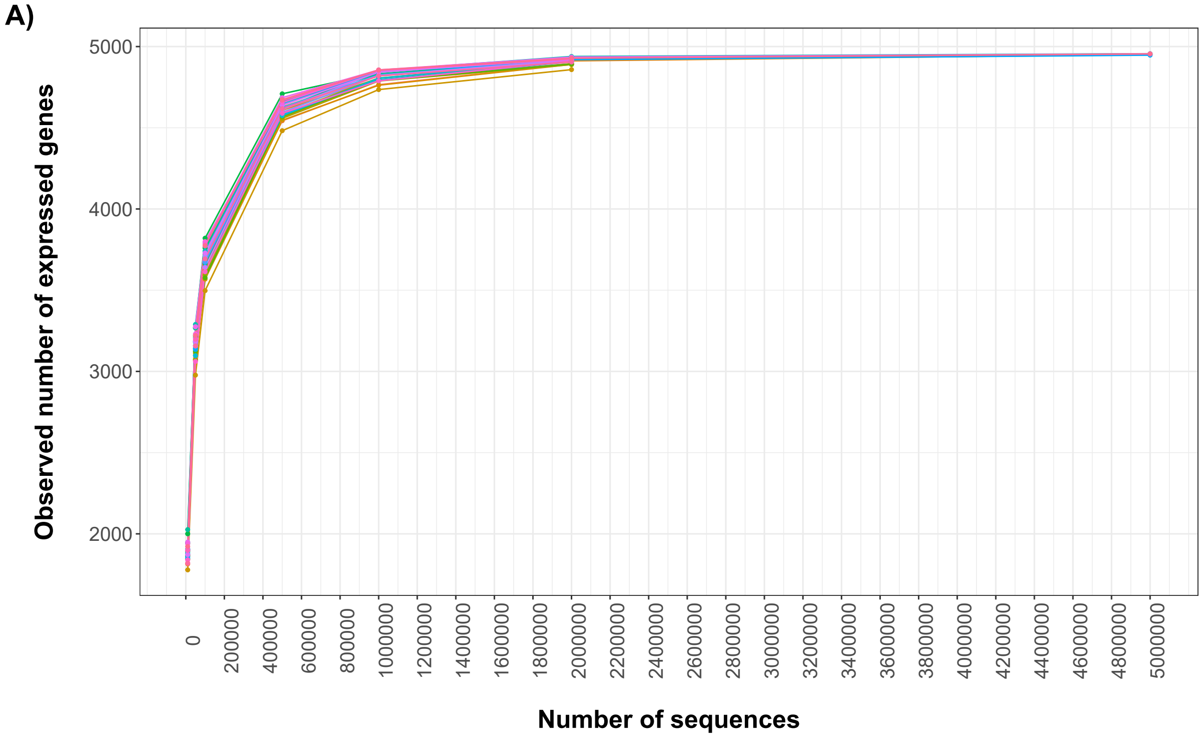
month	gene	log_2Fold Change	function.
	fun3606	-1.39	C. briggsae CBR-FTN-1 protein
	fun836	-1.39	Actin-depolymerising factor homology domain-containing protein
	fun3858	-1.40	C. briggsae CBR-IFTB-1 protein
	fun21047	-1.40	hypothetical protein AOL_s00193g145
	fun40212	-1.41	lea domain-containingprotein
	fun45773	-1.42	Protein unc-87
	fun38371	-1.44	hypothetical protein Tsp_15924, partial
	fun20641	-1.45	hypothetical protein AOL_s00173g249
	fun16481	-1.45	hypothetical protein AOL_s00043g54
	fun16722	-1.45	hypothetical protein AOL_s00054g106
	fun15124	-1.48	hypothetical protein AOL_s00004g152
	fun16843	-1.48	hypothetical protein AOL_s00054g285
	fun40451	-1.51	LMBR1 domain-containing protein
	fun12532	-1.52	FACT complex subunit SSRP1
	fun33079	-1.52	hypothetical protein LOAG_10011
	fun6807	-1.52	collagen triple helix repeat (20 copies) domain-containing protein
	fun1325	-1.54	alpha-2-macroglobulin family protein
	fun41961	-1.56	N,O-diacetyl muramidase
	fun38971	-1.57	hypothetical protein VFPFJ_07854
	fun42239	-1.57	Nematode cuticle collagen, N-terminal domain and Collagen triple helix repeat-containing protein
	fun34180	-1.60	hypothetical protein LOAG_18294
	fun18293	-1.61	hypothetical protein AOL_s00078g582
	fun10692	-1.62	DDHD domain-containing protein
	fun13287	-1.62	GATA transcription factor LreA
