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EXPLORING THE TEMPERATE TREE LEAF MICROBIOME: FROM NATURAL FORESTS TO CONTROLLED EXPERIMENTS AND URBAN ENVIRONMENTS

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ISABELLE LAFOREST-LAPOINTE

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COMPOSITION ET DYNAMIQUES DU MICROBIOME FOLIAIRE DES ARBRES DE LA FORÊT TEMPÉRÉE : DE LA FORÊT NATURELLE AU MILIEU URBAIN

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DEDICATION

Destiny is not a matter of chance;
It is a matter of choice.
It is not a thing to be waited for;
It is a thing to be achieved.
Williams Jennings Bryan

PREFACE

This thesis is organized in four scientific papers written in English. I was responsible for the experimental designs, sampling, laboratory work, bioinformatics processing of genetic sequences, statistical analysis of data, and editing. Steven W. Kembel and Christian Messier participated in the experimental design of all articles. I also did an internship in Jonathan Eisen's lab at the University of California at Davis. I am the first author of each of the four articles.

The first paper, *Host species identity, site and time drive temperate tree phyllosphere bacterial community structure*, was published in the journal *Microbiome* (4: 27) in June 2016. The co-authors are Christian Messier and Steven W. Kembel.

The second paper, *Tree phyllosphere bacterial communities: exploring the magnitude of intra- and inter-individual variation among host species*, was published in the journal *PeerJ* (4: e2367) in August 2016. The co-authors are Christian Messier and Steven W. Kembel.

The third paper, *Leaf bacterial diversity mediates plant diversity-ecosystem function relationships*, has been accepted in *Nature*. The co-authors are Alain Paquette, Christian Messier and Steven W. Kembel.

The last paper, *Tree leaf microbiome changes along a gradient from natural to urban environments*, is in preparation. The co-authors are Christian Messier and Steven W. Kembel.

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LIST OF ABBREVIATIONS & ACRONYMS

A_{mass}	Maximum photosynthetic capacity
ANOVA	Analysis of variance
D_{tol}	Drought tolerance
FD	Functional diversity
FDis	Functional dispersion
H_{max}	Average maximum height
IDENT	International Diversity Experiment Network with Trees
IHI	Index of human influence
LEfSe	Linear discriminant analysis Effect Size
Llo	Leaf longevity
LMA	Leaf mass per area
N_{mass}	Leaf nitrogen mass
NMDS	Non-metric multidimensional scaling
OTU	Operational taxonomic unit
PCA	Principal component analysis
PERMANOVA	Permutational analysis of variance
SLA	Specific leaf area
S_{mass}	Seed mass
SR	Species richness
S_{tol}	Shade tolerance

V_{plot}	Aboveground stem volume
WD	Wood density

ABSTRACT

The plant-associated microbiome is known to influence plant physiology, metabolism and even inter-plant ecological interactions. The aerial surfaces of plants, the phyllosphere, are estimated to measure up to an area of 4×10^8 km^2. Although this habitat is oligotrophic, extremely poor in nutrients and exposed to a rapid and pronounced fluctuation of physical conditions, it harbors a highly diverse community of microorganisms. If previous researches in microbial ecology were limited by culture-dependent methods systematically underestimating microbial population sizes and biodiversity, the recent improvement in culture-independent technologies (i.e. high-throughput sequencing) has greatly contributed to the study of environmental microbial community structure and diversity. Microbes have been shown to participate in animal and plant population regulation, to degrade many pollutants, to contribute to host defense against pathogens and to synthesize compounds vital for plant productivity. In addition, the phyllosphere microbiota has been suggested to contribute substantially to both carbon and nitrogen cycles in terrestrial ecosystems. Trees expose a multitude of surfaces to microorganisms (roots, bark, leaves) enabling the development of tree-microbe interactions that are essential for tree productivity. Therefore, the increasing awareness of the potential roles of phyllosphere microbial communities calls for a greater understanding of their structure and dynamics both in natural and urban ecosystems.

Since most knowledge of tree leaf bacterial communities has been gathered in tropical forests, our first goal was to characterize the community structure and assembly dynamics of leaf bacterial communities in natural temperate forests of Quebec. To do so, we compared the relative influence of host species identity, site and time on phyllosphere bacterial community structure. Our second goal was to assess the amount of variation in the canopy of an individual tree. Therefore, we tested the value of characterizing a tree's complete phyllosphere microbial community through a single sample by measuring the intra-individual, inter-individual and interspecific variation in leaf bacterial communities. Third, we aimed to quantify the relationships among

phyllosphere bacterial diversity, plant species richness, plant functional diversity and identity, and plant community productivity in a biodiversity-ecosystem function experiment with trees. Using a novel tree biodiversity-ecosystem functioning experiment, we tested the hypothesis that leaf bacterial diversity influences positively ecosystem productivity. Finally, tree leaf microbiome has been studied in natural ecosystems but less so in urban settings, where trees act as vectors spreading bacterial cells in the air with possible effects on human health. Thus, we characterized and compared tree leaf bacterial communities in natural and urban environments, as well as along a gradient of increasing anthropogenic pressures.

In summary, the results presented in our first chapter confirm that host species identity is a stronger driver of phyllosphere bacterial community structure than site or time. Our second chapter demonstrates that, although the intra-individual variation in leaf bacterial community structure is smaller than the inter-individual variation, both variations are not statistically different. The third chapter provides evidence of a positive correlation between plant-associated microbial diversity and terrestrial ecosystem productivity, and therefore suggests a new mechanism by which models of biodiversity-ecosystem functioning relationships can be improved. Finally, the fourth chapter shows that bacterial communities from natural and urban environments are clearly distinct in community structure but not in diversity. Our work suggests that feedbacks between human activity and plant microbiomes could shape urban microbiomes.

Although the number of studies that have investigated tree phyllosphere bacterial community is increasing, there are still very few studies that offer a dual characterization of both the natural and urban tree phyllosphere bacterial community structure across multiple host species and drivers. The work presented here therefore offers an original assessment of the dynamics at play in the tree phyllosphere, combining a strong ecological framework, advanced sequencing techniques and sophisticated bioinformatics analyses, consequently making a noteworthy contribution to the field.

Keywords: phyllosphere, microbiome, temperate forest, plant-bacteria interaction, urban ecology.

RÉSUMÉ

À travers les interactions plante-microbe, les microorganismes ont le potentiel d'influencer la physiologie et le métabolisme de leur plante-hôte, voire même les interactions écologiques entre espèces végétales. Les surfaces aériennes des plantes, un habitat connu sous le nom de phyllosphère, représentent une aire totale d'environ 4 x 10^8 km^2. Malgré que cet habitat soit oligotrophique, c'est-à-dire extrêmement pauvre en nutriments et exposé à la fluctuation constante des conditions physiques, une grande diversité de microorganismes y réside. Par le passé, les recherches portant sur la phyllosphère étaient limitées par les méthodes dépendantes de culture, puisque celles-ci sous-estimaient systématiquement la taille et la diversité des populations microbiennes. L'arrivée récente des techniques indépendantes de culture, telles que le séquençage à haut débit, a contribué à l'amélioration de la compréhension de la structure et de la diversité des communautés microbiennes environnementales, tous milieux confondus. L'étude des communautés microbiennes est d'autant plus importante, puisqu'elles participent à la régulation des populations animales et végétales; dégradent plusieurs contaminants; contribuent aux défenses de l'hôte contre les pathogènes; et finalement synthétisent de multiples composés vitaux pour la productivité des plantes-hôtes. De plus, il a été démontré que le microbiome de la phyllosphère contribue significativement aux cycles du carbone et de l'azote au sein des écosystèmes terrestres. Les arbres exposent une multitude de surfaces (racine, écorce, feuille) aux microorganismes, permettant ainsi le développement d'interactions arbre-microbe qui sont essentielles pour la productivité des arbres. Le nombre croissant d'études laisse présager que les communautés microbiennes jouent un rôle crucial pour la santé des plantes-hôtes, soulignant ainsi l'importance d'améliorer la compréhension de la structure et des dynamiques de ces communautés au sein des écosystèmes naturels et urbains.

Plusieurs recherches se sont attardées à l'étude du microbiome des feuilles des arbres en milieu naturel, mais peu d'efforts ont été consacrés à l'étude de ces communautés en milieu urbain, un environnement dans lequel les arbres agissent comme vecteurs de

cellules bactériennes dans l'air, ce qui pourrait influencer indirectement la santé des populations humaines. Puisque la majorité des études du microbiome foliaire des arbres se sont déroulées dans les forêts tropicales, notre premier objectif était de caractériser la structure et les dynamiques d'assemblage des communautés bactériennes de la phyllosphère des arbres de la forêt tempérée du Québec. Pour ce faire, nous avons comparé l'influence relative de l'identité de l'espèce-hôte, de la location géographique et du temps sur la structure des communautés bactériennes foliaires. Notre deuxième objectif était d'évaluer la taille de la variation au sein du feuillage d'un arbre. Ainsi, nous avons testé la robustesse d'un protocole utilisant un échantillon unique pour représenter l'ensemble de la variation des communautés bactériennes foliaires au sein du feuillage d'un individu arborescent en comparant les variations intra-individuelle, interindividuelle et interspécifique. Troisièmement, nous avons quantifié les relations entre la diversité bactérienne des feuilles, plusieurs variables décrivant la communauté végétale locale (la richesse spécifique ainsi que la diversité et l'identité fonctionnelle), ainsi que la productivité de cette communauté végétale dans le cadre d'une expérience de relation fonctionnelle biodiversité-écosystème. En utilisant une expérience de biodiversité innovatrice, nos résultats appuient l'hypothèse voulant que la diversité bactérienne des feuilles soit liée à la productivité végétale locale. Finalement, notre dernier objectif était de caractériser et de comparer les communautés bactériennes de la phyllosphère des arbres en milieu naturel et en milieu urbain, ainsi que le long d'un gradient d'urbanisme.

Somme toute, les résultats de notre premier chapitre confirment le rôle dominant de l'identité de l'espèce-hôte dans la détermination de la composition des communautés bactériennes foliaires. En comparaison, les effets du site et du temps étaient significatifs mais beaucoup plus faibles. Les résultats de notre deuxième chapitre démontrent que, malgré que la variation intra-individuelle des communautés bactériennes foliaires au sein du feuillage d'un arbre soit plus petite que celle entre plusieurs individus (interindividuelle), ces deux variations ne sont pas significativement différentes en taille. Le troisième chapitre fournit une preuve sans précédent de la corrélation positive entre la diversité du microbiome foliaire des plantes et la productivité des écosystèmes terrestres, suggérant ainsi un nouveau mécanisme qui pourrait améliorer le pouvoir explicatif des modèles de relation fonctionnelle biodiversité-écosystème. Finalement, le quatrième chapitre illustre que les communautés bactériennes foliaires des arbres en milieu naturel et urbain diffèrent en composition mais non en diversité. Nos résultats suggèrent donc que les activités anthropogéniques influencent le microbiome urbain des plantes, et que ces changements pourraient agir rétroactivement sur la santé des populations humaines urbaines.

Malgré le nombre grandissant d'études portant sur les communautés bactériennes de la phyllosphère, très peu de ces recherches présentent simultanément une caractérisation des milieux naturels et urbains, et ce pour de nombreuses espèces végétales ainsi que plusieurs facteurs de variation. De la sorte, la thèse de doctorat ci-présente offre une

évaluation originale et innovatrice des dynamiques au sein du microbiome de la phyllosphère, alliant l'utilisation d'une base forte en écologie et de techniques de séquençage avancées, et contribuant ainsi significativement au domaine des interactions plante-microbe.

Mots-clés : phyllosphère, microbiome, forêt tempérée, interaction plante-bactérie, écologie urbaine.

INTRODUCTION

In this thesis I will present a study of the microbial ecology of the leaves (the phyllosphere) of tree species of the temperate forest of Quebec. The aim of this project is to establish essential knowledge of the processes driving phyllosphere microbial community dynamics in a diversity of tree stand structure including natural forest sites, controlled experiments and urban environments.

0.1 Definition of the Phyllosphere

The phyllosphere (Last, 1955, 1965; Ruinen, 1956) habitat is defined as the aerial surfaces of plants, mostly leaves, one of the most widely distributed habitat on earth measuring up to an estimated area of 4×10^8 km^2 (Morris *et al.*, 2002). This habitat is oligotrophic, extremely poor in nutrients and exposed to a rapid and pronounced fluctuation of physical conditions (Lindow & Brandl, 2003). The work of Leveau & Lindow (2001) has demonstrated that the availability of fructose and sucrose for bacterial epiphytes is highly heterogeneous and gets depleted quickly, therefore limiting bacterial population growth. Even under these rough conditions, the phyllosphere harbors a highly diverse community of microorganisms (Lindow & Leveau, 2002; Lindow & Brandl, 2003; Jumpponen & Jones, 2009; Rodriguez *et al.*,

2009), which contributes to host protection and productivity (i.e. Arnold *et al.*, 2003 for fungi; Vogel *et al.*, 2016 for bacteria). A microorganism is defined as any organism (bacteria, archæa, fungi, virus, etc.) having a mass of less than 10^{-5}g and a length of less than 500µm (Martiny *et al.*, 2006). Phyllosphere microorganisms are classified in two groups in function of their colonization strategy: ectophytes reside on the outer surface of leaves whereas endophytes penetrate in the inner leaf tissues (Hallman *et al.*, 1997). Until the 2000's, the study of microbial ecology was limited by culture-dependent methods that underestimated microbial population diversity (Hugenholtz *et al.*, 1998). Recent discoveries in next-generation sequencing and culture-independent methods, such as high-throughput sequencing (Shendure & Ji, 2008), have dramatically improved our knowledge of phyllosphere microbial communities (Yang *et al.*, 2001; Lambais *et al.*, 2006; Yashiro *et al.*, 2011).

0.2 An Overview of the Literature

The phyllosphere microbial community is mainly composed of bacteria and endophytic fungi (Andrews & Harris, 2000; Lindow & Brandl, 2003). Most phyllosphere studies have focused on model organisms such as *Arabidopsis thaliana* (Innerebner *et al.*, 2011; Bodenhausen *et al.*, 2013, 2014; Maignien *et al.*, 2014; Remus-Emsermann *et al.*, 2014; Ryffel *et al.*, 2016; Vogel *et al.*, 2016) or various agriculturally important species such as apple trees (Andrews *et al.*, 2002; Yashiro *et al.*, 2011), maize (Sabaratnam & Beattie, 2003; Beattie & Marcell, 2002; Kadivar & Stapleton, 2003; Peiffer *et al.*, 2013), lettuce (Hunter *et al.*, 2010; Rastogi *et al.*, 2012; Williams *et al.*, 2013; Medina-Martínez *et al.*, 2014; Williams & Marco, 2014), bean (Monier & Lindow, 2003, 2004), rice (De Costa *et al.*, 2006; Knief *et al.*, 2012; Ren *et al.*, 2015), spinach (Lopez-Velasco *et al.*, 2011) and grape (Leveau & Tech, 2011). The

biosphere's microbial diversity supports a great variety of biogeochemical processes fundamental to ecosystem dynamics (Kirchman, 2012). Microbes participate in animal and plant population regulation (Ostfeld *et al.*, 2008), degrade many pollutants (Alexander, 1999), contribute to host defense against pathogens (Fravel, 1988) and synthesize compounds vital for plant productivity (see Friesen *et al.*, 2011 for a review). In terrestrial ecosystems, the phyllosphere microbiota has been suggested to contribute considerably to both carbon (i.e. *Methylobacterium* exploiting plant-produced methanol as a source of energy; Delmotte *et al.*, 2009; Knief *et al.*, 2012; Jo *et al.*, 2015) and nitrogen cycles (i.e. in situ nitrogen fixation therefore increasing its local availability; Abril *et al.*, 2005; Fürnkranz *et al.*, 2008). The rapidly increasing number of plant microbiome studies suggests that improving our comprehension of plant microbiome structure and dynamics holds great potential economically both in the domains of sylviculture (Uroz *et al.*, 2016) and agriculture (Köberl *et al.*, 2012; Berg *et al.*, 2013, 2014), but also in relation to its contribution to human population health in urban agglomerations (Hanski *et al.*, 2012). However, our knowledge of the plant-microbe interactions occurring in the phyllosphere is still limited (but see Hirano & Upper, 2000; Whipps *et al.*, 2008; Vorholt, 2012; Bringel & Couée, 2015; Uroz *et al.*, 2016 for reviews).

0.3 The Importance of Studying the Tree Microbiome

Microbial communities are known to be essential to numerous macro-organisms and their importance for forest ecosystems dynamics has been demonstrated (Furnkranz *et al.*, 2008). The wide distribution of forest ecosystems across the planet combined with the contribution of the forest microbiome to ecosystem processes suggest that the phyllosphere could be driving crucial planet-wide processes (see Uroz *et al.*, 2016 for

a recent review of forest microbiome) such as plant species negative and positive density-dependent patterns, ecosystem nutrient cycling and systemic responses to global change. Forests are complex ecological systems in which trees of various species interact together by ways of competition, facilitation, allelopathy and microbial exchanges. Trees expose a multitude of surfaces to microorganisms (roots, bark, leaves) enabling the development of tree-microbe interaction that are essential for tree productivity both in the rhizosphere (Herre *et al.*, 2007; Berendsen *et al.* 2012) and phyllosphere habitats (Lindow & Brandl, 2003; Fürnkranz *et al.*, 2008). In the rhizosphere, these host-microbe interactions have been intensively studied because of their key role in host productivity (see Berendsen *et al.*, 2012 for a review). Mycorrhizal networks have been demonstrated to facilitate establishment, growth, survival and protection of plants across ecosystems (Horton *et al.*, 1999; Dickie *et al.*, 2004; Teste *et al.*, 2009; Song *et al.*, 2010). Most tree phyllosphere studies have focused on fungal communities (Osono, 2006; Rodriguez & Redman, 2007; Jumpponen & Jones, 2009, 2010; Suda *et al.*, 2009; Cordier *et al.*, 2012a, 2012b; Peñuelas *et al.*, 2012; Hantsch *et al.*, 2013, 2014; Kembel & Mueller, 2014) or pathogens (Gilbert, 2002; Newton *et al.*, 2010) therefore limiting our knowledge of the complex dynamics at play. Nonetheless, studies of the tree phyllosphere bacterial communities are more and more frequent, mainly in tropical forests (Lambais *et al.*, 2006, 2014; Kim *et al.*, 2012; Kembel *et al.*, 2014), temperate forests (Redford & Fierer, 2009; Redford *et al.*, 2010; Jo *et al.*, 2015; Koskella & Parr, 2015; Leff *et al.*, 2015; Meaden *et al.*, 2016), and single species (Finkel *et al.*, 2011, 2012; Peñuelas *et al.*, 2012; Rico *et al.*, 2014).

To date, surveys have shown that the tree phyllosphere community is dominated by a few phyla (*Proteobacteria* representing up to 70 % of the community; gram-negative bacteria) and other sub-groups (*Bacteroidetes*, *Actinobacteria* and *Betaproteobacteria*) (Redford & Fierer, 2009; Finkel *et al.*, 2011; Kim *et al.*, 2012). The phylum

Proteobacteria is mainly represented by two classes: *Alpha-* and *Gamma-proteobacteria* (Whipps *et al.*, 2008; Rastogi *et al.*, 2012), but members of the families *Methylobacteriaceae* and *Sphingomonadaceae* are also commonly found in phyllosphere communities (Knief *et al.*, 2010; Rastogi *et al.*, 2013). These studies display the first censuses and analyses of tree phyllosphere microbial communities. Thus, the aim of the present work will be to make a significant contribution to the general knowledge of temperate tree phyllosphere bacterial community structure, diversity and dynamics.

0.4 Microbes of the Phyllosphere

Survival in the phyllosphere requires an adaptation to the extreme conditions imposed by abiotic and biotic stresses. Microorganisms are unique in many ways including high population growth rates, parasexuality and high rates and extent of dispersal. Due to the harsh biotic and abiotic conditions of life on plant leaf (Lindow & Brandl, 2003), phyllosphere bacterial communities are likely to possess functional traits that confer fitness advantages for an epiphytic life. Phyllosphere microbiota exhibit a high range of metabolic diversity, which allows them to survive in stressful environments where sources of carbon (and other nutrients like sulfur) are limited (Mercier & Lindow, 2000). Although many aspects of phyllosphere microbial metabolism still need to be understood, the first censuses have revealed the presence of various key traits such as phototrophy, methylotrophy and nitrogen fixation. First, Atamna-Ismaeel *et al.* (2012a, 2012b) demonstrated the high abundance of anoxygenic phototrophic bacteria, a class of organisms capable of harvesting light to supplement their metabolic requirements. Indeed, the phyllosphere has been reported to harbor three types of phototrophy that use distinct light spectrum's range: (1) plant chlorophyll-based oxygenic

photosynthesis, (2) bacterial Bchl-based anoxygenic photosynthesis and (3) rhodopsin-based phototrophy (Atamna-Ismaeel *et al.*, 2012a, 2012b). Second, the phyllosphere is also known to harbor a high relative abundance of methylotrophs possessing the ability to grow on formaldehyde, formate and methanol (Corpe & Rheem, 1989; Knief *et al.*, 2008; 2010a, 2010b; Iguchi *et al.*, 2012; Wellner *et al.*, 2011). The methylotroph metabolism has been confirmed by the pioneer proteogenomic work of Delmotte *et al.* (2009), who reported the high relative abundance of proteins involved in methylene tetrahydrofolate and carbon dioxide (both potentially methanol-derived) in phyllosphere bacterial communities. Finally, Fürnkranz *et al.* (2008) and Rico *et al.* (2014) provided evidence of the presence and activity of diazotrophic bacteria respectively on the leaves of various tropical plants and on a Mediterranean tree species, *Quercus ilex*. A recent study also demonstrated that needle endophytes of a *Picea* and *Pinus* tree species contributed to tree host growth by fixing nitrogen (Carrell & Frank, 2014).