	fun38728	-1.63	hypothetical protein VFPFJ_04728
	fun51577	-1.63	tyrosine-protein phosphatase non-receptor type 6
	fun18954	-1.66	hypothetical protein AOL_s00081g21
	fun40141	-1.66	late embryogenesis abundant protein
	fun1055	-1.67	ADF-like domain-containing protein
	fun49578	-1.68	sphingolipid long chain base-responsive protein PIL1
	fun13805	-1.70	Glycoside hydrolase, family 25 and Glycoside hydrolase, catalytic domain and Glycoside hydrolase, superfamily domain-containing protein
	fun5351	-1.71	C. briggsae CBR-VIT-2 protein
	fun25316	-1.73	Hypothetical protein CBG19286
	fun43541	-1.75	Peroxidase superfamily protein
	fun9208	-1.76	CRE-RPL-3 protein
	fun9191	-1.77	CRE-RPL-12 protein
	fun41176	-1.77	MFS transporter, SP family, sugar:H+ symporter
	fun10398	-1.80	cytochrome b-c1 complex subunit 2
	fun4656	-1.82	C. briggsae CBR-RPA-0 protein
	fun4295	-1.82	C. briggsae CBR-NMY-2 protein
	fun20210	-1.85	hypothetical protein AOL_s00112g125
	fun53654	-1.85	Voltage gated chloride channel family protein, partial
	fun11973	-1.86	endo-1,4-beta-glucancase
	fun38901	-1.86	hypothetical protein VFPFJ_06808
	fun50012	-1.88	survival factor 1
	fun41342	-1.90	mitochondrial ATP synthase epsilon chain domain-containing protein
	fun28906	-1.90	hypothetical protein CRE_16583
	fun24243	-1.91	Hypothetical protein CBG11894
	fun50877	-1.92	transcriptional regulator, MarR family