In addition to the variety of metabolism types exhibited by the phyllosphere microbiome, leaf microorganisms produce a range of various secondary metabolites. As an example of secondary metabolite produced by leaf microbiota, a specific strain of *Pseudomonas syringae* has been shown to produce two molecules (coronatine and syringolin) that neutralize the plant's pathogen-triggered mechanism of stomatal closure. The fitness of phyllosphere bacteria has been shown to involve the activation of DNA repair mechanisms including photolyases (Gunasekera & Sundin, 2006), the production of antibiotics and biosurfactants to increase leaf wettability (Schreiber *et al.*, 2005) and of pigments (Jacobs *et al.*, 2005), adding to the potential mechanisms through which phyllosphere microbial communities could impact their host. Plants produce five commonly known hormones (abscidic acid, auxin, cytokinin, ethylene and gibberellin) for which microorganisms are able to produce secondary metabolites inferring with hormone's production, therefore potentially influencing plant growth

and fitness (Vorholt, 2012). Many of phyllosphere microbiota, including the *Methylobacterium*, have been shown to impact positively on plant health and development (Abanda-Nkpwatt *et al.*, 2006; Innerebner *et al.*, 2011). Research on phyllosphere bacterial diversity has shown various potential mechanisms through which it can influence host productivity. Such mechanisms include (1) inducing plant-resistance mechanisms that improve host resistance to pathogens by increasing the competition for niches, depleting nutrient pools and increasing the production of antibiotic molecules (Innerebner *et al.*, 2011; Rastogi *et al.*, 2012; Raghavendra & Newcombe, 2013; Turner *et al.*, 2013; Ritpitakphong *et al.*, 2016); (2) influencing phytohormones production such as auxin like molecules (i.e. indole-3-acetic acid, IAA; Glickmann *et al.*, 1998; Brandl *et al.*, 2001) and cytokinin (Brandl & Lindow, 1998; Manulis *et al.*, 1998); and 3) increasing atmospheric N fixed by leaf bacterial communities and therefore increasing local nitrogen availability in the system (Carrell & Frank, 2014; Moyes *et al.*, 2016). Therefore, the phyllosphere microbiome is potentially essential for single plant's fitness and growth, suggesting that these microorganisms drive key processes for plant ecosystems such as forests (Furnkranz *et al.*, 2008). Considering the variety of microbial metabolisms and secondary metabolites shaping a complex multi-trophic network and that microbes have the capacity to evolve at a velocity unmatched by macro-organisms, it raises the question of how these organisms will adapt to the current environmental changes and how this adaptation will retroactively impact the plant hosts.

0.5 Of Ecological Theories and Microbial Communities

Microbial communities, due to their high complexity and diversity, are excellent model systems to test ecological and evolutionary theories (Jessup *et al.*, 2004; Prosser *et al.*,

2007). The two main theories employed to explain the patterns of community assembly and diversity are the niche theory and the neutral theory. The niche theory, based on Gause's law of competitive exclusion, states that coexistence in local areas requires species to occupy distinct niches (Lotka, 1910; Volterra, 1926; Gause, 1934; Hardin, 1960; MacArthur & Levins, 1967; Goel *et al.*, 1971). This theory suggests that species diversity is maintained by strong niche differences stabilizing the interactions of highly unequal competitors (Chesson, 2000a, 2000b; Chase & Leibold, 2003; Ackerly *et al.*, 2006). On the other hand, the neutral theory assumes that the long-term fitness of trophically similar species is equal and that species distribution behave like a random walk, therefore creating an unstable community (Hubbell, 2001; Rosindell *et al.*, 2011). Instead of deterministic factors like density-dependence or competition driven processes, stochastic factors, such as dispersal and immigration, become the key mechanism of community assembly (Gravel *et al.*, 2011). In an environment prone to stochasticity (large random variation in conditions), greater niche distance would be required to preserve species coexistence (Gravel *et al.*, 2006). Though these two theories are the most widely known, various ecological theories have been used to explain the complex dynamics behind microbial community structure including the lottery hypothesis (Sale, 1976). This hypothesis states that species similar in trophic capacities can coexist through chance recolonization of unoccupied patches in a temporally and spatially stochastic environment (Chesson & Warner, 1981). Researches on microbial ecology aiming to identify the main drivers of phyllosphere bacterial community structure and dynamics will thus provide key information on the prevalence of deterministic or stochastic factors in this habitat.

0.6 Dynamics of Tree Phyllosphere Microbial Communities

Host species identity has been shown to be the main driver of phyllosphere bacterial community structure (Redford & Fierer, 2009; Kim *et al.*, 2012; Kembel *et al.*, 2014; Kembel & Mueller, 2014). These large differences between neighbor species suggest that specific plant characteristics (leaf physical properties, secondary metabolite production, etc.) shape phyllosphere microbial community structure (Knief *et al.*, 2010; Kembel *et al.*, 2014). These characteristics, defining the physical conditions and nutrients limitations of leaf microbial community habitat, might allow host-species to select key microbial species that play a fundamental role in structuring phyllosphere microbial community (Vorholt, 2012). Consequently, tree species could differ in their microbiota selection, resulting in a variation in phyllosphere microbial community function and composition across host species. However, the diversity of phyllosphere microbial communities is also known to differ across forest ecosystems, decreasing from tropical forest to artic vegetation (Arnold & Lutzoni, 2007). Whether this gradient of microbial biodiversity is the result of environmental heterogeneity, of dispersal history or of forest ecosystem selection forces still needs to be determined (Berendsen *et al.*, 2012). However, shared community operational taxonomic units (OTUs) are known to decay with distance (Green *et al.*, 2004), showing that microbes are not randomly distributed but exhibit spatially predictable, aggregated patterns. Drivers linked to site dispersal history, such as geographical location, have been demonstrated to exert a long-term impact similar to a distance-decay relationship on local microbial pools available to colonize the phyllosphere (Finkel *et al.*, 2011, 2012). Therefore, both host species identity and site geographical location could be key drivers of tree phyllosphere bacterial community structure and diversity.

0.7 The Influence of Host Tree Functional Traits

Functional traits define tree species' ecological strategies, ranging from an acquisitive strategy (fast growth species with low wood density, low investment in leaf nutrients and dry mass in leaves, short-lived leaves) to a retentive strategy (low growth species with high wood density, high nutrients investment, leaf dry mass and long-lived leaves) (Wright *et al.*, 2004). The principal tree functional traits can be categorized in the leaf (Wright *et al.*, 2004) and wood (Chave *et al.*, 2009) economics spectra: cuticle structure and composition (leaf wettability), leaf chemical composition (nitrogen and potassium; photosynthetic capacity of the leaf), micro-topography of the leaf, leaf mass per unit area and wood density. Epiphytic fungi colonization has been shown to be higher in density along leaf veins and around natural microscopic lesions (Andrews *et al.*, 2002). Traits related to leaf photosynthetic capacity including leaf mass per area, leaf dry matter content and leaf nutrients concentrations are known determinants of phyllosphere microbial community composition in tropical forest of Panama (Kembel *et al.*, 2014). Levels of soluble carbohydrates were also found to influence the microbial community of the leaf habitat (Hunter *et al.*, 2010). Traits related to plant stature (height and diameter) and growth-mortality trade off (wood traits, growth and mortality rates) could also influence the phyllosphere microbial community through correlations with other aspects of plant ecological strategy. Therefore, functional traits could be key determinants of the phyllosphere microbial community since they define the physicochemical conditions (host morphology and physiology) of phyllosphere microbial community habitat (Hunter *et al.*, 2010). The effect of each of these determinants could vary, having a distinct influence on nutrients availability on the leaf and thus on the composition of phyllosphere microbial community (Vorholt, 2012). Leaf microscopic structure and composition differs between host tree species, individuals and leaves. The variation in these traits is controlled by trees' genetic background and environment factors. For model organisms such *Arabidopsis thaliana,* plant genotypes have also been shown to influence phyllosphere microbial community structure through modifications of cuticle formation genes (Bodenhausen *et al.*, 2014) or mutations in cuticular wax biosynthesis (Reisberg *et al.*, 2013). A diverse and

enriched environment, creating a multitude of varying niches might increase nutrient availability, and the leaf's capacity to harbor a highly diverse or dense microbial community. As a result, a higher local diversity in tree species has the potential to increase the exchange rates between different phyllosphere microbial communities and thus can influence community dynamics. Therefore, the functional differences among plant species might play a key role in the structure of phyllosphere microbial assemblages. It is thus of great interest to investigate how these leaf micro-characteristics influence the composition of phyllosphere microbial community.

0.8 Spatial and Temporal Dynamics

Factors influencing local and regional changes in physicochemical conditions such as seasonal variation in temperature and precipitation can influence tree phyllosphere bacterial communities (Jackson & Denney, 2011; Peñuelas *et al.*, 2012; Rico *et al.*, 2014) such as it was demonstrated for lettuce (Rastogi *et al.*, 2012; Medina-Martínez *et al.*, 2014). Precipitation and temperature drive phyllosphere fungal community assembly in terms of abundances and species (Cordier *et al.*, 2012b). For example, precipitation could influence the growth of phyllosphere microbial communities through differences in the process of quorum-sensing. This process, defined as the production and perception of small diffusible signal molecules mediating cell-density-dependent gene expression, has been shown to be faster on dry leaves than on wet leaves (Dulla & Lindow, 2008). Climatic conditions influence the stress level imposed on tree-hosts and therefore could also have an impact on the phyllosphere microbial community composition. Likewise, variation across temporal scales is a recognized determinant of microbial biodiversity mainly because of the ability of microorganisms to adapt to rapid changes in their environmental conditions (Prosser *et al.*, 2007). Since

microbes are capable of rapid growth and short generation times, phyllosphere microbial communities are subject to changes during the growing season following changes in temperature and precipitation (Cordier *et al.*, 2012). Temporal succession patterns have been observed in the *Populus deltoides* phyllosphere (Redford & Fierer, 2009). Bacterial diversity has been observed to be lower during drought episodes (dry and hot weather) and higher when climatic conditions were humid and mild (Ercolani 1991). Furthermore, there are seasonal conditions that contribute to propagule growth or production and therefore create a dynamic seasonal fluctuation in the phyllosphere microbial community (Wilson & Caroll, 1994; Hata *et al.*, 1998; Kaneko *et al.*, 2003; Osono, 2008). During the growth season, leaching across leaf cuticle could also increase leaf support capacity and thus phyllosphere microbial diversity. Insect herbivory (Humphrey *et al.*, 2014) and leaf location in the canopy (Jacobs & Sundin, 2001; Kadivar & Stapleton, 2003) could also drive changes in phyllosphere community structure through differential host plant resistance to herbivores and resistance to UV-B radiation exposition respectively. Further examination of the relative importance of these drivers is required to improve our understanding of the complex dynamics shaping phyllosphere microbial community structure and dynamics.

0.9 Natural vs. Urban Environments

Phyllosphere microbial community dynamics of natural forests might be quite different than microbial dynamics in urban stands. Urban forest environments differ strikingly from natural environments mainly since biotic and abiotic stresses are increased. Urban trees are submitted to multiple anthropogenic stresses of different length and intensity leading to photosynthetic biomass loss and tree lesions (Sieghardt *et al.*, 2005). These stresses have been shown to affect plant survival (Mittler, 2006; Niinemets, 2010a,

2010b) and induce numerous physiological responses. Accordingly, numerous anthropogenic activities have the capacity to influence the composition of phyllosphere microbial community on urban trees. Several studies have suggested that urban areas retain only a limited quantity of biodiversity (Blair, 1999; Cincotta & Engelman, 2000; Mckinney, 2002), whereas other empirical studies have suggested that these areas could support diverse assemblies of organisms (Kühn *et al.*, 2004; Wania *et al.*, 2006). Whereas urban species diversity could be considered "high", the actual urban functional and phylogenetic diversity could be quite diminished, as many urban habitats are characterized by closely related species that are also functionally similar (Knapp *et al.*, 2008; Nock *et al.* 2013). Therefore, the potential modification of diversity by urban conditions might affect phyllosphere microbial community composition and dynamics, possibly retroactively impacting urban trees fitness and productivity. In addition, the diversity of vegetation in our neighborhood, also linked to the phyllosphere microbial community diversity, has recently been linked to human immune reactions and asthma (Hanski *et al.*, 2012). Urban vegetation, by means of the microbial communities they support, could play an unexpected role in public health. It is thus of great importance to demystify the dynamics of beneficial microbes and pathogens on urban trees and eventually their impacts on our health.

The "urban heat island" phenomenon describes the general increase of temperature in city areas compared to rural and natural areas (Oke, 1973). This trend results from the increase of non-penetrating surfaces (Hart & Sailor, 2009) and the decrease of vegetation cover (Jenerette *et al.*, 2011) in cities. Temperature increase in urban areas, already influencing vegetation phenology (Roetzer *et al.*, 2000; White *et al.*, 2002; Zhang *et al.*, 2004), will become more extensive with city growth and the progress of global warming (Kalnay & Cai, 2003). In addition to the increase in local temperature, urban habitats have been found to be biogeochemically distinct from natural habitats. Numerous studies have observed anthropogenic activities to increase leaf

macronutrients (Nitrogen, Potassium, Sulfur), micronutrients (Boron, Manganese, Selenium) and trace elements (Cadmium, Lead, Zinc) for urban trees (Pouyat & McDonnell, 1991; Kaye *et al.*, 2006; Jumponnen & Jones, 2010). Stresses on urban trees are definitely different than the stresses on natural stand trees. Furthermore, urban trees frequently suffer from limited access to water and nutrients (Wiersum & Harmanny, 1983; Fluckiger & Braun, 1999) and root development limitation (see Day *et al.*, 2010 for a review). Stress intensification on trees could reduce tree defense, which could also lead to an increased presence of herbivores (Mattson & Haack, 1987). For animal communities, the impact of urban habitat through determinants such as habitat connectivity and resource accessibility has been demonstrated (Gomes *et al.*, 2011; Schnitzler *et al.*, 2011; Bennett & Gratton, 2012). However, the impact of urban conditions on tree phyllosphere community still remains undescribed and hard to predict since the combination of all previously introduced urban conditions could have many diverging impacts.

Adding to the combination of the intensified "urban heat island" phenomenon and the enrichment of a naturally oligotrophic environment, urban trees could also be threatened by both increased herbivory linked to urban heat (Meineke *et al.*, 2012) and an increase in pathogen or insect presence in the future. Thermal accumulation could influence enzymatic processes, affecting microbial communities directly, and also increase the presence of insect ectotherms (Briere *et al.*, 1999), which are known disease vectors (Lounibos, 2002). Insect pest abundance increase in urban areas when compared to rural areas (Bennett & Gratton, 2012; y Gomez & Van Dyck, 2012) is suggested to be the result of changes in host plant quality and natural enemy efficacy (Raupp *et al.*, 2010). In addition to the higher presence of dispersal vectors, a higher nutrient concentration in urban phyllosphere could trigger an increase in microbial community density, as total growth has been directly linked to the initial concentration of limiting nutrients (Monod, 1949). However, nutrients abundance alone cannot

predict accurately the diversity and abundance of microbial communities, since microorganisms possess a plethora of strategies to acquire resources like motility, antibiotic production and coordinated behavior (Hibbing *et al.*, 2010). Therefore, urban phyllosphere bacterial communities could be disturbed by urban conditions causing changes in (1) host plant health and functional traits, influencing (2) microbe-microbe interactions, which could lead to (3) modifications of plant-microbe associations.

0.10 Presentation of the Thesis

The main purpose of this Ph.D. project is to integrate microbial ecology and tree-microbe interactions in the study of natural, experimental, and urban ecosystems of the temperate forest of Quebec. The following work will be structured in four chapters focusing on (1) the identity and drivers of the phyllosphere bacterial communities of the natural temperate forest of Quebec; (2) intra-individual vs. inter-individual variation in tree phyllosphere bacterial communities; (3) the influence of plant neighborhood identity, richness, and diversity on tree phyllosphere bacterial communities and their influence on plant community productivity; and finally, (4) urban tree phyllosphere bacterial community structure and dynamics.

To reach theses goals, we will make use of recent DNA sequencing techniques which have many advantages on culture-dependent techniques, but also have the consequence to create biases in the microbial communities detected. In the last 10 years, the number of studies exploiting high-throughput sequencing techniques (i.e. Illumina sequencing), to study microbial communities has grown exponentially. This success can be attributed to the precise description of community composition obtained with a minimal amount of work and cost-per-sequence when compared to older technologies

(Tedersoo *et al.*, 2010). High-throughput sequencing of bacterial communities typically uses the hyper-variable regions of phylogenetically informative 16S rRNA gene. The output of a sequencing run is a list of thousands sequences for each sample that includes the targeted microbial DNA present in the sample. These sequences can be analyzed to obtain information on taxonomic identity, relative abundances and diversity of community structure. Each sample is assigned a barcode tag (unique identifier) that is added to a primer (previously selected by the investigator in function of the samples) used for amplification. Although the power of these technologies is considerable, they are sensitive to biases that can be caused during the PCR amplification of 16S rRNA amplicons (Claesson *et al.*, 2010) or by bacterial species not having the same number of genomic copies of the marker gene (Chaffron *et al.*, 2010). One of the main challenges of this Ph.D. thesis will therefore be to address these challenges accordingly to ensure the production of a robust body of work. To do so, the protocols in this work were designed to minimize biases and errors at all stages of sequencing and data analysis (Kozich *et al.*, 2013) and to test the sensitivity of the statistical analyses employed to assess community structure and diversity.

0.11 Chapter 1: Natural Temperate Forest

In the first chapter, we explore the ecological drivers of variation in phyllosphere bacterial community composition of temperate trees. A conceptual understanding of the metacommunity ecology of microbes brings us to reflect on Bass Becking and Beijerinck's question (DeWit & Bouvier, 2006): "Is everything everywhere? And if not, does the environment select?" In this view, this chapter aims to characterize the dynamics of microbial spatial distributions in forest ecosystem, merging forest, microbial and community ecology.

Objectives

(1.1) to identify the phyllosphere bacteria of temperate forest trees;

(1.2) to detect the patterns of associations between host taxa and bacteria;

(1.3) to quantify the relative influence of three drivers on phyllosphere bacterial community composition: host species identity, site and sampling time.

Hypotheses

- ✓ **H1.1** A greater part of the variation in phyllosphere microbial community assembly is explained by host species identity rather than by climatic differences or site location; because microbial communities are sensible to host-genotype particularities, secondary metabolite production and plant-microbe interaction co-evolution.
- ✓ **H1.2** Phyllosphere microbial community diversity will be higher on angiosperm than on gymnosperms because of the increased amount of nutrient compounds leaking from broadleaves which have a thinner cuticle.
- ✓ **H1.3** Phyllosphere microbial community composition fluctuates during the growth season, following a pattern of development from the colonization to the end of the growth season (from first to the final microbiome) and also due to sensibility to environmental conditions (lower densities during droughts and higher density in mildest and wetter episodes).

0.12 Chapter 2: Intra-individual vs. Inter-individual Variation

In the second chapter, we reflect on the various methodology employed in tree phyllosphere researches and ask the question: "Is one leaf sample enough to characterize a full tree canopy?" Our main goal is to characterize the relative importance of intra-individual variation in phyllosphere communities across multiple species, and compare this variation to inter-individual and interspecific variation of phyllosphere epiphytic bacterial communities.

Objectives

- **(2.1)** compare the intra-individual, inter-individual and interspecific variation of phyllosphere bacterial communities;
- **(2.2)** characterize the composition of epiphytic phyllosphere bacterial communities at different canopy locations for five tree species;
- **(2.3)** make practical recommendations for the sampling of tree phyllosphere bacterial communities.

Hypotheses

- ✓ **H2.1** The magnitude of intra-individual variation will be smaller than inter-individual and interspecific variation;
- ✓ **H2.2** Canopy location will be a significant driver of phyllosphere bacterial community structure because of variation in abiotic conditions (e.g. radiation, wind), and changes in ecophysiological and morphological leaf characteristics.

0.13 Chapter 3: Biodiversity Experiment with Trees

In the third chapter, our main aim is to quantify the relationships among phyllosphere bacterial diversity, plant species richness, plant functional diversity and identity, and

plant community productivity in a biodiversity-ecosystem function experiment with trees. This chapter will allow us to extend our work from the natural forest ecosystem to study leaf bacterial communities in experimental settings.

Objectives

(3.1) to compare the relative influence of host species identity and diversity on host-level phyllosphere bacterial community structure and diversity;

(3.2) to evaluate the hypothesis that effects mediated through phyllosphere bacterial diversity explain an important part of the influence of plant diversity and identity on plant community productivity.

Hypotheses

- ✓ **H3.1.** Host species identity, plant species richness and plant functional diversity of immediate tree neighbors increase the diversity of the microbial species in the local pool, therefore increasing phyllosphere community diversity and driving community structure.
- ✓ **H3.2** A higher leaf bacterial diversity will be positively linked with plant community productivity through a variety of mechanisms, including (1) improving host resistance to pathogens; (2) influencing plant hormone production; and (3) augmenting local nitrogen availability.

0.14 Chapter 4: The Urban Environment

In the fourth chapter, because the phyllosphere microbial community dynamics of natural forests might be quite different than microbial dynamics in urban stands, we aim to improve our understanding of the urban tree microbiome. This chapter will also

aim to improve our understanding of the progressive changes that occur in leaf bacterial communities when the environmental anthropogenic pressures increase.

Objectives

(4.1) to compare the bacterial communities present in tree phyllosphere bacterial communities of natural forests and the urban environment;

(4.2) to describe the changes in tree phyllosphere bacterial community structure and diversity along a gradient of increasing urban intensity.

Hypotheses

- ✓ **H4.1** Urban stress on trees in urban agglomerations (nutrient enrichment, heat increase, physical stress, etc.) will change phyllosphere bacterial community structure and reduce diversity, in comparison with natural temperate forest stands.
- ✓ **H4.2** Increasing urban intensity will gradually influence the abundance of the main taxonomical groups of bacteria usually present in the natural temperate forest.

CHAPTER I

HOST SPECIES IDENTITY, SITE AND TIME DRIVE TEMPERATE TREE PHYLLOSPHERE BACTERIAL COMMUNITY STRUCTURE

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Isabelle Laforest-Lapointe, first author
Centre d'étude de la forêt, Département des sciences biologiques, Université du Québec à Montréal, C.P. 8888, Succ. Centre-Ville, Montréal, Canada, H3C 3P8.
Laforest-lapointe.isabelle@courrier.uqam.ca

Christian Messier
Centre d'étude de la forêt, Département des sciences biologiques, Université du Québec à Montréal, C.P. 8888, Succ. Centre-Ville, Montréal, Canada, H3C 3P8.; Institut des Sciences de la Forêt Tempérée, Université du Québec en Outaouais, 58 rue Principale, Ripon, Canada, J0V 1V0.
Messier.christian@uqam.ca

Steven W. Kembel
Centre d'étude de la forêt, Département des sciences biologiques, Université du Québec à Montréal, C.P. 8888, Succ. Centre-Ville, Montréal, Canada, H3C 3P8.
Kembel.steven_w@uqam.ca

1.1 Abstract

Background: The increasing awareness of the role of phyllosphere microbial communities in plant health calls for a greater understanding of their structure and dynamics in natural ecosystems. Since most knowledge of tree phyllosphere bacterial communities has been gathered in tropical forests, our goal was to characterize the community structure and assembly dynamics of phyllosphere epiphytic bacterial communities in temperate forests in Quebec, Canada. We targeted five dominant tree species: *Acer saccharum*, *Acer rubrum*, *Betula papyrifera*, *Abies balsamea* and *Picea glauca*. We collected 180 samples of phyllosphere communities on these species at four natural forest sites, on three separate occasions during the growing season.

Results: Host functional traits (i.e. wood density, leaf nitrogen content) and climate variables (summer mean temperature and precipitation) were strongly correlated with community structure. We highlight three key findings: (1) temperate tree species share a "core microbiome"; (2) significant evolutionary associations exist between groups of bacteria and host species; and (3) a greater part of the variation in phyllosphere bacterial community assembly is explained by host species identity (27 %) and species-site interaction (14 %), than by site (11 %) or time (1 %).

Conclusion: We demonstrated that host species identity is a stronger driver of temperate tree phyllosphere bacterial communities than site or time. Our results suggest avenues for future studies on the influence of host functional traits on phyllosphere community functional biogeography across terrestrial biomes.

Key words: Phyllosphere, bacteria, plant-bacteria interaction, microbiome, temperate forest.

1.2 Introduction

Microorganisms colonize aerial tree surfaces (bark, leaves), enabling interactions that are essential for plant growth and fitness (Lindow & Brandl, 2003; Herre *et al.,* 2007; Fürnkranz *et al.,* 2008). Aerial plant surfaces (mostly leaves), a habitat known as the phyllosphere, are estimated to sum up to 4×10^8 km^2 on Earth (Morris *et al.,* 2002), which is almost equivalent to the total surface of the earth. The phyllosphere habitat is extremely poor in nutrients and exposed to a rapid and pronounced fluctuation of physical conditions (Lindow & Brandl, 2003). Tree phyllosphere microbial communities are mainly composed of bacteria and endophytic fungi (Lindow & Brandl, 2003; Andrews & Harris, 2000). These communities are extremely diverse (Lambais *et al.,* 2006; Jumpponen & Jones, 2009; Rodriguez *et al.,* 2009; Redford *et al.,* 2010) and contribute to host protection and productivity (Arnold *et al.,* 2003; Vorholt, 2012). Although our knowledge of plant-microbe interactions on tree leaf surfaces is still limited (but see Vorholt, 2012; and Müller, 2012 for reviews), most studies have focused on endophytic fungi (Rodriguez *et al.,* 2009; Osono, 2006; Suda *et al.,* 2009) and pathogens (Gilbert, 2002; Newton *et al.,* 2010) limiting our knowledge of the complex dynamics at play for other organisms. Studies of the tree phyllosphere are more and more frequent, with most studies focusing on tropical forests (Kim *et al.,* 2012; Kembel *et al.,* 2014; Kembel & Mueller, 2014).

Bacteria exhibit a wide range of metabolic diversity, which allows them to survive in stressful environments where sources of energy are limited (Mercier & Lindow, 2014). Although many aspects of phyllosphere bacterial metabolism and functional traits are poorly understood, the first censuses have revealed the presence of anoxygenic phototrophic bacteria (Atamna-Ismaeel *et al.,* 2012a, 2012b). Many bacteria abundant in the phyllosphere, such as *Methylobacterium*, have been shown to positively

influence plant health and development (Abanda-Nkpwatt *et al.,* 2006; Innerebner *et al.,* 2011) mainly through the production of secondary metabolites interacting with host hormone production and influencing plant growth and health (Vorholt, 2012). While high-throughput sequencing techniques provide more information on plant-bacteria interactions, there is still no clear understanding of host-bacteria association patterns across multiple host species. For example, individual trees have been shown to share part of their dominant bacterial community (Kembel *et al.,* 2014), yet little is known about this 'core' microbiome, the group of bacterial taxa shared among multiple communities sampled from the same habitat (Shade & Handelsman, 2012). Understanding the drivers of phyllosphere bacterial diversity is the first step toward developing management strategies that encourage a healthy phyllosphere microbial community structure favoring tree health and function.

Phyllosphere bacterial community composition is the result of a combination of dispersal history, host selection (Redford *et al.,* 2010; Kim *et al.,* 2012), growth and survival in the face of environmental conditions and competition (Vorholt, 2012; Redford & Fierer, 2009). Hypotheses for the ecological processes structuring phyllosphere communities have included lottery models of colonization (Burke *et al.,* 2011), as well as filtering models whereby environmental attributes act as a filter restricting the bacterial taxa that are able to persist on the leaf (Knief *et al.,* 2010a, 2010b). Although drivers of phyllosphere microbial assembly have been quantified in previous studies both for fungi (Osono, 2008; Cordier *et al.,* 2012a, 2012b) and bacteria (Redford *et al.,* 2010; Knief *et al.,* 2010a, 2010b; Finkel *et al.,* 2011), most of these studies evaluated only a single potential driver of phyllosphere community structure.

In this study, we explore the ecological drivers of variation in leaf bacterial community composition of temperate trees, taking into account the influence of multiple drivers. Our objectives are (1) to identify the epiphytic bacteria present in the phyllosphere of

temperate forest trees; (2) to detect the patterns of associations between host taxa and bacteria; and (3) to quantify the relative influence of three drivers on phyllosphere bacterial community composition: host species identity, site and sampling time. We selected five common temperate tree species present at all sites to obtain a fair representation of Quebec's temperate forests, including both angiosperms and gymnosperms: *Abies balsamea* (Balsam fir), *Acer rubrum* (Red maple), *Acer saccharum* (Sugar maple), *Betula papyrifera* (Paper birch) and *Picea glauca* (White spruce). We collected 180 samples of phyllosphere communities on these species at four natural forest sites (*see Annexes A and B*), three times during the growing season. Bacterial community structure was determined through High-throughput Illumina sequencing of the bacterial 16S rRNA gene (Claesson *et al.*, 2010).

1.3 Methods

1.3.1 Study Site

The study plots are located in four natural temperate forest stands in Quebec (*see Annex A*): Sutton (45°6'46"N; 72°32'28"W), Abitibi (48°9'45"N; 79°24'4"W), Gatineau (45°44'50"N; 75°17'57"W) and Bic (48°20'1"N; 68°49'3"W). Distances between sites range from 295 km (Sutton and Gatineau) to 765 km (Abitibi and Bic) (*see Annex B*). This region is characterized by a cold and humid continental climate with temperate summer. We obtained monthly climate data from Canada's public weather database (Canada Weather Database) (*see Annex A*).

1.3.2 Bacterial Community Collection

We sampled at each site on three occasions during the 2013 growing season (June, July and August) from three individuals for each tree species, a total of 180 samples. For each randomly chosen tree, we clipped 50–100 g of shade leaves at mid-canopy height (1-2 m above the bottom of the tree's canopy) into sterile roll bags with surface-sterilized shears. For bacterial community collection and amplification, we used the protocols described by Kembel *et al.* (2014). We collected microbial communities from the leaf surface by agitating the samples in a diluted Redford buffer solution. We resuspended cells in 500 µL of PowerSoil bead solution (MoBio, Carlsbad, California). We extracted DNA from isolated cells using the PowerSoil kit according to the manufacturer's instructions and stored at -80 °C.

1.3.3 DNA Library Preparation and Sequencing

We used a two-stage PCR approach to prepare amplicon libraries for the high-throughput Illumina sequencing platform. The use of combinatorial primers for paired-end Illumina sequencing of amplicons reduced the number of primers while maintaining the diversity of unique identifiers (Gloor *et al.,* 2010). First, to avoid PCR contamination by chloroplast DNA amplification, we targeted the V5–V6 region of the bacterial 16S rRNA gene using cyanobacteria-excluding primers (16S primers 799F-1115R (Redford *et al.,* 2010; Redford & Fierer, 2009; Chelius & Triplett, 2001)) following protocols described by Kembel *et al.* (2014). These chloroplast-excluding primers have been widely employed in studies of phyllosphere bacteria in order to avoid contamination by host plant DNA (Rastogi *et al.,* 2010), and their use is justified

for, while they exclude both plant chloroplasts and Cyanobacteria sequences, Cyanobacteria are known to be rare in tree phyllosphere communities (Vorholt, 2012; Delmotte *et al.*, 2009). Using cleaned PCR product as a template, a second PCR was performed with custom HPLC-cleaned primers to further amplify 16S products and complete the Illumina sequencing construct (PCRII_for: 5′-AAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGC; PCRII_rev: 5′-ATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACG). We cleaned the resulting product using MoBio UltraClean PCR cleanup kit. We isolated a ~445 bp fragment by electrophoresis in a 2 % agarose gel, and recovered DNA with the MoBio GelSpin kit. We prepared multiplexed 16S libraries by mixing equimolar concentrations of DNA, and sequenced the DNA library using Illumina MiSeq 250 bp paired-end sequencing at Genome Quebec.

We processed the raw sequence data with PEAR (Zhand *et al.*, 2014) and QIIME (Caporaso *et al.*, 2010) pipelines to merge paired-end sequences to a single sequence of length of approximately 350 bp, eliminate low quality sequences (mean quality score <30 or with any series of 5 bases with a quality score <30), and de-multiplex sequences into samples. We eliminated chimeric sequences using the Uclust and Usearch algorithms (Edgar, 2010). Then, we binned the remaining sequences into operational taxonomic units (OTUs) at a 97 % sequence similarity cutoff. We determined the taxonomic identity of each OTU using the BLAST algorithm and Greengenes database (DeSantis *et al.*, 2006) as implemented in QIIME (Caporaso *et al.*, 2010).

1.3.4 Host plant trait data

We obtained data on host plant functional traits (*see Annex C*) including drought tolerance (D_{tol}), average maximum height (H_{max}), leaf nitrogen mass (N_{mass}), seed mass (S_{mass}), shade tolerance (S_{tol}), specific leaf area (SLA), and wood density (WD) from a global database collected by Abrams and Kubiske (1990), Burns and Honkala (1990), Farrar (1996), Shipley and Vu (2002), Wright *et al.* (2004), Niinemets and Valladares (2006), Chave *et al.* (2009) and USDA (2009).

1.3.5 Biomarker Analysis

We tested for the significant associations between bacterial taxa and host species, host taxonomy (angiosperms vs. gymnosperms), and sites using the Linear Discriminant Analysis Effect Size (LEfSe) algorithm (Segata *et al.,* 2011). The LEfSe algorithm aims to discover biomarkers (genes, pathways, or taxa) of different sample groups employing the linear discriminant analysis to approximate the effect size of each biomarker identified. A significant association between bacterial clades and a specific group (i.e. a host tree species) will be detected when there is consistently higher relative abundance of the clade in the group's samples. Among the bacterial clades detected as statistically and biologically relevant, the strongest scores identify which clades have the greatest explanatory power for differences between communities (Segata *et al.,* 2011).

1.3.6 Statistical analyses

Because PCR and sequencing errors could lead to spurious OTU identification (Acinas *et al.,* 2005), we created a database excluding OTUs represented by less than 20 sequences to eliminate rare OTUs. Analyses were performed on both the full database and the database with rare OTUs excluded to assess the results' sensibility to rarefaction. The number of sequences per sample ranged from 4 574 to 86 280. From a database of 3 868 892 quality sequences, we rarefied each sample to 4 000 sequences, with 38 samples excluded from subsequent analyses due to insufficient sequence reads as a result of extraction or sequencing errors, totalizing 668 000 sequences from 142 samples representing 5 tree species. Rarefaction and all subsequent statistical analyses were repeated 100 times. Results did not differ qualitatively across iterations of the rarefaction and we therefore present only the result of a single random rarefaction. We performed analyses with the ape (Paradis *et al.* 2004), ggplot2 (Wickham, 2009), picante (Kembel *et al.,* 2010), and vegan (Oksanen *et al.,* 2007) packages in R (R Development Core Team 2013).

We quantified the phylogenetic variation in bacterial community structure among samples with the weighted UniFrac index, an abundance-weighted measure of the phylogenetic differentiation among bacterial communities (Lozupone *et al.,* 2006). To illustrate patterns of bacterial community structure, we performed a nonmetric multidimensional scaling (NMDS) ordination of Bray–Curtis dissimilarities and weighted UniFrac distances among all samples. We identified relationships between bacterial community structure, host species identity, time, and site by conducting a permutational multivariate analysis of variance (PERMANOVA, (Anderson, 2001)) on the community matrix. We singled out functional traits and climate variables that are significant drivers of leaf community structure through a PERMANOVA. We employed a blocking randomization to account for the non-independence of observations across species and sites. The functional trait PERMANOVA was blocked by site and the climate variable PERMANOVA was blocked by species to correct for

the absence of intra-site and intra-specific variation in our trait and climate data. To visualize the changes in bacterial communities with respect to different variables, we tested for correlations between these variables and community scores on the NMDS ordination axes while applying the Bonferroni correction for multiple comparisons to our significance threshold (Hochberg, 1988; Bland & Altman, 1995). The cutoffs for significant correlations ($\alpha = 0.05$) were adjusted to $P < 0.007$ (functional traits) and $P < 0.025$ (climate data). To quantify the influence of host taxonomic levels on bacterial community structure, we performed a nested PERMANOVA (levels: angiosperm/gymnosperm, family, genus, species).

We estimated phyllosphere bacterial alpha diversity using the Shannon index calculated from OTU relative abundances for each community. We performed an analysis of variance (ANOVA) and subsequent post-hoc Tukey's tests to test for differences in diversity across species, time, and site. To account for the repeated measures taken on individual trees in our data, we constructed a linear mixed model fitted by maximum likelihood. This model sought to estimate the power of tree identity as a random factor in driving microbial community diversity in comparison with host species identity, site and sampling time.

1.4 Results

1.4.1 Sequences, OTUs and taxonomy

Sequencing identified 15 873 bacterial operational taxonomic units (OTUs, sequences binned at 97 % similarity) in phyllosphere samples, an average of 517 ± 16 OTUs (mean ± standard error) per tree sampled. Most of these bacterial taxa were rare, with

52.6 % of bacterial OTUs occurring only on a single tree. Each tree sampled revealed additional bacterial taxa as shown by a collector's curve of the number of OTUs per sample (*see Annex D*). Four of the nine most abundant bacterial classes belonged to the phylum *Proteobacteria*: *Alpha-* (68 % of all sequences), *Beta-* (6 %), *Gamma-* (5 %), and *Deltaproteobacteria* (3 %); three belonged to the phylum *Bacteroidetes*: *Cytophagia* (4 %), *Sphingobacteria* (1 %), and *Saprospirae* (1 %); and finally the classes *Acidobacteria* (6 %) and *Actinobacteria* (5 %) were also abundant.

We detected a 'core microbiome' (Shade & Handelsman, 2012), defined as OTUs present on 99 % or more of all trees sampled, of 19 bacterial OTUs belonging to 2 phyla, 4 classes, and 7 families. This core microbiome represented less than 0.001 % of the bacterial taxonomic diversity but more than 42.7 % of sequences (*see Annex E*). The most abundant core microbiome OTUs included representatives of *Methylocystaceae* (two OTUs at 17.8 % and 4 % relative abundance), *Beijerinckia* (two OTUs at 4.0 % and 1.2 %), *Sphingomonas* (two OTUs at 2.4 % and 1.2 %), *Acidobacteriaceae* (2.3 %), *Oxalobacteraceae* (2.3 %), and *Acetobacteraceae* (1.2 %) (*see Annex E*). Most of the abundant OTUs showed significant associations with host species identity, site and sampling time (Table 1.1).

Table 1.1 Linear models of the relationship between each of the 19 core microbiome OTU abundance and time, site and host species identity. Numbers represent the coefficient of factors.

TAXONOMY (FAMILY)	OTU number	TIME		SITE			SPECIES				Model total R2 (%)
		July	August	Bic	Gatineau	Sutton	ACRU	ACSA	BEPA	PISP	
Acetobacteraceae	3293	NS	NS	NS	NS	NS	NS	NS	-0.71**	-0.53*	18
	7913	NS	NS	-0.80**	-1.77***	-1.12***	NS	-0.71**	0.66**	-0.91***	45
	20300	NS	NS	NS	NS	NS	-1.04***	-0.49*	-2.19***	NS	46
	30571	NS	NS	NS	-1.91***	NS	NS	NS	-0.79**	-1.01***	58
	33295	NS	NS	NS	NS	NS	0.6777*	NS	-0.68*	NS	19
Acidobacteriaceae	4366	NS	NS	-1.01***	-1.42***	-1.06***	NS	-1.17***	-0.99***	-0.84**	32
	30762	NS	NS	-0.94***	-1.06***	-0.63***	NS	-1.09***	-0.91***	-0.70**	30
	37541	NS	NS	-1.47***	-2.47***	-0.77**	1.33***	NS	1.30***	-0.99***	55
	42054	NS	0.51*	-0.71**	-1.31***	-0.56*	-1.55***	-2.02***	-0.68*	-0.72**	44
	45264	NS	NS	NS	-1.72***	-0.58**	-1.61***	-1.80***	-1.78***	-0.52*	60
Beijerinckiaceae	17267	NS	NS	-0.55*	-0.97***	-0.66**	1.60***	0.74**	NS	NS	39
	43328	NS	NS	NS	-0.74**	NS	0.92***	NS	NS	NS	26
Cystobacterineae	45353	-0.67**	NS	-1.68***	-1.69***	-1.54***	1.61***	NS	1.72***	NS	50
Methylocystaceae	6292	NS	NS	NS	-0.66**	NS	1.24***	NS	NS	-0.49*	34
	32918	NS	NS	0.68*	-1,45***	NS	-1,83***	-1,70***	-2,29***	-0,69*	55
	38758	NS	NS	NS	-0.72**	NS	1.28***	0.67**	NS	NS	38
Oxalobacteraceae	26524	NS	NS	NS	NS	NS	1.53***	1.95***	NS	NS	32
Sphingomonadaceae	11233	NS	0.81**	NS	0.99**	0.99**	NS	NS	-1.96***	NS	42
	20227	NS	NS	-0,88**	-1,26***	-1,36***	NS	NS	NS	NS	22

Significance levels for each variable are given by: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, $P > 0.1$.