Table D.6 continued from previous page

month	gene	log_2Fold Change	function.
	fun43157	-1.93	PAP2 superfamily protein
	fun12786	-1.94	Fibronectin, type III domain and Immunoglobulin-like fold domain-containing protein
	fun4294	-1.98	C. briggsae CBR-NMY-1 protein
	fun43989	-1.99	phosphorylcholine phosphatase
	fun44095	-2.00	plasma membrane intrinsic protein 2E
	fun16327	-2.00	hypothetical protein AOL_s00043g301
	fun39643	-2.06	intracellular kinase
	fun25322	-2.09	Hypothetical protein CBG19303
	fun24337	-2.10	Hypothetical protein CBG12375
	fun29175	-2.13	hypothetical protein CRE_18364
	fun30151	-2.14	hypothetical protein CRE_24917
	fun17952	-2.14	hypothetical protein AOL_s00076g9
	fun38552	-2.17	hypothetical protein VFPFJ_02467
	fun43178	-2.19	Paralysed Arrest at Two-fold
	fun44430	-2.19	pre-rRNA processing protein
	fun15356	-2.20	hypothetical protein AOL_s00004g481
	fun6803	-2.21	collagen col-34
	fun5303	-2.23	C. briggsae CBR-UNC-54 protein
	fun6802	-2.24	Collagen alpha-5(IV) chain
	fun54295	-2.26	zona pellucida-like domain protein
	fun42235	-2.32	nematode cuticle collagen domain protein
	fun38073	-2.36	Hypothetical protein SRAE_2000067400
	fun51515	-2.38	tyrosinase
	fun13223	-2.45	galectin
	fun445	-2.46	50S ribosomal protein L21
	fun9163	-2.49	CRE-RNR-2 protein
	fun29734	-2.50	hypothetical protein CRE_22106