1.4.2 Biomarker analysis

At the OTU level, four OTUs were significantly associated with host species: two OTUs from *Acetobacteraceae* associated with both conifer species; one OTU from *Cystobacterineae* associated with *Acer saccharum*; and finally one OTU from *Rickettsiaceae* associated with *Acer rubrum* (Table 1.1, *see Annex F*). At the species level, 147 bacterial species were significantly associated with host species (Figure 1.1a, *see Annex F*). Overall, the *TM7* group was significantly associated with *Acer rubrum*; the *Firmicutes*, *Bacilli*, and *Betaproteobacteria* were associated with *Acer saccharum*; the *Proteobacteria*, *Alphaproteobacteria* and *Chlamydiae* with *Betula papyrifera*; the *Armatimonadetes* and *Acidobacteria* with *Abies balsamea*; and finally the *Actinobacteria*, *Bacteroidetes*, *Chloroflexi* and *FSP* were significantly associated with *Picea glauca*. At a broader taxonomic scale, 129 bacterial species were significantly associated with the gymnosperms and 79 with the angiosperms (Figure 1.1b, *see Annex G*). In short, the *Armatimonadetes*, *Actinobacteria*, *Bacteroidetes*, *Acidobacteria*, *TM7*, *TM6*, *Deltaproteobacteria*, *OD1*, *Fusobacteria*, and *FBP* were associated with the gymnosperms; whereas the groups *Chlamydiae*, *Proteobacteria*, *Gammaproteobacteria*, *Alphaproteobacteria* and *Firmicutes* were associated with angiosperms.

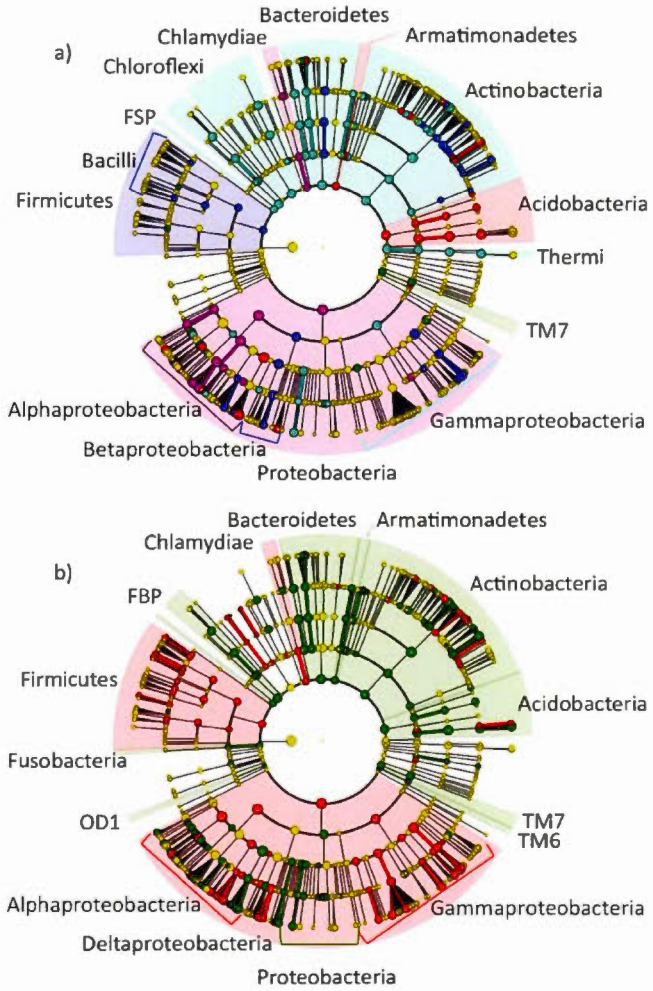

Figure 1.1 Cladogram of significant associations between phyllosphere bacterial taxon and host identity (linear discrimination algorithm LEfSe). a) color indicates association with a host species (green: *Acer rubrum*; blue: *Acer saccharum*; purple: *Betula papyrifera*; red: *Abies balsamea*; turquoise: *Picea glauca*) (b) green indicates an association with gymnosperms (*Abies balsamea* and *Picea glauca*) and red with the angiosperms (*Acer rubrum*, *Acer saccharum,* and *Betula papyrifera*). The circles, parentheses, and shadings indicate with which host-group the bacterial taxonomic group is associated.

1.4.3 Drivers of Variation in Phyllosphere Community Composition and Diversity

An analysis of variation in community structure (PERMANOVA on Bray-Curtis distances) explained by different factors showed that Gymnosperm/Angiosperm groups explained 13.4 % (P = 0.001), host taxonomic family explained 9.3 % (P = 0.001); host genus explained 2.21 % (P = 0.002), and finally host species explained 2.1 % (P = 0001). Host taxonomic levels thus explained 24.8 % of the variation in phyllosphere bacterial community structure. Host species identity, the interaction between species and site, site, and time, explained respectively 27.2 %, 13.8 %, 10.9 %, and 1.5 % of the variation in leaf bacterial community structure (PERMANOVA on Bray-Curtis distances) for a total of 53.4 % (Figure 1.2 and Table 1.2). These factors showed similar trends when explaining the variation in leaf bacterial phylogenetic community structure (PERMANOVA on weighted UniFrac distances) thus here we present only the results of analyses based on Bray-Curtis dissimilarities. The best model from the linear mixed models of variation in bacterial alpha diversity explained by different factors (model: Shannon Diversity ~ (1 | TREE) + Species + Site + Time; fit by maximum likelihood) showed that tree identity explains 13 % of the variance in bacterial community alpha diversity (ΔAIC = 1.2). Only species, site, and their interactions significantly affected microbial diversity. The Abitibi site was significantly less diverse than the three other sites. Conifer species (*Pinus* and *Abies*) showed a significantly higher alpha-diversity than the three deciduous species (Figure 1.3).

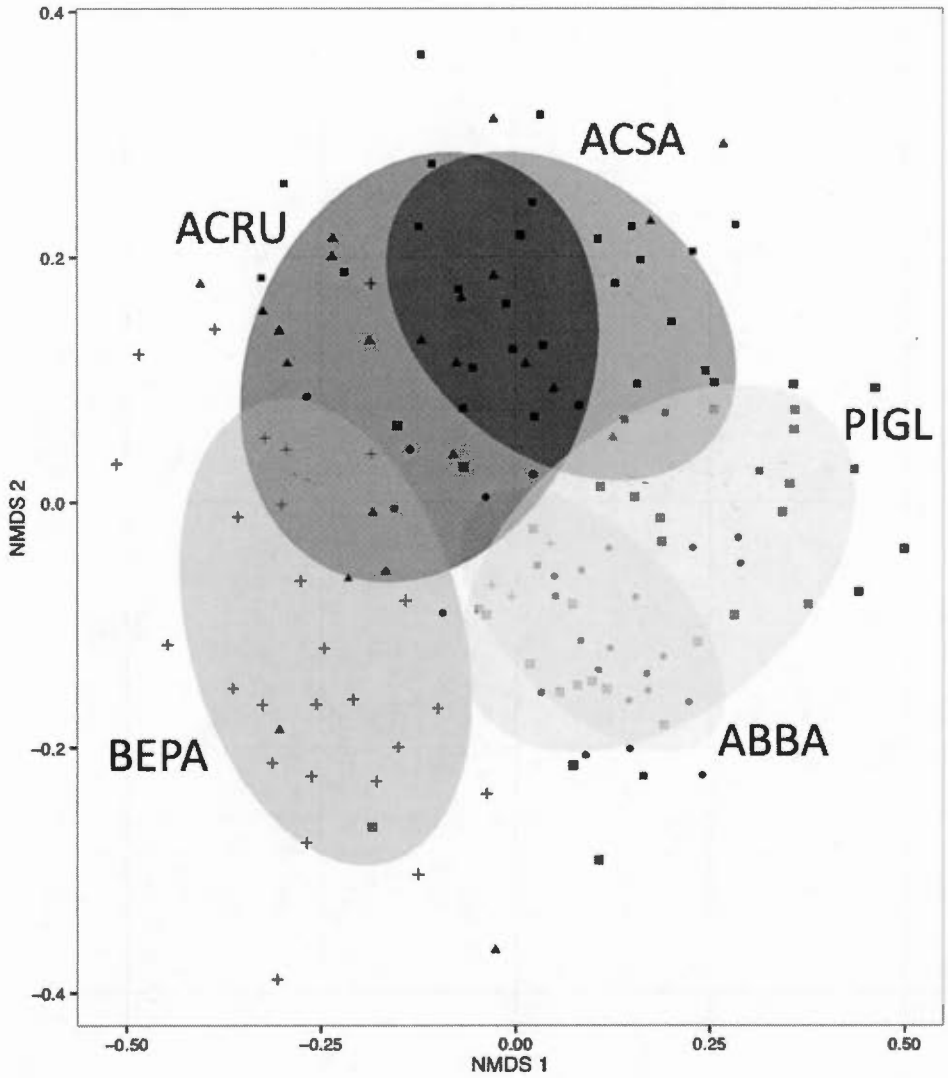

Figure 1.2 Non-metric multidimensional scaling (NMDS) ordination of variation in bacterial community structure of temperate tree phyllosphere based on Bray-Curtis distances among samples. Samples (points) are shaded based on host species identity (ABBA for *Abies balsamea*; ACRU for *Acer rubrum*; ACSA for *Acer saccharum*; BEPA for *Betula papyrifera*; and PIGL for *Picea glauca*); ellipses indicate 1 standard deviation confidence intervals around samples from each host species.

Table 1.2 Bacterial community structure variation of the 142 samples explained by various factors (permutational ANOVA on Bray-Curtis dissimilarities).

Variable		Bray-Curtis dissimilarities	
		R^2 (%)	Pr(>F)
Single Factor	Species	27.16	0.001***
	Site	10.90	0.001***
	Time	1.46	0.008**
2nd order interaction	Species*Site	13.75	0.001***
	Site*Time	NS	NS

The model explained 53 %. Significance levels for each variable are given by: * P <0.05; ** P <0.01; *** P <0.001; NS, P> 0.1.

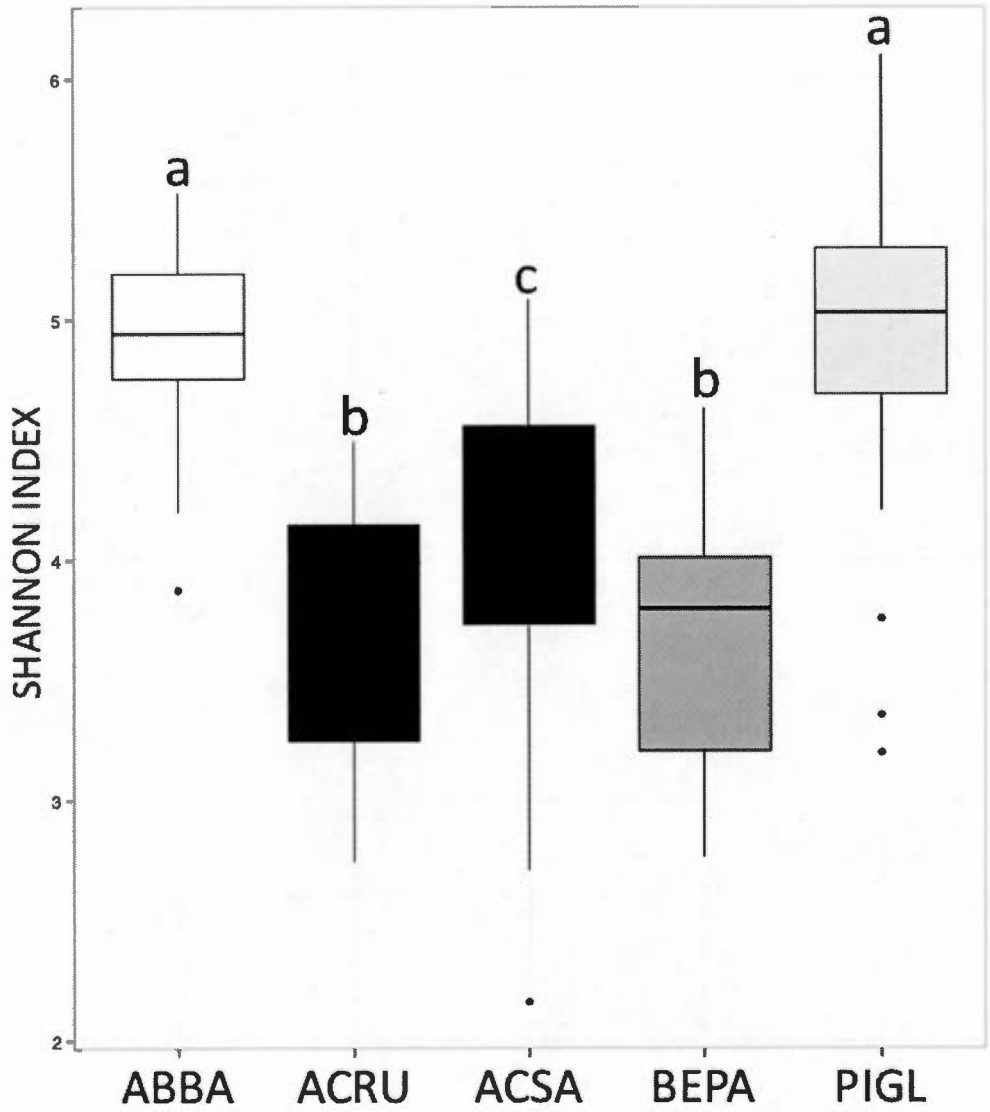

Figure 1.3 Shannon diversity indices of phyllosphere bacterial communities for different host species. Boxplots are shaded by host species (ABBA for *Abies balsamea*; ACRU for *Acer rubrum*; ACSA for *Acer saccharum*; BEPA for *Betula papyrifera*; and PIGL for *Picea glauca*). Only the pairs BEPA-ACRU and PIGL-ABBA are not significantly different following a post-hoc test of Tukey multiple comparisons of means at a 95 % family-wise confidence level.

Four functional traits were significant drivers of phyllosphere bacterial community structure (PERMANOVA on Bray-Curtis distances): nitrogen content of leaves (N_{mass}; P = 0.001), specific leaf area (SLA; P = 0.001), wood density (WD, P = 0.001) and seed mass (S_{mass}; P = 0.001). The relative abundances of *Acidobacteria*, *Chlamydia*, *Deinococci*, *Fimbriimonadia* and *Saprospirae* were significantly correlated (P <0.001) with traits related to the leaf economics spectrum (N_{mass} and SLA). These bacterial classes were more abundant on the leaves of tree species that have lower leaf nitrogen concentrations and higher leaf dry matter content (Figure 1.4). The relative abundances of *Actinobacteria*, *Alphaproteobacteria*, *Bacilli*, *Betaproteobacteria*, *Clostridia*, *Cytophagia* and *Gemmatimonadetes* were significantly correlated (P <0.001) with traits related to wood density (Figure 1.4). Climate variables were weakly but significantly correlated with phyllosphere bacterial community structure (total precipitation: 1.8 % of variance explained (P <0.002), mean monthly temperature: 1.2 % of variance explained (P <0.006)).

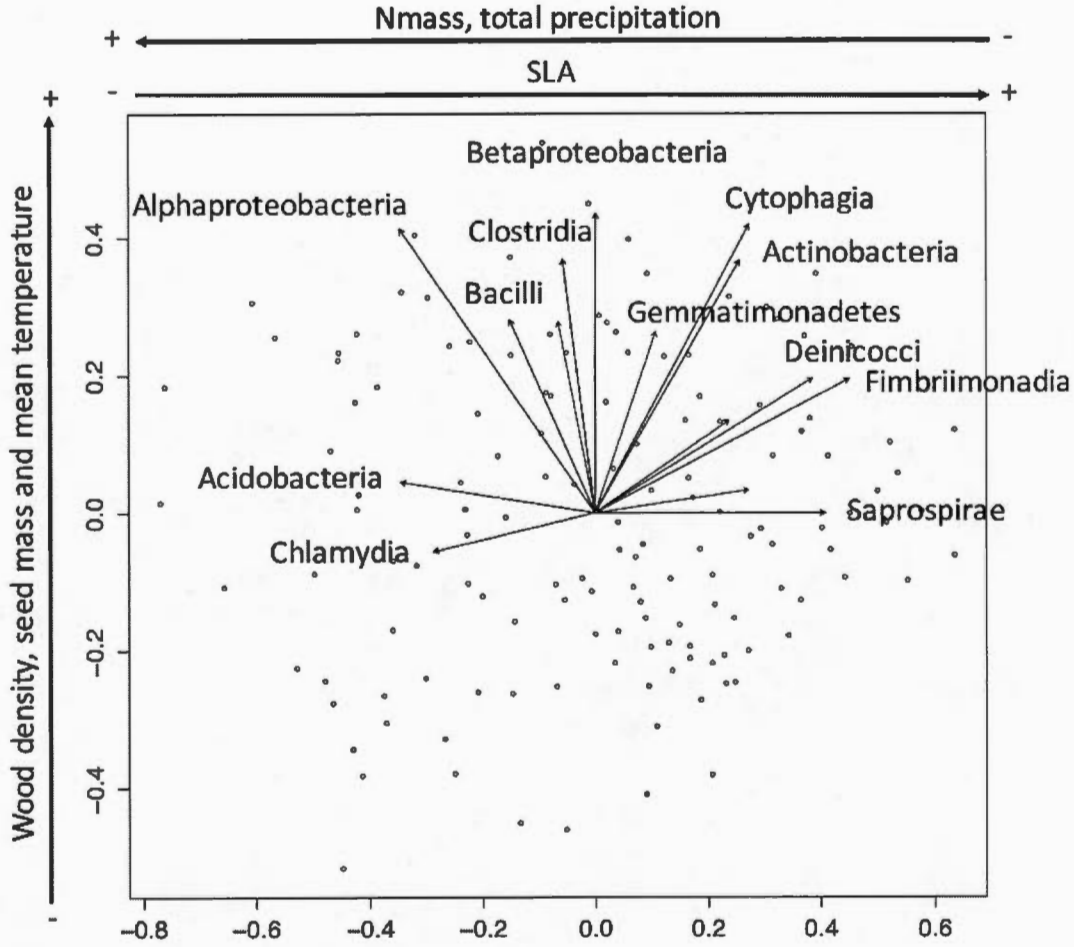

Figure 1.4 Non-metric multidimensional scaling (NMDS) ordination of variation in bacterial community structure of temperate tree phyllosphere. Ordination based on Bray-Curtis dissimilarities among samples. Points represent samples and blue arrows represent the significant ($p < 0.001$) correlations between NMDS axes versus the relative abundances of bacterial classes in communities. Arrows outside plot margins indicate host plant traits and climatic variables with significant ($p < 0.007$ for functional traits and $p < 0.025$ for climatic data) correlations with sample scores on each ordination axis.

1.5 Discussion

In terms of the taxonomic composition of phyllosphere communities, temperate leaf communities seem to differ slightly from past reports of tropical and temperate phyllosphere community structure. Natural temperate phyllosphere communities in Quebec forests were dominated by *Alphaproteobacteria* (68 % of all sequences), contrasting with 27 % (Kim *et al.,* 2012) and 22.8 % (Kembel *et al.,* 2012) of sequences in tropical tree species and 24.5 % in suburban temperate stands (Redford *et al.,* 2010). Due to the necessity of using chloroplast-excluding primers to prevent contamination of samples by plant DNA (Rastogi *et al.,* 2010), we were unable to quantify the abundance of Cyanobacteria in the temperate forest phyllosphere. However, metagenomic studies have demonstrated that Cyanobacteria are typically rare in the vascular plant phyllosphere (Delmotte *et al.,* 2009; Vorholt, 2012), and by using the same chloroplast-excluding 16S primer employed by previous studies (Redford & Fierer, 2009; Redford *et al.,* 2010; Kembel *et al.,* 2014) we were able to eliminate primer taxonomic bias as an explanation of differences in clade abundances among studies.

In contrast with Redford *et al.* (2010), we detected the presence of a core phyllosphere microbiome, a group of bacterial taxa shared among multiple communities sampled from the same habitat and thought to play key ecological roles (Shade & Handelsman, 2012). The core microbiome was composed of 19 OTUs representing 42.7 % of all sequences present in more than 99 % of samples, even when study sites were hundreds of kilometers apart. Assuming that bacterial OTUs represent ecologically or evolutionarily coherent units (Schmidt *et al.,* 2014), this finding suggests that bacteria from a similar metacommunity colonize tree leaves across Quebec's temperate forests by dispersal through a variety of vectors (i.e. air, rain, soil) (Bulgarelli *et al.,* 2012),

homogenizing the epiphytic phyllosphere community structure across broad geographic distances.

Despite the presence of a core microbiome of abundant taxa, individual trees also showed unique communities that varied predictably across species, sites and time, suggesting a role for selection- or niche-based mechanisms during community assembly. Linear models testing the association between core microbiome OTUs versus host species identity, site, and time explained 18 to 60 % of the variation in phyllosphere bacterial community structure (Table 1.1), confirming these three drivers' roles in shaping phyllosphere community structure. In addition, biomarker analyses confirmed the existence of host selective mechanisms on phyllosphere community structure as shown by associations between numerous bacterial taxa and different host species and sites (Figure 1.1).

At the tree species level, *Abies balsamea* (balsam fir) tended to associate with the order *Sphingomonadales*, as with the families *Acidobacteraceae, Solibacteraceae* and *Frankiaceae.* The three first groups mentioned above are common in soils (Janssen, 2006; Kim *et al.,* 2006), and the *Frankiaceae* are nitrogen-fixing bacteria that colonize plant roots (Normand, 2006). This finding is in line with other studies showing that conifers select a different microbiome than other plant species: for example, they harbor less ice nuclei active bacteria (Lindow & Arny, 1978). In contrast, *Betula papyrifera* (paper birch) was associated with the family *Rhodospirillaceae* (*Rhodospirillales:Alphaproteobacteria).* This bacterial family is mostly composed of purple nonsulfur bacteria that produce energy through photosynthesis (Biebl *et al.,* 1981). Photosynthesis could be a key adaptation to the phyllosphere habitat, an environment where simple carbon sources are scarce and highly variable (Lindow & Brandl, 2003; Vorholt, 2012). Tree-bacteria associations were also observed at the angiosperm vs. gymnosperm level (Figure 1.3), likely driven by the influence of the

numerous plant functional trait differences between these clades (Kembel *et al.,* 2014; Lambais *et al.,* 2014).

Host species identity was the main driver of phyllosphere bacterial community structure among trees ($R^2 = 27$ %) when compared to site and time. As shown in other studies, each tree species harbors a distinctive phyllosphere bacterial community (Redford *et al.,* 2010; Kim *et al.,* 2012, Lambais *et al.,* 2014), but our results highlight for the first time the relative influence of site ($R^2 = 11$ % for site alone and $R^2 = 14$ % for site-species interaction) and time ($R^2 = 1$%) for multiple tree species. In accordance with the findings of Kembel *et al.* (2014) in tropical forests, temperate phyllosphere epiphytic bacterial community structure was correlated with both traits linked to plant-resource uptake strategies such as leaf nitrogen content and leaf mass per area (Wright *et al.,* 2004), and traits linked to the wood density/growth/mortality tradeoff such as wood density (Wright *et al.,* 2010). This confirms that phyllosphere bacterial communities are shaped by the ecological strategies of their plant hosts. These similarities also suggest that the factors driving the functional biogeography of plant-microbe associations in the phyllosphere are similar across temperate and tropical biomes, as we found a similar set of traits influencing phyllosphere community structure in temperate forests versus those described for tropical forests (Kembel *et al.,* 2014). Although many insights have been gained from individual tree microbiome studies in tropical and temperate biomes, meta-analyses controlling for methodological differences will be needed to better understand plant-microbe associations across terrestrial biomes and environmental gradients.

Consistent with the idea of environmental selective pressure on phyllosphere communities due to abiotic conditions such as temperature and precipitation, climate differences between sites (monthly precipitation and mean monthly temperature) were correlated with variation in phyllosphere bacterial community structure. In addition,

the effect of sampling time and the interaction between sampling time and site on phyllosphere community structure suggests that phyllosphere communities undergo a succession during the growth season. As previously demonstrated for individual host tree species by Redford & Fierer (2009) for bacterial communities and by Jumpponen & Jones (2009) for fungal communities, leaf communities were temporally dynamic. However, the variance explained by sampling time was small relative to the importance of host species and site, suggesting that once a community of bacteria successfully colonizes a leaf, temporal changes are not enough to overcome the influence of host species identity and site on community assembly. In the temperate forest we studied, growth season had a significant impact on community structure at two sites at the beginning and end of the growth season: the months of June and August. To minimize phyllosphere community structure variation due to sampling time, leaf sampling in these forests should be completed in July once leaves are fully mature but before senescence begins in August.

We found consistent evidence that community composition and alpha diversity differed between coniferous (gymnosperm) versus broadleaved (angiosperm) tree species. Our results show that several functional traits characteristic of tree ecological strategy explained differences in leaf community structure. However, additional leaf functional traits not measured here (i.e. increased leaf cuticle thickness and wax composition of gymnosperms) could also play a key role by limiting carbon compound availability and humidity at the leaf surface (Redford *et al.,* 2010; Vorholt, 2012). Because our sampling did not exclusively target the new needles of conifers, a study of succession on conifer needles will really be needed to determine if the diversity is caused by the particular selective power of the host species, or by the longer accumulation through leaf life span of the bacterial community on conifer leaves.

1.6 Conclusion

In this study, we describe for the first time natural temperate tree phyllosphere bacterial communities across multiple tree species while exploring the influence of host species identity, site and time of sampling on phyllosphere community structure. In addition, we performed the first simultaneous evaluation of the importance of key dispersal-related and niche-based drivers such as host species identity (phylogeny, co-evolution, functional traits), geographical location (dispersal history and abiotic conditions) and time of sampling (abiotic conditions) on tree phyllosphere bacterial communities. Our key findings include: (1) that temperate host-species share a "core microbiome"; (2) that there are significant associations between groups of bacteria and host species; and finally (3) that a greater part of the variation in phyllosphere bacterial community assembly is explained by host species identity rather than by site or time.

1.7 Acknowledgments

We sincerely thank Travis Dawson, Sophie Carpentier and Gabriel Jacques for their great support in the field and laboratory.

CHAPTER II

TREE PHYLLOSPHERE BACTERIAL COMMUNITIES: EXPLORING THE MAGNITUDE OF INTRA- AND INTER-INDIVIDUAL VARIATION AMONG HOST SPECIES

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Isabelle Laforest-Lapointe, first author
Centre d'étude de la forêt, Département des sciences biologiques, Université du Québec à Montréal, C.P. 8888, Succ. Centre-Ville, Montréal, Canada, H3C 3P8.
Laforest-lapointe.isabelle@courrier.uqam.ca

Christian Messier
Centre d'étude de la forêt, Département des sciences biologiques, Université du Québec à Montréal, C.P. 8888, Succ. Centre-Ville, Montréal, Canada, H3C 3P8.; Institut des Sciences de la Forêt Tempérée, Université du Québec en Outaouais, 58 rue Principale, Ripon, Canada, J0V 1V0.
Messier.christian@uqam.ca

Steven W. Kembel
Centre d'étude de la forêt, Département des sciences biologiques, Université du Québec à Montréal, C.P. 8888, Succ. Centre-Ville, Montréal, Canada, H3C 3P8.
Kembel.steven_w@uqam.ca

2.1 Abstract

Background. The diversity and composition of the microbial community of tree leaves (the phyllosphere) varies among trees and host species and along spatial, temporal, and environmental gradients. Phyllosphere community variation within the canopy of an individual tree does exist, however the importance of this variation relative to among-tree and among-species variation is poorly understood. Sampling techniques employed for phyllosphere studies include picking leaves from one canopy location to mixing randomly selected leaves from throughout the canopy. In this context, our goal was to characterize the relative importance of intra-individual variation in phyllosphere communities across multiple species, and compare this variation to inter-individual and interspecific variation of phyllosphere epiphytic bacterial communities in a natural temperate forest in Quebec, Canada.

Methods. We targeted five dominant temperate forest tree species including angiosperms and gymnosperms: *Acer saccharum*, *Acer rubrum*, *Betula papyrifera*, *Abies balsamea* and *Picea glauca*. For one randomly selected tree of each species, we sampled microbial communities at six distinct canopy locations: bottom-canopy (1-2 m height), the four cardinal points of mid-canopy (2-4 m height), and the top-canopy (4-6 m height). We also collected bottom-canopy leaves from five additional trees from each species.

Results. Based on analysis of bacterial community structure measured via Illumina sequencing of the bacterial 16S gene, we demonstrate that 65 % of the intra-individual variation in leaf bacterial community structure could be attributed to the effect of inter-individual and inter-specific differences while the effect of canopy location was not significant. In comparison, host species identity explains 47 % of inter-individual and inter-specific variation in leaf bacterial community structure followed by individual identity (32 %) and canopy location (6 %).

Discussion. Our results suggest that individual samples from consistent positions within the tree canopy from multiple individuals per species can be used to accurately quantify variation in phyllosphere bacterial community structure. However, the considerable amount of intra-individual variation within a tree canopy asks for a better understanding of how changes in leaf characteristics and local abiotic conditions drive spatial variation in the phyllosphere microbiome.

Key words: Phyllosphere, plant-bacteria interaction, microbiome, temperate forest, intraindividual variation, interspecific variation, inter-individual variation, bioindicator

2.2 Introduction

The phyllosphere microbiota represents the communities of microorganisms including bacteria, archaea, and eukaryotes such as fungi that are associated with plant leaves (Inácio *et al.,* 2002; Lindow & Brandl, 2003). Phyllosphere microbes influence host fitness through a variety of mechanisms such as plant hormone production and protection from pathogen colonization (Innerebner *et al.,* 2011; Ritpitakphong *et al.,* 2016). As a result of their effect on host plant fitness, leaf microorganisms can influence plant population dynamics and community diversity (Clay & Holah, 1999; Bradley *et al.,* 2008) as well as ecosystem functions including water (Rodriguez *et al.,* 2009) and nutrient cycling (van der Heijden *et al.,* 2008; McGuire & Treseder, 2010; Allison & Treseder, 2011). Tree microbial phyllosphere communities have been studied in tropical (Lambais *et al.,* 2006, 2014; Kim *et al.,* 2012; Kembel *et al.,* 2014; Kembel & Mueller, 2014), temperate (Jumpponen & Jones, 2009; Redford & Fierer, 2009; Redford *et al.,* 2010; Jackson & Denney, 2011) and Mediterranean forests (Peñuelas *et al.,* 2012), along altitudinal gradients (Cordier *et al.,* 2012a, 2012b), and in deserts (Finkel *et al.,* 2011, 2012). In order to understand the structure and function of phyllosphere microbial communities, studies typically either assume that a single sample of leaves from a plant canopy is representative of the phyllosphere community of the entire tree or host species (Lambais *et al.,* 2006; Kim *et al.,* 2012; Kembel *et al.,* 2014), or control for spatial structure in phyllosphere community structure by mixing leaves from multiple canopy locations (Redford & Fierer, 2009; Redford *et al.,* 2010; Jumpponen & Jones, 2009, 2010; Finkel *et al.,* 2011, 2012; Cordier *et al.,* 2012a, 2012b). In this study, our aim was to quantify the relative importance of intra-individual versus inter-individual and inter-specific variation in the structure of temperate tree phyllosphere communities, across multiple host species.

Host genetic factors (Bodenhausen *et al.,* 2014; Horton *et al.,* 2014) and taxonomic identity (Redford *et al.,* 2010; Kembel *et al.,* 2014) are important drivers of phyllosphere bacterial community structure. Most studies of phyllosphere communities across different host species have assumed within-plant and within-species variation in phyllosphere community structure to be negligible, and looked passed intra-individual and inter-individual variation (but see Redford *et al.,* 2010 and Leff *et al.,* 2015). In tree phyllosphere studies, samples are usually taken from shade leaves either at the bottom of the canopy or at mid-canopy height near the trunk. However, the technique to sample phyllosphere communities vary between studies, ranging from studies that sampled leaves from a specific canopy location (i.e. Kembel *et al.,* 2014; Kembel & Mueller, 2014) to taking multiple leaves from around the canopy at the same height (i.e. Redford & Fierer, 2009; Redford *et al.*, 2010; Jackson & Denney, 2011). However, Leff *et al.,* 2015 demonstrated for a single tree species (*Ginkgo biloba*) that there is intra-individual variation in phyllosphere community structure within the canopy of a single tree. The relative importance of this within-individual variation versus inter-individual and inter-specific variation, and the degree to which a sample of leaves from a canopy are representative of the microbiome of an individual or a species, is not well understood.

A multitude of factors could influence microbial community structure on leaves within a tree canopy. Leaf position in the canopy defines the degree of exposure to ultraviolet radiation and wind and therefore community structure could change depending on the position of the leaves sampled. Exposure to ultraviolet radiation has been shown to increase the diversity of the maize leaf microbial community (Kadivar & Sapleton, 2003) and anoxygenic phototropic bacteria have been detected in the phyllosphere of *Tamarix nilotica* (Atamna-Ismaeel *et al.,* 2012a, 2012b). This phenomenon could also be caused by leaf morphological and ecophysiological attributes associated with high light availability (thicker leaves, lower specific leaf area, lower water content, higher

total chlorophyll, higher photosynthetic activity rate; Lichtenthaler *et al.,* 2007). Variation in atmosphere conditions within the canopy (i.e. increased exposure to wind and gas exchange levels) modifies local leaf humidity conditions potentially influencing leaf epiphytic bacterial communities by inhibiting or favoring the growth of particular groups (Medina-Martìnez *et. al* 2015). Wind exposure could reduce leaf moisture and induce a stomata closure (Grace *et al.,* 1975), which could impact the diffusion of nutrients and reduce the size of microbial aggregates (Leveau & Lindow, 2001; Miller *et al.,* 2001).

In this study, we aim to (1) compare the intra-individual, inter-individual and interspecific variation of phyllosphere bacterial communities; (2) characterize the composition of epiphytic phyllosphere bacterial communities at different canopy locations for five tree species; and (3) make practical recommendations for the sampling of tree phyllosphere bacterial communities. We hypothesized that (1) the magnitude of intra-individual variation will be smaller than inter-individual and interspecific variation, (2) that canopy location will be a significant driver of phyllosphere bacterial community structure because of variation in abiotic conditions (e.g. radiation, wind), and changes in ecophysiological and morphological leaf characteristics.

2.3 Methods

2.3.1 Study Site & Host-Tree Species

The two study sites are located in a natural temperate forest stand in Gatineau (45°44'50"N; 75°17'57"W) and Sutton (45°6'46"N; 72°32'28"W) Quebec, Canada.

These sites are characterized by a cold and humid continental climate with temperate summer. A total of six individuals (three at each site) from each of five tree species common to temperate forests and dominant in the canopy were sampled to provide representatives of both angiosperms and gymnosperms: *Abies balsamea* (Balsam fir), *Acer rubrum* (Red maple), *Acer saccharum* (Sugar maple), *Betula papyrifera* (Paper birch) and *Picea glauca (*White spruce).

2.3.2 Bacterial community collection

We sampled phyllosphere communities from trees on August 29, 2013 as part of another experiment (Laforest-Lapointe *et al.,* 2016a). Sampling was carried out one week after the last rainfall event. We defined three strata within the canopy: bottom-canopy (1-2 m height), mid-canopy (2-4 m height), and top-canopy (4-6 m height). 30 individuals were randomly selected by picking random geographic coordinates and finding the closest individual at this location. For the first tree sampled from each species, we clipped 50-100 g of leaves at the four cardinal points at mid-canopy height, plus a single sample at bottom-canopy and top-canopy heights, into sterile roll bags with surface-sterilized shears. We also sampled bottom-canopy leaves from two other randomly chosen trees from each species. For bacterial community collection and amplification, we used the protocols described by Kembel *et al.* (2014). We collected microbial communities from the leaf surface by five minutes of horizontal mechanical agitation of the samples in a diluted Redford buffer solution. We resuspended cells in 500 µL of PowerSoil bead solution (MoBio, Carlsbad, California). We extracted DNA from isolated cells using the PowerSoil kit according to the manufacturer's instructions and stored at -80 °C.

2.3.3 DNA library preparation and sequencing

We used a two-step PCR approach to prepare amplicon libraries for the high-throughput Illumina sequencing platform. The use of combinatorial primers for paired-end Illumina sequencing of amplicons reduced the number of primers while maintaining the diversity of unique identifiers (Gloor *et al.,* 2010). First, we amplified the V5–V6 region of the bacterial 16S rRNA gene using chloroplast-excluding primers in order to eliminate contamination by host plant DNA (16S primers 799F-1115R (Redford *et al.,* 2010; Chelius & Triplett, 2001)) following protocols described by Kembel *et al.* (2014). We cleaned the resulting product using MoBio UltraClean PCR cleanup kit. We isolated a ~445 bp fragment by electrophoresis in a 2 % agarose gel, and recovered DNA with the MoBio GelSpin kit. We prepared multiplexed 16S libraries by mixing equimolar concentrations of DNA, and sequenced the DNA library using Illumina MiSeq 250 bp paired-end sequencing at Genome Quebec.

We processed the raw sequence data with PEAR (Zhang *et al.,* 2014) and QIIME (Caporaso *et al.,* 2010) software to merge paired-end sequences to a single sequence of length of 350 bp, eliminate low quality sequences (mean quality score <30 or with any series of 5 bases with a quality score <30), and de-multiplex sequences into samples. We eliminated chimeric sequences using the Uclust and Usearch algorithms (Edgar, 2010). Then, we binned the remaining sequences into operational taxonomic units (OTUs) at a 97 % sequence similarity cutoff using the Uclust algorithm (Edgar 2010) and determined the taxonomic identity of each OTU using the BLAST algorithm (Greengenes reference set) as implemented in QIIME (Caporaso *et al.,* 2010). The number of sequences per sample ranged from 6 256 to 75 412. From these 1 499 777 sequences, we rarefied each sample to 5 000 sequences and repeated analyses on 100

random rarefactions. Re-analysis did not quantitatively change results and so we report only the result of the analysis of a single random rarefaction. We included the resulting 275 000 sequences in all subsequent analyses.

2.3.4 Statistical analyses

We created a database excluding OTUs represented fewer than 3 times to minimize the presence of spurious OTUs caused by PCR and sequencing errors (Acinas *et al.*, 2005). We identified the OTUs that were present on all samples to define the "core microbiome" (Shade & Handelsman, 2012). Then we tested for significant associations between bacterial taxa and host species, and canopy location using the Linear Discriminant Analysis Effect Size (LEfSe) algorithm (Segata *et al.*, 2011). This analysis allows the recognition of significant individual host-microbe associations and evaluates the strength of associations between organisms from different groups (Segata *et al.*, 2011).

We performed analyses with the ape (Paradis *et al.*, 2004), picante (Kembel *et al.*, 2010), and vegan (Oksanen *et al.*, 2007) packages in R (R Development Core Team 2013) and ggplot2 (Wickham, 2009) for data visualization. We quantified the taxonomic variation in bacterial community structure among samples with respectively the Bray-Curtis dissimilarity. To illustrate patterns of bacterial community structure, we performed a nonmetric multidimensional scaling (NMDS) ordination of Bray–Curtis dissimilarity. We identified relationships between bacterial community structure, host species identity, and sample canopy location by conducting a permutational multivariate analysis of variance (PERMANOVA, Anderson, 2001) on the community matrix. We employed a blocking randomization to account for the non-

independence of observations among sites. To decompose the total variation in the community matrix explained by host species identity and canopy location, we performed a partial redundancy analysis (RDA; Legendre & Legendre, 1998). This technique measures the amount of variation that can be attributed exclusively to each set of explanatory variables. We performed three permutational tests of multivariate homogeneity of group dispersions (Levene's test for variances' homogeneity multivariate equivalent; Anderson, 2006; Anderson *et al.,* 2006): one to test if variance in intra-individual canopy bacterial communities was equal between individuals (30 samples from five trees sampled at six canopy locations); a second to compare interspecific variation between species (30 bottom-canopy samples from 30 different trees); and finally a third to test per-species intra- and inter-individual variation (all 55 samples). We estimated phyllosphere bacterial alpha diversity using the Shannon index calculated from OTU relative abundances for each community. We performed an analysis of variance (ANOVA) and subsequent post-hoc Tukey's tests to compare differences in diversity across species. The authors declare that the experiment comply with the current laws of the country in which the experiment was performed.

2.4 Results

2.4.1 Sequences, OTUs and taxonomy

High-throughput Illumina sequencing of the bacterial 16S rRNA gene (Claesson *et al.,* 2010) identified 5 005 bacterial operational taxonomic units (OTUs, sequences binned at 97 % similarity) in the phyllosphere of five temperate tree species, an average of 1055 ± 57 OTUs (mean ± SE) per tree sampled. Most of these bacterial taxa were relatively common across samples, with only 3.4 % of OTUs occurring on a single tree

and 0.8 % of OTUs occurring on all trees. The OTUs present on all samples represent the "core microbiome": the microbial taxa shared among multiple communities sampled from the same habitat (Shade & Handelsman, 2012). In this study, the core microbiome consisted of 42 OTUs (Table 2.1) representing 61 % of all sequences, of which 72 % were *Alphaproteobacteria*, 9 % *Cytophagia*, 7.8 % *Betaproteobacteria*, 5 % *Acidobacteria*, 2 % *Gammaproteobacteria* and 2 % *Actinobacteria*. The most abundant order was *Rhizobiales* (49 %) from which 77 % of sequences were assigned to the family *Methylocystaceae*. While there was some variation in the most abundant classes both across the five tree species and among canopy locations (Figure 2.1 and 2.2), the class *Alphaproteobacteria* was always the dominant taxon, with relative abundances ranging from 42 % on *P. glauca* to 84 % on *B. papyrifera* (Figure 2.1).

Table 2.1 Taxonomy and relative abundance of the 42 OTUs constituting the tree phyllosphere bacterial core microbiome in Quebec temperate forests (present in all 55 samples).