	fun2363	-2.55	bacteriophage N adsorption protein A c-term domain-containing protein
	fun1733	-2.56	apolipoprotein/apolipophorin
	fun17120	-2.63	hypothetical protein AOL_s00054g626
	fun33881	-2.63	hypothetical protein LOAG_17054
	fun18681	-2.64	hypothetical protein AOL_s00080g161
	fun6790	-2.68	Col_cuticle_N domain-containing protein
	fun41150	-2.75	MFS hexose transporter
	fun21065	-2.85	hypothetical protein AOL_s00193g173
	fun31127	-2.89	hypothetical protein CRE_30323
	fun23603	-3.04	Hypothetical protein CBG07436
	fun1337	-4.06	alpha-amylase inhibitor/lipid transfer/seed storage family protein precursor

APPENDIX E

SUPPLEMENTARY FIGURES - CHAPTER III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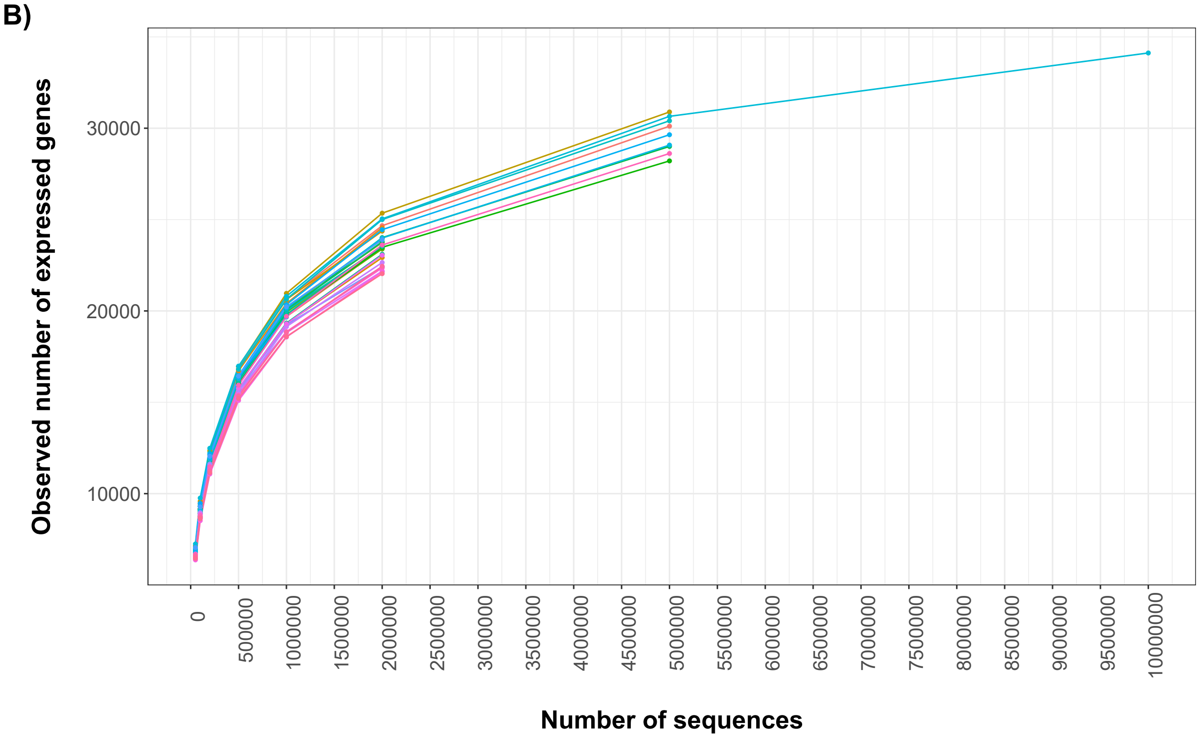
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Observed number of expressed genes
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1000000
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Number of sequences

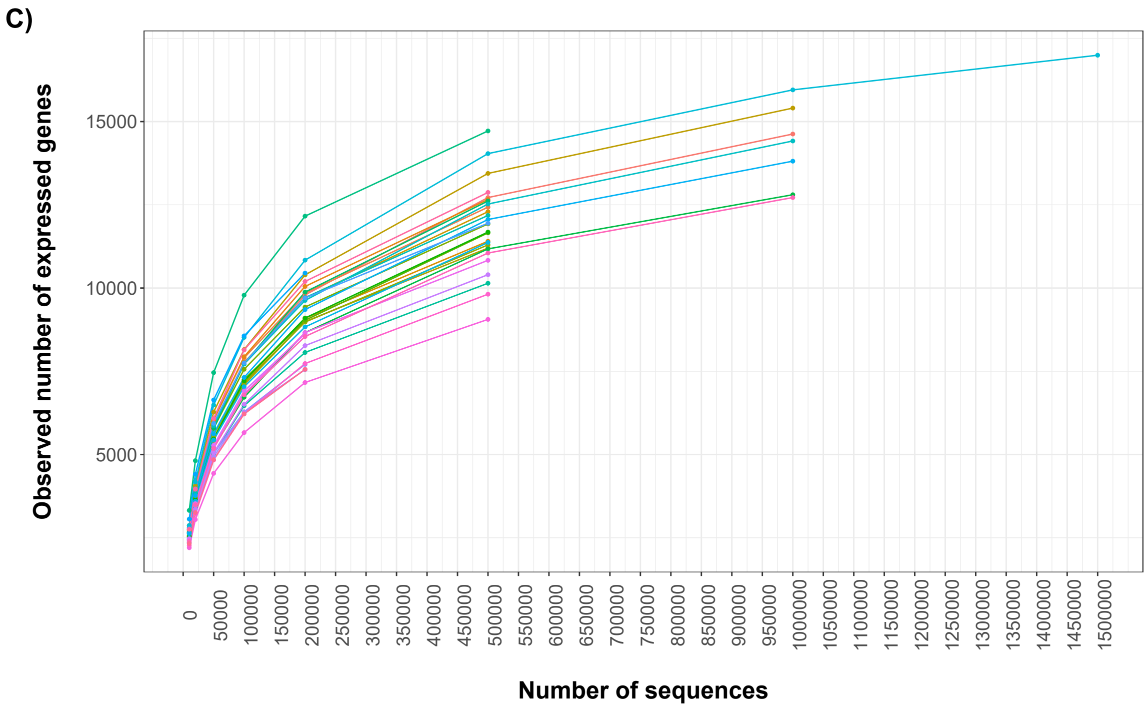
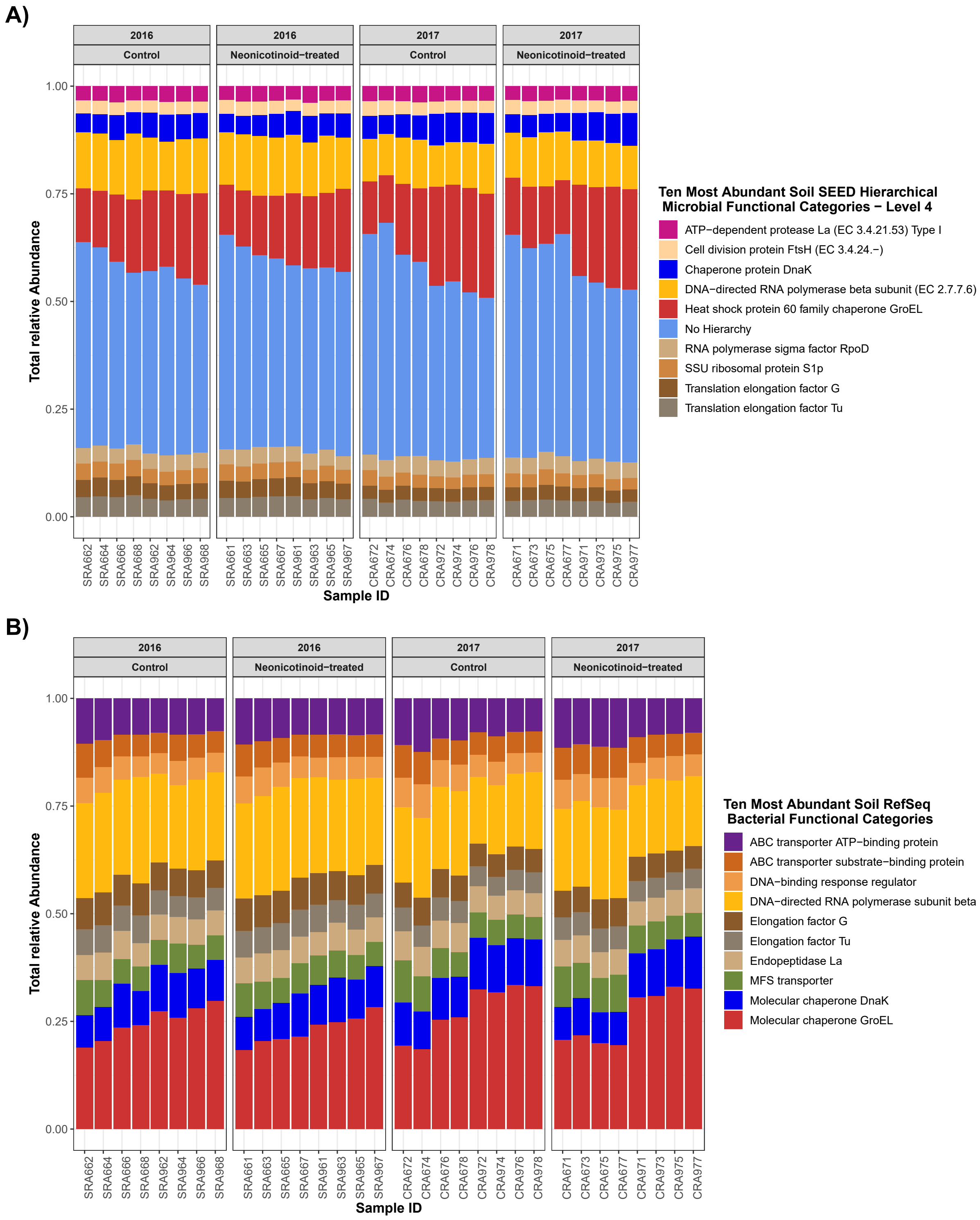


Figure E.1: Rarefaction curves of the soil microbial gene expression. Rarefaction curves for SEED hierarchical microbial (level4; A), RefSeq bacterial (B) and RefSeq eukaryotic (C) gene expression according to the observed number of expressed genes in soil samples of a two-year soybean/corn rotation in