CLASS	ORDER	FAMILY	GENERA	SPECIES	%
Acidobacteriia	Acidobacteriales	Acidobacteriaceae	Bryocella	elongata	0.5
			4 NAs		4.8
Actinobacteria	Actinomycetales	Frankiaceae	NA		1.3
		Microbacteriaceae	Frondihabitans	cladoniiphilus	0.5
Cytophagia	Cytophagales	Cytophagaceae	Hymenobacter	2 NAs	9.0
Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Mucilaginibacter	daejeonensis	0.5
			NA		0.2
Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA		1.5
	Rhizobiales	Beijerinckiaceae	Beijerinckia	2 NAs	8.9
		Methylobacteriaceae	Methylobacterium	2 NAs	2.3
		Methylocystaceae	7 NAs		38.1
	Rhodospirillales	Acetobacteraceae	6 NAs		11.2
	Rickettsiales	NA	NA		0.10
		Rickettsiaceae	Rickettsia	NA	0.6
	Sphingomonadales	Sphingomonadaceae	Sphingomonas	6 NAs	7.9
				wittichii	1.7
				wittichii	0.1
Betaproteobacteria	Burkholderiales	Oxalobacteraceae	2 NAs		7.8
Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	Bdellovibrio	NA	0.2
	Myxococcales	Cystobacterineae	NA		0.7
Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Erwinia	NA	0.7
	Pseudomonadales	Pseudomonadaceae	Pseudomonas	fragi	1.3

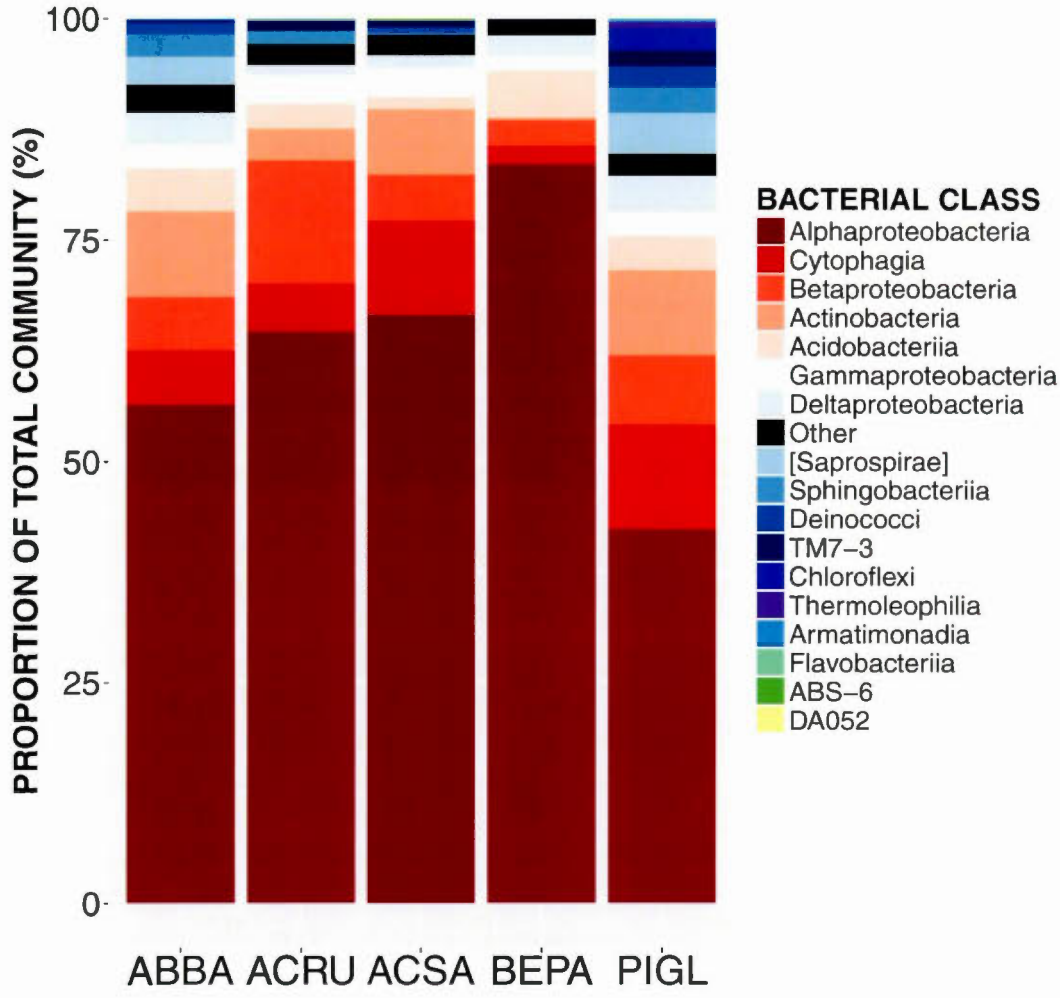

Figure 2.1 Relative abundance of sequences from bacterial taxonomic classes in the phyllosphere microbiome of temperate tree species in a Quebec forest. (ABBA: *Abies balsamea*; ACRU: *Acer rubrum*; ACSA: *Acer saccharum*; BEPA: *Betula papyrifera*; PIGL: *Picea glauca*).

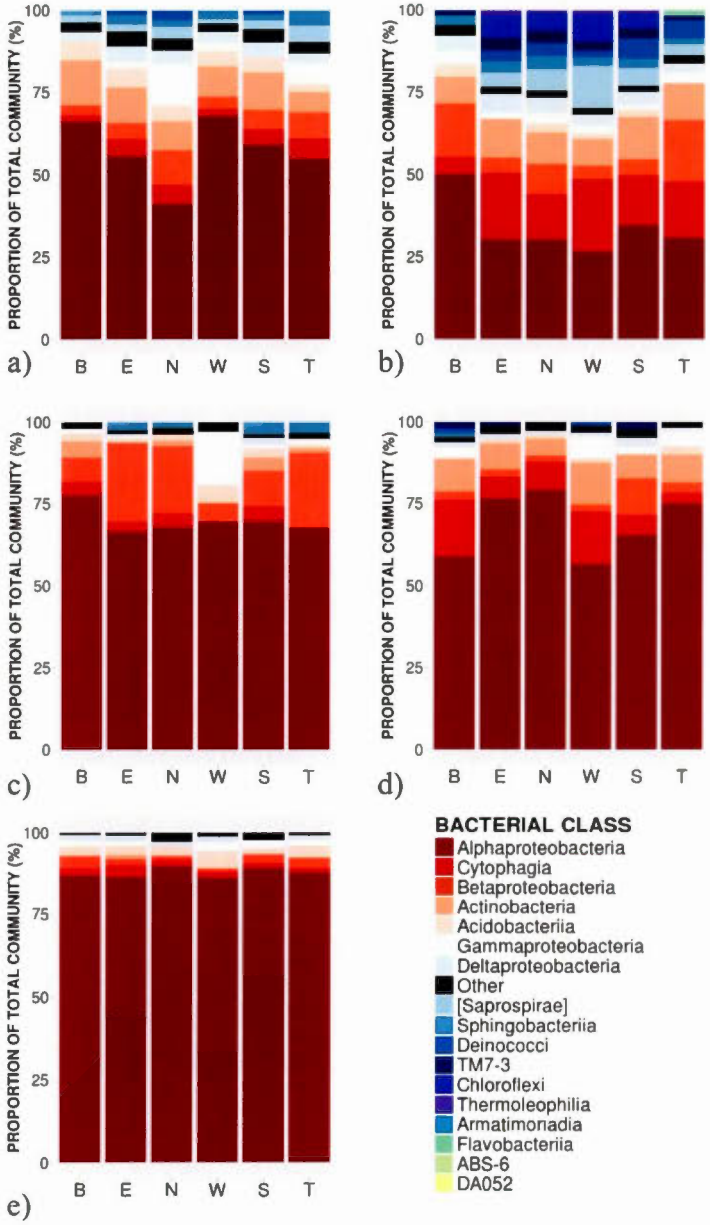

Figure 2.2 Relative abundance of bacterial classes in the phyllosphere at six canopy locations (B:Bottom, E:East, N:North, W:West, S:South T:Top) for one individual of the five temperate tree species under study. a) *Abies balsamea*; b) *Picea glauca*; c) *Acer rubrum*; d) *Acer saccharum*; and e) *Betula papyrifera*.

2.4.2 Intra-individual vs. Inter-Individual and Interspecific variation

Host species identity and individual identity effects could not be distinguished statistically due to the fact that analyses of intra-individual variation were based on a single individual per species. This host species/individual effect explained 65 % of variation in phyllosphere bacterial taxonomic community structure while the impact of canopy location was not statistically significant (PERMANOVA on Bray-Curtis dissimilarities; Table 2.2). We then tested whether canopy position had an effect on community structure after accounting for the variation explained by host species/individual using a partial redundancy analysis (RDA) on bacterial community structure constrained by host species identity. The RDA showed that when differences in bacterial community structure driven by host species identity were accounted for, sample canopy location explained 22 % of the remaining variation in community structure. In comparison, in the dataset with 30 different individuals, host species identity explained only 47 % of variation in phyllosphere bacterial community structure (PERMANOVA on Bray-Curtis dissimilarities; Table 2.2). When considering intra-individual and inter-individual samples, host species identity (R^2 = 47 %) was the strongest driver of variation in phyllosphere bacterial community structure closely followed by individual identity (R^2 = 32 %) and finally by canopy location (R^2 = 6 %; PERMANOVA on Bray-Curtis dissimilarities; Table 2.2). Community composition of samples clustered based both on the individual (Figure 2.3a) and species (Figure 2.3b) from which they were collected (non-metric multidimensional scaling (NMDS) based on Bray-Curtis distances among samples).

Table 2.2 Variation in phyllosphere bacterial community structure explained by various drivers: host species identity, sample location within the tree canopy and individual identity. PERMANOVA on Bray-Curtis dissimilarities.

				Variables R^2 (%)		
Dataset	**Scope**	**Nb samp.**	**Nb ind./species**	**Canopy location**	**Host species identity**	**Individual identity**
#1	Intra-individual	30	1	8*	65**	
#2	Inter-individual and interspecific	30	6	*na*	**47**	*na*
#3	Intra- and inter-individual, and interspecific	60	6	**6**	**47**	**32****

*The effect of canopy location was not significant after accounting for individual identity.

**Host species identity and individual identity are confounded as there were no replicates per species.

***Individual identity was nested in host species identity. *na*: non applicable.

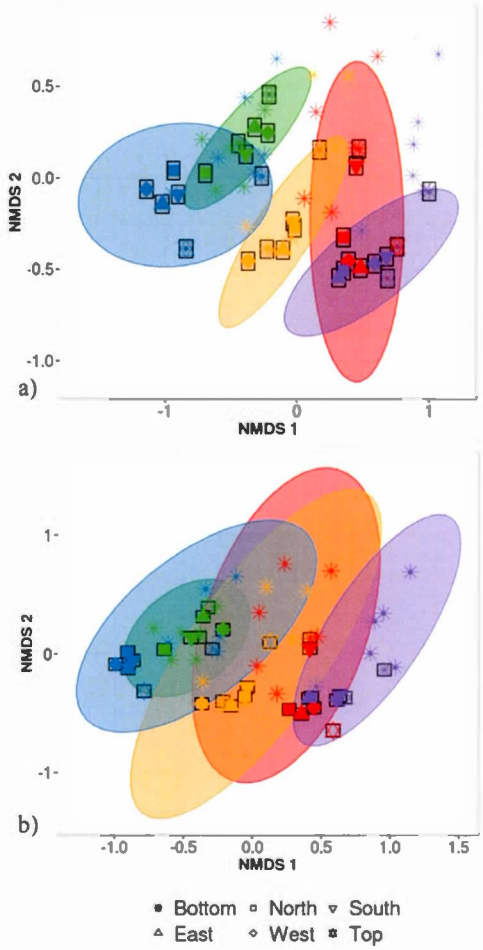

Figure 2.3 Non-metric multidimensional scaling (NMDS) ordination of within-individual variation in bacterial community structure across 55 phyllosphere samples from Quebec temperate forest trees. Ellipses indicate 1 standard deviation confidence interval around of a) intra-individual samples and b) inter-individual samples. Gray boxes indicate the 30 samples that came from individuals sampled at six different canopy locations. The other 25 samples came from 5 more individuals per host species. Symbols indicate sample position in the tree canopy; colours indicate by host species identity (green: *Abies balsamea*; red: *Acer rubrum*; orange: *Acer saccharum*; purple: *Betula papyrifera*; blue: *Picea glauca*). Stress value was 0.16.

The first permutational multivariate test of variance homogeneity (an analogue of Levene's test of homogeneity of variances) on intra-individual phyllosphere communities indicated a significant difference between *P.glauca* and *B. papyrifera* (Tukey's post hoc test; P = 0.03). The second test of the homogeneity of inter-individual variance between host species showed that *P. glauca*'s variance in community structure (mean distance to centroid = 0.34) was higher than *A. saccharum* (0.25; P<0.01) and *A. rubrum* (0.26; P<0.05) while all other comparisons were not significant. Finally, the third test between per species intra-individual and inter-individual variation indicated one significant difference in variation for *B. papyrifera* (P = 0.005; Figure 2.4).

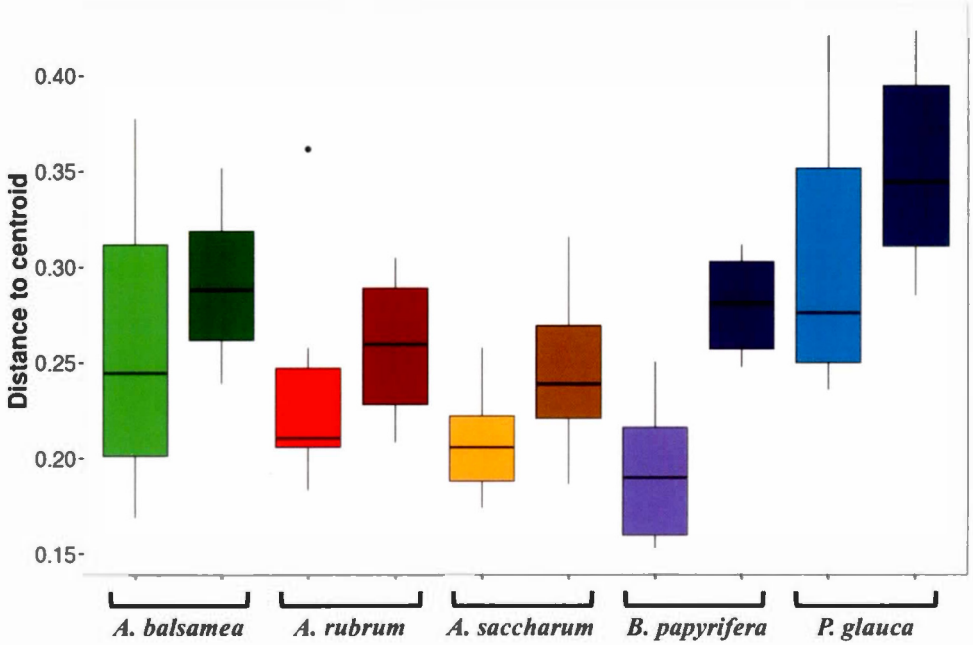

Figure 2.4. Permutation test for homogeneity of multivariate dispersions in leaf bacterial communities between per species intra- and inter-individual samples. Colours indicate host species identity (green for *Abies balsamea*; red for *Acer rubrum*; orange for *Acer saccharum*; purple for *Betula papyrifera*; and blue for *Picea glauca*); shading indicate intra- (pale color) and inter-individual (dark color) variance respectively.

The alpha-diversity of leaf bacterial community differed significantly across host species identity but not across canopy locations. Post-hoc Tukey honestly significant differences tests confirmed that Shannon alpha-diversity is higher on conifer species (4.9 ± standard error (SE) of 0.04 for *A. balsamea* and 5.3 ± SE 0.04 for *P. glauca*) than on angiosperm species (3.7 ± SE 0.06 for *A. rubrum*, 4.1 ± SE 0.05 for *A. saccharum* and 3.6 ± SE 0.09 for *B. papyrifera*).

2.4.3 Bacterial Indicator Taxa

The LEfSe analysis successfully identified indicator taxonomic groups associated with different host species, but not across different canopy locations (Table 2.3). The conifers, *A. balsamea* and *P. glauca*, had the highest number of associated bacterial indicator taxa (46 and 188 respectively). The strongest bio-indicators of *A. balsamea* were the *Frankiaceae* family and multiple taxonomic levels of the phylum *Acidobacteria*: *Acidobacteria*, *Acidobacteriales* and *Acidobacteriaceae*. For *P. glauca*, the strongest bioindicators were multiple taxa from the *Bacteroidetes* phylum (*Cytophagia*, *Cytophagales*, *Cytophagaceae*, *Spirosoma* and *Saprospirae*, *Saprospirales*, *Chitinophagaceae*), and from the *Actinobacteria*, *Chloroflexi*, and *Deltaproteobacteria*. In contrast, *B. papyrifera* showed an overrepresentation of 24 bacterial taxa including the phylum *Proteobacteria*, the class *Alphaproteobacteria* and several of its orders (*Rhodospiralles*, *Rickettsiales*, *Caulobacterales*). Finally, the two *Acer* species (*A. rubrum and A. saccharum*) were associated with 19 and 32 indicators respectively, including the order *Rhizobiales*: *A. rubrum* being associated with the family *Methylocystaceae* and *A. saccharum* with the order *Methylobacteriaceae*.

Table 2.3 Bacterial taxa identified as bio-indicators of different host species in Quebec temperate forests. The LEfSe analysis was performed on 30 samples: 6 individuals per species. Only the top five bio-indicators are shown. Significance are given by: * P <0.05; ** P <0.01; *** P <0.001; NS, P>0.05.

HOST SPECIES IDENTITY	BACTERIAL TAXA	EFFECT SIZE
Abies balsamea	Actinobacteria.Actinobacteria.Actinomycetales.Frankiaceae	4.34***
	Acidobacteria	4.30***
	Acidobacteria.Acidobacteriia.Acidobacteriales.Acidobacteriaceae	4.27***
	Acidobacteria.Acidobacteriia.Acidobacteriales	4.27***
	Acidobacteria.Acidobacteriia	4.27***
Acer rubrum	Proteobacteria.Alphaproteobacteria.Rhizobiales.Methylocystaceae	5.13***
	Proteobacteria.Betaproteobacteria	4.79***
	Proteobacteria.Betaproteobacteria.Burkholderiales	4.79***
	Proteobacteria.Betaproteobacteria.Burkholderiales.Oxalobacteraceae	4.77***
	Proteobacteria.Alphaproteobacteria.Rickettsiales.Rickettsiaceae	3.81***
Acer saccharum	Proteobacteria.Alphaproteobacteria.Rhizobiales	5.18***
	Bacteroidetes.Cytophagia.Cytophagales.Cytophagaceae.Hymenobacter	4.48***
	Proteobacteria.Alphaproteobacteria.Rhizobiales.Beijerinckiaceae	4.47***
	Proteobacteria.Alphaproteobacteria.Rhizobiales.Beijerinckiaceae.Beijerinckia	4.47***
	Actinobacteria.Actinobacteria.Actinomycetales.Microbacteriaceae	4.33***
Betula papyrifera	Proteobacteria.Alphaproteobacteria	5.39***
	Proteobacteria	5.28***
	Proteobacteria.Alphaproteobacteria.Rhodospirillales	5.26***
	Proteobacteria.Alphaproteobacteria.Rhodospirillales.Acetobacteraceae	5.25***
	Proteobacteria.Alphaproteobacteria.Rickettsiales	4.13***
Picea glauca	Bacteroidetes	4.97***
	Bacteroidetes.Cytophagia.Cytophagales	4.74***
	Bacteroidetes.Cytophagia	4.74***
	Actinobacteria	4.73***
	Bacteroidetes.Cytophagia.Cytophagales.Cytophagaceae	4.73***

2.5 Discussion

In this study, we demonstrate for multiple host species that there is a significant amount of intra-individual variation in phyllosphere bacterial community structure (Figure 2.3a). While the mean distance to centroid is always smaller for intra- than for inter-individual variation (Figure 2.4), this distance was only statistically significant for *B. papyrifera.* This result therefore provides partial support for our first hypothesis, stating that magnitude of intra-individual variation would be smaller than inter-individual and interspecific variation. When analyzing all samples, we found host species identity to be a stronger determinant of phyllosphere bacterial community structure than individual identity (Table 2.2). However, this result could be biased by the fact that we sampled a single individual for multiple canopy location. The importance of host species identity as a driver of phyllosphere community structure agrees with past studies of tropical (Kim *et al.,* 2012; Kembel *et al.,* 2014; Lambais *et al.,* 2014) and temperate trees (Redford *et al.,* 2010). Previous studies have quantified intra- and inter-individual variation in phyllosphere bacterial community structure, but these studies mixed leaves from within tree canopies without quantifying intra-individual variation (Redford *et al.,* 2010) or explored intra-individual variation for a single host species (Leff *et al.,* 2015). Our results show that after taking host species identity into account, there exist detectable differences in microbial community structure within tree canopies, at least in natural forest settings.

In terms of the taxonomic composition of the tree phyllosphere, each tree species can be characterized by a particular combination of most abundant classes across all canopy locations, consistent with other studies of the phyllosphere microbiome (Redford *et al.,* 2010; Kembel *et al.,* 2014; Laforest-Lapointe *et al.,* 2016a). Amongst the potential mechanisms that could explain host species selective power on their phyllosphere

bacterial communities, ecological strategies could play a role by impacting leaf abiotic conditions. *B. papyrifera*, a shade intolerant species (Krajina *et al.,* 1982; Burns & Honkala, 1990) exposed to sunlight in the upper part of the forest canopy, exhibited the smallest alpha diversity with a dominance of *Alphaproteobacteria* (Figure 2.2e) and also the smallest amount of intra-individual variation (Figure 2.4). In contrast, both conifer host species, growing below a deciduous canopy, exhibited the highest diversity in their community structure. While ultraviolet radiation could be driving the observed differences in leaf alpha diversity across species, our results provide no evidence of a significant and consistent difference in the alpha-diversity among canopy locations. However, because we sampled only one individual per species, canopy location effects remain to be quantified across multiple individuals of the same species. As shown by the multivariate test of homogeneity of variance, the intra-individual variation in phyllosphere community structure is not different from the variation observed at the inter-individual level. Future phyllosphere studies characterizing the relative influence of potential key factor such as random colonization via vectors such as the atmospheric air flow (Barberán *et al.,* 2014) or animals (Scheffers *et al.*, 2013), competition between bacterial populations (Vorholt, 2012); or intra-individual variation in leaf functional traits (Hunter *et al.*, 2010; Reisberg *et al.*, 2012) are needed to understand the dynamics driving intra-individual variability in bacterial community structure.

In conclusion, our results demonstrate that there exists considerable intra-individual variation in phyllosphere community structure, and that the magnitude of this variation is smaller but not statistically different from the magnitude of inter-individual variation. When designing a study of tree phyllosphere bacterial communities, if quantifying interspecific variation is the goal, then samples from a consistent location within the tree canopy for individual trees are sufficient to quantify the majority of the variation in community structure. However, future studies and especially studies focusing on a single host species should acknowledge that there can be significant intra-

individual variation in phyllosphere community structure, and sampling plans should explicitly select leaves at different positions within the canopy to describe spatial structure of the overall community composition for individual trees.

2.6 Acknowledgments

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CHAPTER III

LEAF BACTERIAL DIVERSITY MEDIATES PLANT DIVERSITY – ECOSYSTEM FUNCTION RELATIONSHIPS

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Isabelle Laforest-Lapointe, first author
Centre d'étude de la forêt, Département des sciences biologiques, Université du Québec à Montréal, C.P. 8888, Succ. Centre-Ville, Montréal, Canada, H3C 3P8.
Laforest-lapointe.isabelle@courrier.uqam.ca

Alain Paquette
Centre d'étude de la forêt, Département des sciences biologiques, Université du Québec à Montréal, C.P. 8888, Succ. Centre-Ville, Montréal, Canada, H3C 3P8.
alain.paquette@gmail.com

Christian Messier
Centre d'étude de la forêt, Département des sciences biologiques, Université du Québec à Montréal, C.P. 8888, Succ. Centre-Ville, Montréal, Canada, H3C 3P8.; Institut des Sciences de la Forêt Tempérée, Université du Québec en Outaouais, 58 rue Principale, Ripon, Canada, J0V 1V0.
Messier.christian@uqam.ca

Steven W. Kembel
Centre d'étude de la forêt, Département des sciences biologiques, Université du Québec à Montréal, C.P. 8888, Succ. Centre-Ville, Montréal, Canada, H3C 3P8.
Kembel.steven_w@uqam.ca

3.1 Abstract

Research on biodiversity-ecosystem functioning has demonstrated links between plant diversity and ecosystem functions such as productivity (Tilman & Downing, 1996; Isbell *et al.,* 2012; Tilman *et al.,* 2012). At other trophic levels, the plant microbiome has been shown to influence host plant fitness and function (Vorholt, 2012; Vandenkoornhuyse *et al.,* 2015), and host-associated microbes have been hypothesized to influence ecosystem function through their role in defining the extended phenotype of host organisms (Turner *et al.,* 2013; Bringel & Couée, 2015; Müller *et al.,* 2016). However, the importance of the plant microbiome for ecosystem function has not been quantified in the context of the known importance of plant diversity and traits. Using a novel tree biodiversity-ecosystem functioning experiment, we provide strong support for the hypothesis that leaf bacterial diversity is linked with ecosystem productivity even after accounting for the role of plant diversity; and we show that host species identity, functional identity and functional diversity are the main determinants of leaf bacterial community structure and diversity. Our study provides evidence of a positive correlation between plant-associated microbial diversity and terrestrial ecosystem productivity, and, in a parallel fashion, a new mechanism by which models of biodiversity-ecosystem functioning relationships can be improved.

Key words: biodiversity-ecosystem functioning, leaf bacterial diversity, plant productivity, functional diversity, species richness, leaf bacterial communities.

Introduction

The identification of the mechanisms promoting and maintaining primary production in terrestrial ecosystems is a central question in ecology, especially in the context of anthropogenic global change (Pawson *et al.,* 2013; Hautier *et al.,* 2015), and increasing biodiversity loss (Cardinale *et al.,* 2012; Allan *et al.,* 2015). After years of research on biodiversity-ecosystem functioning, the importance of diversity in driving ecosystem productivity and services has been demonstrated in many ecosystems (Isbell *et al.,* 2011; Tilman *et al.,* 2012; Liang *et al.,* 2016). These studies have shown that plant species richness, functional diversity and functional identity (Flynn *et al.,* 2011; Gross *et al.,* 2014) are among the key factors driving terrestrial ecosystem productivity; however, recent work suggests that these relationships could differ among trophic levels (O'Connor *et al.,* 2016).

The development of high-throughput sequencing technologies has revolutionized our understanding of microbial ecology, and furthermore led to calls for consideration of host-associated microbial communities as part of the host's extended phenotype or 'holobiont'[4] with potential effects on host ecology and evolution. Plant-associated microbial communities play direct roles in ecosystem functioning through effects on carbon (Delmotte *et al.,* 2009; Knief *et al.,* 2012; Jo *et al.,* 2015) and nitrogen cycles (Knief *et al.,* 2012; Saikkonen *et al.,* 2015; Moyes *et al.,* 2016). They also influence ecosystem function indirectly through their effects on host plant health and productivity via numerous mechanisms (Vorholt, 2012; Bringel & Couée, 2015) such as modifying plant hormone production (Schauer & Kutschera, 2011; Bodenhausen *et al.,* 2014) and increasing host resistance to abiotic and biotic stress (Zamioudis & Pieterse, 2012). Healthy hosts have been shown to harbor a greater diversity of microorganisms than hosts infected by pathogens in systems including the human gut (Giloteaux *et al.,* 2016;

Khanna *et al.*, 2016) and plant root (Haas & Défago, 2005; Mendes *et al.*, 2011; Berendsen *et al.*, 2012) and leaf (Agler *et al.*, 2016) microbiomes. There is accumulating evidence that higher leaf bacterial diversity influences host productivity through a variety of mechanisms, including (1) inducing plant-mediated resistance by improving host resistance to pathogens through increasing competition for niches, depleting nutrient pools and enhancing the production of antibiotic molecules (Rastogi *et al.*, 2012; Raghavendra & Newcombe, 2013; Ritpitakphong *et al.*, 2016;); (2) influencing plant hormone production (i.e. auxins (Glickmann *et al.*, 1998; Brandl *et al.*, 2001) and cytokinins (Brandl & Lindow, 1998; Manulis *et al.*, 1998)); and (3) augmenting nitrogen availability through atmospheric nitrogen fixation by leaf bacterial communities (Carrell & Frank, 2014; Moyes *et al.*, 2016). Despite their potential importance in mediating plant biodiversity-ecosystem function relationships, the role of microbial communities in driving ecosystem productivity and function has never been evaluated in an experimental context that allows direct quantification of the association between plant-associated microbes and ecosystem function.

In this study we quantified the relationships among leaf bacterial diversity, plant species richness, plant functional diversity and identity, and plant community productivity in a biodiversity-ecosystem function experiment with trees. We first compared the relative influence of host species identity and diversity on host-level leaf bacterial community structure and diversity. We then evaluated the hypothesis that effects mediated through leaf bacterial diversity explain an important part of the influence of plant diversity and identity on productivity. We hypothesized (1) that host species identity and functional diversity will be the strongest driver of leaf bacterial community structure and diversity on individual trees; and (2) that a higher leaf bacterial diversity will be positively linked with plant community productivity. We tested these hypotheses by measuring leaf bacterial community structure on 620 trees from 19 species in a common field garden biodiversity experiment near Montreal,

Canada where tree species richness and functional diversity were manipulated in a replicated design with 1 - 12 tree species grown together for 5 years in 4 x 4 meter experimental plots (Figure 3.1).

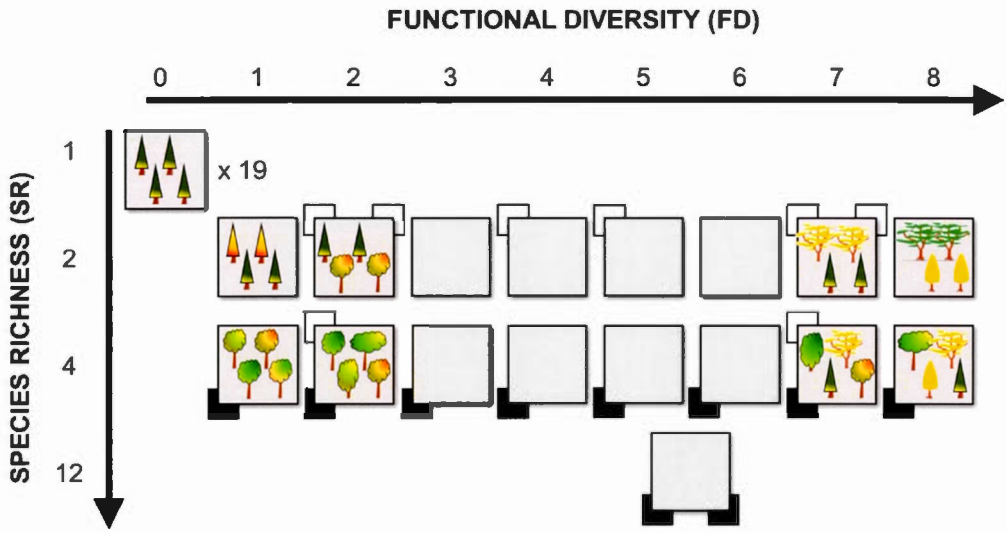

Figure 3.1 The IDENT experiment near Montreal, Canada. A total of 54 community mixtures involving 19 tree species replicated four times were established in spring 2009, including gradients of species richness (SR; 1, 2, 4 and 12) and functional diversity (FD; 8 initial levels). The FD of all possible mixtures was ordered into 8 bins from which communities to be planted were chosen (Tobner *et al.*, 2014). Smaller white squares placed as exponents denote additional plots at some FD levels (different communities producing similar FD values). Exotic species were included as monocultures and in mixtures of 4 and 12 with native species in equal proportion, denoted as subscript black squares.

Methods

3.3.1 Experiment Description

The common garden experiment is located at Sainte-Anne-de-Bellevue, near Montreal, Quebec, Canada (45°26° N, 73°56°W, 39 m.a.s.l.) where the mean annual temperature and mean annual precipitation are 6.2 °C and 963 mm respectively (http://climate.weatheroffice.gc.ca). This experiment was established in 2009 as part of the 'International Diversity Experiment Network with Trees' (IDENT) present in North America and Europe (Tobner *et al.,* 2014), and the TreeDivNet (Verheyen *et al.,* 2016). The experiment is organized in a randomized block (4) design that includes densely planted (50 cm spacing) trees in 8 x 8 plots (16 m^2) in monocultures of 19 temperate and boreal tree species, 14 two-species mixtures, 18 four-species mixtures and three 12-species mixtures of a set of 12 native and 7 exotic species (Figure 3.1). The study site is a flat agricultural field intensively managed for decades. The soil consists of a 20-70 cm deep sandy layer overtopping clay. Microtopography (the difference in elevation between plot centers) was measured to account for slight differences in drainage (Tobner *et al.,* 2016). The experiment is surrounded by a buffer of random tree species from the same pool. At the end of the 2014 season, tree height ranged from 1.3-5.7 m with a mean of 3.2 m while diameter at 5 cm from ground ranged between 20-60 mm with a mean of 38 mm. At the beginning of that season, tree mortality since establishment was below 4 %. Species mixtures were established to create functional diversity gradients over each of the fixed and independent species richness levels. Additional species combinations were also established at some functional diversity levels to increase resolution (see Figure 3.1 and *Annex H* for the complete design).

3.3.2 Functional Diversity, Functional Identity and Productivity

We measured functional diversity using the functional dispersion (FDis) index (Laliberté & Legendre, 2010) calculated as the mean distance of each species to the centre of mass of all species in a multidimensional trait space. We quantified functional identity using the first axis of a principal component analysis (PCA) on community weighted mean traits (Lavorel *et al.,* 2008) based on planted relative abundances, explaining 80 % of variation in traits among species (Figure 3.2). We obtained data on host plant functional traits including maximum photosynthetic capacity (A_{mass}), leaf longevity (Llo), leaf mass per area (LMA), leaf nitrogen content (N_{mass}), and wood density (WD) from global databases (Table 3.1). To estimate total plant community productivity, we measured the diameter and height of each 13,824 trees at the end of the sixth growth year (2014) since planting and then estimated the aboveground stem volume (V_{plot}) with the following formula:

$$V_{plot} = \sum_{i}^{n} (D_i^2 \times H_i)$$

where D_i represents tree *i* diameter and H_i tree *i* height. Plot volume was calculated only for the inner 36 trees, leaving out trees from the outer rows of each plot, to minimize edge effects.

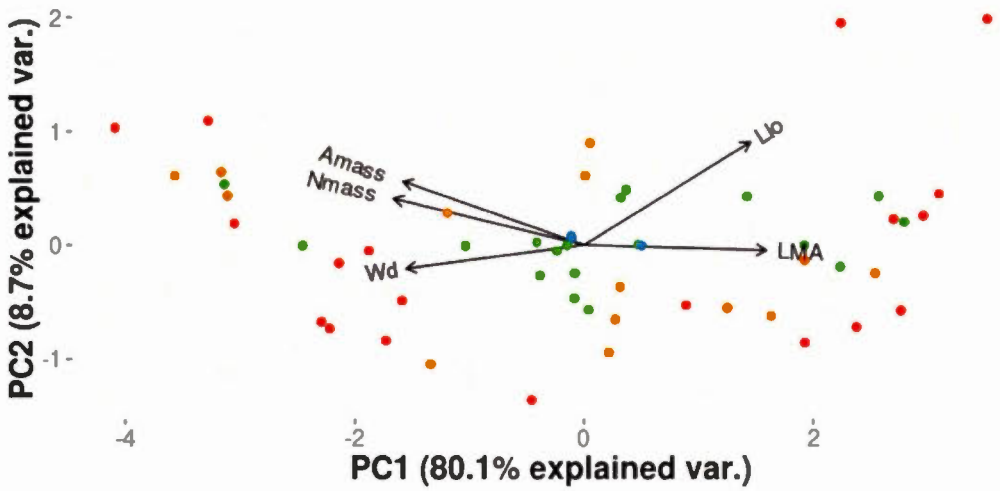

Figure 3.2 Principal component analysis on functional traits community weighted means. Traits are: maximum photosynthetic capacity (A_{mass}), nitrogen content of leaves (N_{mass}), leaf longevity (Llo), wood density (WD) and leaf mass per area (LMA). Colors represent plot species richness levels (red for one species, orange for two, green for four and blue for 12).

Table 3.1 Host species functional traits. Traits are maximum photosynthetic capacity (A_{mass}), drought tolerance (D_{tol}), leaf longevity (Llo), leaf mass per area (LMA), leaf nitrogen content (N_{mass}), seed mass ($Seed_{mass}$), shade tolerance ($Shade_{tol}$), water tolerance (W_{tol}), and wood density (WD) from global databases (Wright *et al.*, 2004; Niinemets & Valladares, 2006; Dickie, 2008; Chave *et al.*, 2009). Tolerance indices are based on a 0 (no tolerance) to 5 (maximal tolerance) scale.

Host Species	Functional Traits								
	A_{mass} ($\mu mol * m^{-2} * s^{-1}$)	D_{tol} (scale 0-5)	Llo (month)	LMA ($g * m^{-2}$)	N_{mass} (%)	$Seed_{mass}$ (g/1000)	$Shade_{tol}$ (scale 0-5)	$Water_{tol}$ (scale 0-5)	WD ($g * cm^{-3}$)
Abies balsamea	12.0	1.0	110	151.0	1.66	7.6	5.0	2.0	0.33
Acer platanoides	83.1	2.7	6	50.6	1.99	139.0	4.2	1.5	0.52
Acer rubrum	111.2	1.8	5.6	71.1	1.91	26.5	3.4	3.1	0.49
Acer saccharum	84.6	2.3	5.5	70.6	1.83	55.2	4.8	1.1	0.56
Betula alleghaniensis	206.0	3	5.5	46.1	2.20	0.9	3.2	2.0	0.55
Betula papyrifera	195.0	2	3.6	77.9	2.31	0.4	1.5	1.3	0.48
Larix decidua	71.6	2.3	6	93.9	2.05	7.1	1.5	1.1	0.47
Larix laricina	59.4	2	6	120	1.36	2.0	1.0	3.0	0.49
Picea abies	29.3	1.8	103.2	235.2	1.19	7.0	4.5	1.2	0.37
Picea glauca	35.6	2.9	50	302.9	1.28	2.4	4.2	1.0	0.33
Picea omorika	NA	2.8	NA	NA	1.03	2.9	4.7	1.0	0.36
Picea resinosa	24.0	3	36	294.1	1.17	8.0	1.9	1.0	0.41
Picea rubens	NA	2.5	103.2	304.7	1.15	3.3	4.4	2.0	0.37
Pinus strobus	43.8	2.3	20	121.9	1.42	17.0	3.2	1.0	0.34
Pinus sylvestris	36.9	4.3	27.9	254.6	1.33	6.0	1.7	2.6	0.42
Quercus robur	85.1	3	6	68.5	2.37	3378.0	2.5	1.9	0.56
Quercus rubra	148.6	2.9	6	84.2	2.06	3143.0	2.8	1.1	0.56
Thuya occidentalis	32.2	2.7	33	223	1.02	1.4	3.5	1.5	0.30
Tilia cordata	NA	2.8	4.8	49.1	2.13	50.9	4.2	1.83	0.42

3.3.3 Bacterial Community Sampling and DNA extraction

On July 2nd 2014, we collected one 50-100 g sample of leaves per host species per plot for a total of 620 samples. For bacterial community collection and amplification, we used previously described protocols (Kembel *et al.,* 2014). In laboratory all samples were uniformly trimmed to 50 g mass. We collected microbial communities from leaf surfaces by agitating the samples in 100 mL of diluted Redford buffer solution for five minutes. We re-suspended cells in 500 µL of PowerSoil bead solution (MoBio, Carlsbad, California). We extracted DNA from isolated cells using the PowerSoil kit according to the manufacturer's instructions and stored at -80 °C. All samples were amplified using the same one-step PCR step and normalized with primers designed to attach a 12 base pair barcode and Illumina adaptor sequence to the fragments during PCR (Fadrosh *et al.,* 2014). We used chloroplast-excluding primers targeting the V5–V6 region [799F and 1115R (Redford *et al.,* 2010)] of the 16S rRNA gene. These primers contained a heterogeneity spacer along with the Illumina linker sequence (Forward (799F): 5' - CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCT TCCGATCT xxxxxxxxxxxx HS AACMGGATTAGATACCCKG – 3', Reverse (1115R): 5' - AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCT TCCGATCT xxxxxxxxxxxx HS – AGGGTTGCGCTCGTTG - 3') where x represents barcode nucleotides and HS represents a 0-7 base pairs heterogeneity spacer. Each sample was submitted to a single 25 µL PCR reaction containing 5 µL 5xHF buffer (Thermo Scientific), 0.5 µL dNTPs (10 µM), 0.5 µL forward primer (10 µM), 0.5 µL reverse primer (10 µM), 0.75 µL DMSO, 0.25 µL Phusion HotStart II polymerase (Thermo Scientific), 1 µL DNA, and 16.5 µL molecular-grade water. The reaction was performed using: 30 s initial denaturation at 98 °C, 35 cycles of 15 s at 98 °C, 30 s at 60 °C, and 30 s at 72 °C, with a final 10-minute elongation at 72 °C. The samples were

processed with an Invitrogen Sequalprep PCR Cleanup and Normalization Kit (Frederick, MD) to be then pooled with equal concentration and then sequenced. Samples were sequenced on the Illumina MiSeq platform. We processed the raw sequence data with PEAR (Zhang *et al.,* 2010) and QIIME (Caporaso *et al.,* 2010) pipelines to merge paired-end sequences to a single sequence of length of approximately 350 bp, eliminate low quality sequences (mean quality score <30 or with any series of five bases with a quality score <30), and de-multiplex sequences into samples. We eliminated chimeric sequences using the Uclust and Usearch algorithms (Edgar, 2010). Then, we binned the remaining sequences into operational taxonomic units (OTUs) at a 97 % sequence similarity cutoff. After filtering OTUs that were represented by less than 20 sequences, our database contained 6,834 OTUs. The number of sequences per sample ranged from 4,006 to 40,900. From a database of 8,965,472 quality sequences, we rarefied each sample to 3,500 sequences, with 14 samples excluded from subsequent analyses due to insufficient sequence reads as a result of extraction or sequencing errors, totaling 2,121,000 sequences from 606 samples. We determined the taxonomic identity of each OTU using the BLAST algorithm and Greengenes database (DeSantis *et al.,* 2006) as implemented in QIIME (Caporaso *et al.,* 2010). We performed analyses with the ape (Paradis *et al.,* 2004), picante (Kembel *et al.,* 2010), and vegan (Oksanen *et al.,* 2007) packages in R (R Development Core Team 2013).