l'Acadie, Quebec, Canada. Each line and color represent one soil sample. The maximum sequencing coverage (x-axis: number of expressed genes) is 5,000,000 reads with cutoffs at 10,000, 50,000, 100,000, 500,000, 1,000,000 and 2,000,000 reads for SEED hierarchical microbial functional expressed genes (level4), 5,000,000 reads with cutoffs at 10,000,000 reads with cutoffs at 50,000, 100,000, 200,000, 500,000, 1,000,000, 2,000,000 and 5,000,000 reads, and 10,000,000 reads for RefSeq bacterial functional expressed genes, and 1,500,000 reads with cutoffs at 10,000, 20,000, 50,000, 100,000, 200,000, 500,000 and 1,000,000 reads for RefSeq eukaryotic expressed genes.


A)
2016
Control
2016
Neonicotinoid−treated
2017
Control
2017
Neonicotinoid−treated
Total relative Abundance
1.00
0.75
0.50
0.25
0.00
SRA662
SRA664
SRA666
SRA668
SRA962
SRA964
SRA966
SRA968
SRA661
SRA663
SRA665
SRA667
SRA961
SRA963
SRA965
SRA967
CRA672
CRA674
CRA676
CRA678
CRA972
CRA974
CRA976
CRA978
CRA671
CRA673
CRA675
CRA677
CRA971
CRA973
CRA975
CRA977
Sample ID
Ten Most Abundant Soil SEED Hierarchical Microbial Functional Categories − Level 4
ATP−dependent protease La (EC 3.4.21.53) Type I
Cell division protein FtsH (EC 3.4.24.−)
Chaperone protein DnaK
DNA−directed RNA polymerase beta subunit (EC 2.7.7.6)
Heat shock protein 60 family chaperone GroEL
No Hierarchy
RNA polymerase sigma factor RpoD
SSU ribosomal protein S1p
Translation elongation factor G
Translation elongation factor Tu
B)
2016
Control
2016
Neonicotinoid−treated
2017
Control
2017
Neonicotinoid−treated
Total relative Abundance
1.00
0.75
0.50
0.25
0.00
SRA662
SRA664
SRA666
SRA668
SRA962
SRA964
SRA966
SRA968
SRA661
SRA663
SRA665
SRA667
SRA961
SRA963
SRA965
SRA967
CRA672
CRA674
CRA676
CRA678
CRA972
CRA974
CRA976
CRA978
CRA671
CRA673
CRA675
CRA677
CRA971
CRA973
CRA975
CRA977
Sample ID
Ten Most Abundant Soil RefSeq Bacterial Functional Categories
ABC transporter ATP−binding protein
ABC transporter substrate−binding protein
DNA−binding response regulator
DNA−directed RNA polymerase subunit beta
Elongation factor G
Elongation factor Tu
Endopeptidase La
MFS transporter
Molecular chaperone DnaK
Molecular chaperone GroEL

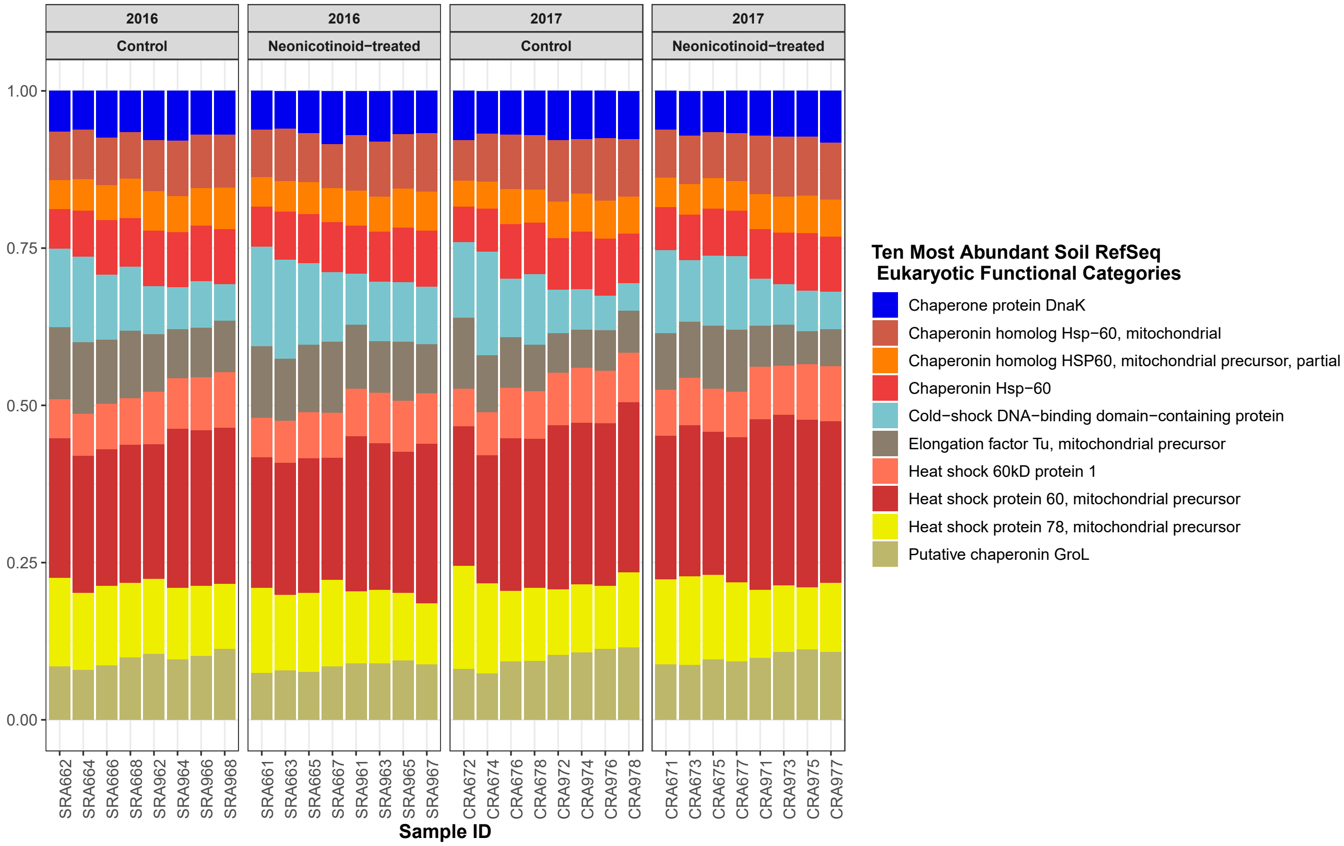


Figure E.2: Most abundant microbial functional categories. Ten most abundant soil SEED hierarchical microbial functional categories (levels 4:A), RefSeq bacterial functional categories (B), and RefSeq eukaryotic functional categories (C) in a two-year soybean/corn crop rotation in L'Acadie, Quebec, Canada. Each stack bar represents one soil sample. Mutual functional categories among the three gene profiles are represented with the same colors.

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