3.3.4 Statistical Analyses

We quantified plot alpha-bacterial diversity using the Shannon diversity index on all samples from each plot combined. At the tree level, we used a PERMANOVA (Bray-Curtis dissimilarities) to identify the main drivers of leaf bacterial community structure.

In the PERMANOVA, functional diversity and functional identity are continuous variables whereas host species identity is a categorical variable with 19 levels. The interaction between host species identity and species richness was not significant (P = 0.23) and was therefore removed from the model. We performed and a linear mixed-model to test the impact of the same drivers on bacterial diversity (Shannon index). The model formula is:

leaf diversity ~

host species identity+functional diversity+functional identity+(1|block/plot)

where fixed effects included leaf bacterial diversity, functional diversity and functional identity as continuous variables and host species identity as a 19 levels factor. Random effects were plot (54 levels) and block (4 levels) both being factors. Species richness was not significant (P = 0.30) and was thus removed from the model. We compared the strength of the variables in the linear mixed model by an ANOVA type II test and computed a marginal pseudo-R^2 for the model (Nakagawa & Schielzeth, 2013; Johnson 2014).

At the plot-level, we built a structural equation model to test for the direct and indirect effects of host tree identity and diversity on leaf bacterial diversity and plant community productivity (Figure 3.3-3.5). Prior to fitting the structural equation model, variables were transformed to achieve normality. Productivity and species richness were log-transformed while functional diversity and leaf bacterial diversity were both rank transformed. In all analyses, we started with the fully specified model and eliminated the least non-significant relationship until none remained. The following two covariances were removed from the *a priori* model (Figure 3.3): the covariances between plant functional identity and both plant species richness (P = 45) and plant functional diversity (P = 90). The correlations between microtopography with both plant community productivity (P = 0.27) and leaf bacterial diversity (P = 0.99) were

not significant and therefore were excluded from the final model. Variables' explanatory power is inferred from their respective R^2 (PERMANOVA, Table 3.2), F values (ANOVA on linear mixed-model, Table 3.3) or standardized regression coefficient (Structural equation model, Figure 3.3-3.5) rather than p-values. For the PERMANOVA and linear-mixed model, we blocked by block and plot identity to account for any non-random difference in local conditions. For the structural equation modeling, we tested the influence of soil microtopography on plant community productivity and leaf bacterial diversity (Figure 3.3), which was not significant and so was removed from the final model.

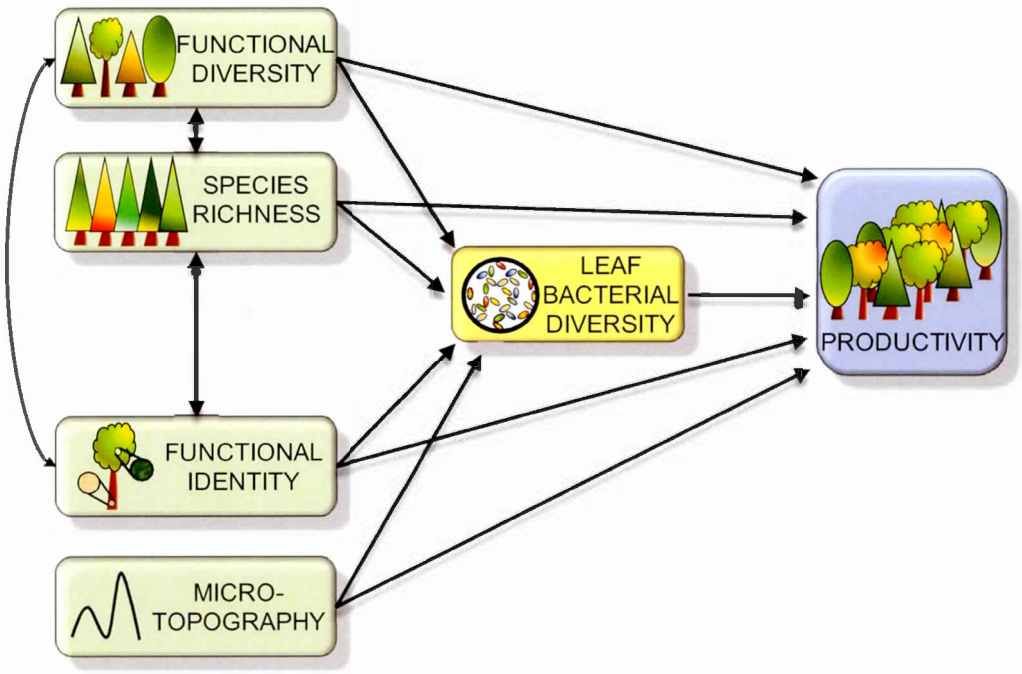

Figure 3.3 *A priori* structural equation model. Factors are species richness, functional identity, functional diversity and plot microtopography (elevation at plot center, cm) as determinants of leaf bacterial diversity and plant community productivity. Green boxes indicate exogenous variables (diversity indices and plot microtopography), whereas responses are in yellow for plot-level leaf bacterial diversity and blue for plant community productivity.

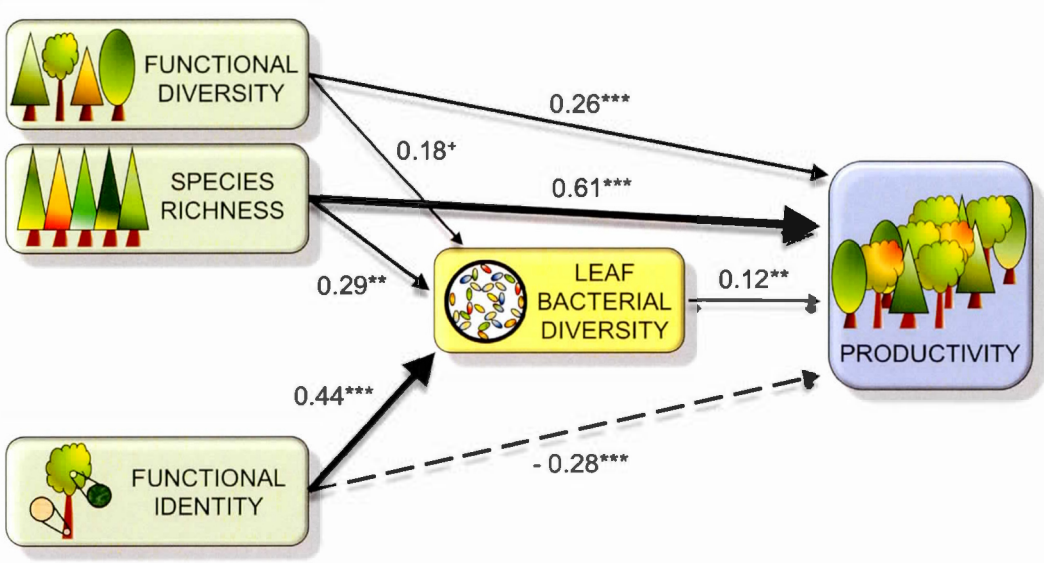

Figure 3.4 Structural equation model of plant diversity and identity explaining leaf bacterial diversity and plant community productivity. The path analysis (n = 216, χ^2 = 1.451, P = 0.484, df = 2; RMSEA P = 0.644) explains 41 % of the variance in leaf bacterial diversity and 85 % of the variance in plot productivity (4 replicates of 54 tree species monocultures or combinations). Green boxes indicate plot-level plant diversity indices, yellow for plot-level leaf bacterial diversity and blue for plant community productivity. Numbers adjacent to arrows and arrow width indicate the effect-size of the relationships. Significance levels are given by: $^{+}$ P <0.1; * P <0.05; ** P <0.01; *** P <0.001. Continuous and dashed arrows indicate positive and negative relationships respectively.

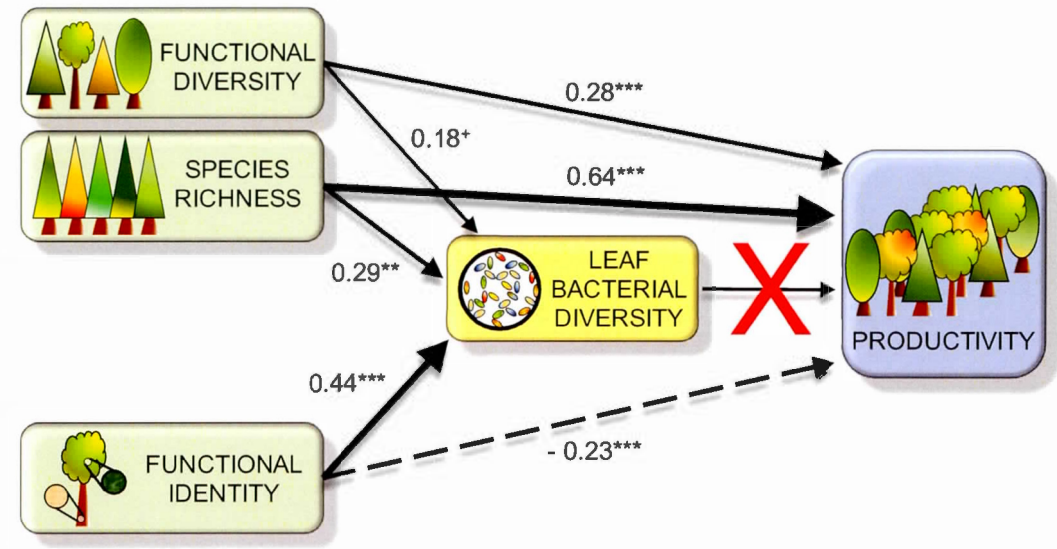

Figure 3.5 Alternative structural equation model excluding the link between leaf bacterial diversity and plant community productivity. After deletion of this link, the path analysis (n = 216, χ^2 = 11.906, P = 0.008, df = 3; RMSEA P = 0.044) is unstable and inferior to the model with the leaf bacterial diversity-plant community productivity link included. Green boxes indicate plot-level plant diversity indices, yellow for plot-level leaf bacterial diversity and blue for plant community productivity. Numbers adjacent to arrows and arrow width indicate the effect-size of the relationships. Significance levels are given by: $^{+}$ P <0.1; * P <0.05; ** P <0.01; *** P <0.001. Continuous and dashed arrows indicate positive and negative relationships respectively.

Table 3.2 Bacterial community structure (Bray-Curtis dissimilarity) explained by various factors (PERMANOVA). The model explains a total of 36 % of the variation in bacterial community structure (Bray-Curtis dissimilarity) in 606 samples of leaf bacterial communities from trees.

Variables	F-value	Df	R^2	P(>F)
Host species identity	12.58	18	26.6	0.001
Functional identity	13.95	1	1.6	0.001
Functional diversity	11.15	1	1.3	0.001
Species richness	1.68	1	0.2	0.055
Host species identity * Functional identity	1.67	18	3.5	0.001
Host species identity * Functional diversity	1.18	18	2.5	0.021

Table 3.3 Variance in tree-level leaf bacterial diversity (Shannon diversity) explained by different variables (ANOVA on linear mixed model). Block and treatment (nested in block) were included as random effects. The model explains 53 % of the marginal variation (only due to fixed effects) in leaf alpha diversity in 606 samples of leaf bacterial communities from trees.

Variables	F-value	Df_n	Df_d	P(>F)
Host species identity	37.99	18	535	<0.0001
Functional identity	26.16	1	474	<0.0001
Functional diversity	21.90	1	302	<0.0001

Type II ANOVA with Kenward-Rodger approximation of degree of freedom on linear-mixed model.

Results

The strongest driver of leaf bacterial community structure at the tree-level was host species identity (PERMANOVA; $F = 12.68$, $R^2 = 26.6$ %, $P = 0.001$; Table 3.2), in accordance with previous studies (Redford *et al.,* 2010; Laforest-Lapointe *et al.,* 2016a). Although their relative influence was much smaller, plant functional identity ($R^2 = 1.7$ %) and diversity ($R^2 = 1.3$ %) were also significant drivers of leaf bacterial community structure and interacted with host species identity to shape bacterial communities on leaves. Likewise, host species identity ($F_{18,535} = 38.0$, $P < 0.0001$) was the strongest determinant of leaf bacterial diversity (linear mixed model on leaf bacterial diversity; marginal $R^2 = 53$ %; Table 3.3), followed by functional identity ($F_{1,474} = 26.2$, $P < 0.0001$) and functional diversity ($F_{1,302} = 21.9$, $P < 0.0001$). These results suggest that host species identity plays a dominant role in determining leaf microbial community structure even after accounting for changes in plot-level plant functional diversity, identity and species richness. In addition, our results support the idea that plant-associated microbial communities vary predictably with host plant ecological strategy (Kembel *et al.,* 2014), and thus potentially impact host growth and ecosystem productivity.

The diversity of bacterial communities on tree leaves explained significant amounts of variation in plant community productivity (0.12; $P = 0.002$; Figure 3.4) even when accounting for the effects of all other variables (structural equation model; $\chi^2 = 1.451$, $P = 0.484$; Figure 3.4). Removing the link between leaf bacterial diversity and community productivity in the structural equation model yielded an unstable model ($\chi^2 = 11.906$, $P = 0.008$; Supplementary Information), providing further evidence for the importance of leaf bacterial diversity for plant community productivity. At the plot-level, plant species richness (0.61; $P < 0.001$), functional identity (-0.28; $P < 0.001$) and

functional diversity (0.26; P <0.001) had a strong impact on productivity in the model, with plant species richness being the strongest determinant of plant community productivity (R^2 = 85 %; Figure 3.4). In addition, plant species richness (0.29; P = 0.003), functional identity (0.44; P <0.001) and functional diversity (0.18; P = 0.08) also drove leaf bacterial diversity, explaining 41 % of the variance in bacterial diversity between plots. These results offer empirical evidence that leaf bacterial diversity is positively related to terrestrial ecosystem productivity even after accounting for other explanatory factors, and that biodiversity-ecosystem functioning relationships in plant communities could in part be driven by positive interactions involving other trophic levels. Here we reveal that plant-associated microbial diversity is related with plant community productivity, explaining a portion of the variation in productivity that would otherwise have been attributed to plant diversity and functional traits, both adding to the explanatory power of the model of plot productivity and mediating the tree diversity-identity effect on productivity.

Discussion

Many studies have hypothesized that niche complementarity is one of the principal mechanisms that explains positive biodiversity-ecosystem function relationships (Sapijanskas *et al.,* 2014; Tobner *et al.,* 2016), through more efficient capture of resources with increasing species diversity and complementarity (Yachi & Loreau, 1999; Fargione *et al.,* 2007). Recent food web studies have introduced the idea of trophic complementarity, a concept based on complementarity occurring either through differential resource use, predation by distinct predators, or both (Poisot *et al.,* 2013). Here, we provide unprecedented evidence that leaf bacterial diversity could play a role in stimulating plant community productivity. Our work concurs with previous studies

that demonstrated the influence of leaf bacterial diversity on plant community productivity through mechanisms such as fixation of atmospheric nitrogen or protection from pathogen infection (Raghavendra & Newcombe, 2013; Ritpitakphong *et al.,* 2016; Wei *et al.,* 2016). The demonstration of causality between diversity and productivity is a common concern raised in biodiversity-ecosystem functioning studies, and since we did not manipulate leaf bacterial diversity experimentally it is not possible to state definitely that it caused the observed increase in plant community productivity. However, our findings suggest that adding a multi-trophic component to studies of biodiversity-ecosystem functioning in plant communities is a promising avenue to better understand complementarity mechanisms, by improving models of plant ecosystem productivity and suggesting the need for future research oriented toward system-level and multi-trophic experiments.

Given the capacity of microbes to respond rapidly to environmental changes (Lau & Lennon, 2012), studying how the effect of microbial communities on plant productivity interacts with global change and intensified anthropogenic pressures will be crucial to optimize or maintain primary production. Using one of the most extensive studies of tree leaf bacterial communities to date, our results suggest that considering plant-associated microbial diversity can improve models of biodiversity-ecosystem functioning and should therefore be considered in future experiments.

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CHAPTER IV

TREE LEAF MICROBIOME CHANGES ALONG A GRADIENT FROM NATURAL TO URBAN ENVIRONMENTS

This article is in preparation

Isabelle Laforest-Lapointe, first author
Centre d'étude de la forêt, Département des sciences biologiques, Université du Québec à Montréal, C.P. 8888, Succ. Centre-Ville, Montréal, Canada, H3C 3P8.
Laforest-lapointe.isabelle@courrier.uqam.ca

Christian Messier
Centre d'étude de la forêt, Département des sciences biologiques, Université du Québec à Montréal, C.P. 8888, Succ. Centre-Ville, Montréal, Canada, H3C 3P8.; Institut des Sciences de la Forêt Tempérée, Université du Québec en Outaouais, 58 rue Principale, Ripon, Canada, J0V 1V0.
Messier.christian@uqam.ca

Steven W. Kembel
Centre d'étude de la forêt, Département des sciences biologiques, Université du Québec à Montréal, C.P. 8888, Succ. Centre-Ville, Montréal, Canada, H3C 3P8.
Kembel.steven_w@uqam.ca

4.1 Abstract

Tree leaf associated microbiota has been studied in natural ecosystems but less so in urban settings, where anthropogenic pressures on trees could impact microbial communities and modify their interaction with their hosts. Additionally, trees act as vectors spreading bacterial cells in the air in urban environments due to the high density of microbial cells on aerial plant surfaces. Characterizing urban tree leaf bacterial communities is thus key to understand their impact on urban tree health and on the overall urban microbiome. In this study, we aimed (1) to characterize and compare changes in phyllosphere bacterial communities of three tree species in natural forest and urban environments; and (2) to describe the changes in tree phyllosphere bacterial community structure and diversity along a gradient of increasing urban intensity. Our results show that the bacterial communities from these two environments are clearly distinct in community structure but not in diversity. As anthropogenic pressures increase, urban leaf communities show a reduction in the abundance of the most dominant class, *Alphaproteobacteria*. In conclusion, we find that urban trees possess characteristic microbial communities when compared to natural forest trees, and our results suggest that feedbacks between human activity and plant microbiomes could shape urban microbiomes.

Key words: Urban ecology, urban microbiome, microbial ecology, indicator species, phyllosphere, plant-microbe interactions, temperate tree, urban gradient, anthropogenic pressures.

4.2 Introduction

While the human population in urban centers is estimated to increase by two to four billion this century (United Nations, 2015), the focus of public health research is shifting from the benefits of plant communities (air quality, physical activity, social cohesion, and stress reduction; Hartig *et al.,* 2014) to the potential roles of the urban microbiota. The positive influence of urban vegetation on human physical health has been demonstrated many times (Maas *et al.,* 2006; Richardson & Mitchell, 2010) but it could also play an unexpected role by means of the microbial communities they support and their contribution to the urban diversity. Studies using high-throughput sequencing techniques are rapidly improving our understanding of the urban microbiome, defined as the ensemble of microbial organisms residing or transiting in the urban environment (King, 2014). Land use type (e.g. forest, rural, urban) has been shown to impact air microbial communities (Burrows *et al.,* 2009; Bowers *et al.,* 2011) and recent work has demonstrated that the local vegetation drives the airborne bacterial community composition and abundance in urban (Mhuireach *et al.,* 2016) and natural settings (Lymperopoulou *et al.,* 2016). Most urban microbiome research has been done on the built-environment (indoor space of human-built structure; but see Afshinnekoo *et al.,* 2015; Mhuireach *et al.,* 2016; Tischer *et al.,* 2016), improving our understanding of urban microbial communities but leaving much to be defined especially in the non-built environmental microbiome. In addition, the surrounding plant community has been suggested to influence the microbial community of key buildings frequented by the human population (i.e. hospitals, schools and homes; Kembel *et al.,* 2012; Meadow *et al.,* 2014a, 2014b). Therefore, characterizing the assembly and dynamics of the urban plant microbiome is crucial to strengthen our understanding of the urban microbiome.

The phyllosphere, mainly the leaf surfaces of plants, is estimated to sum up to to 4 x 10^8 km^2 on Earth (Morris *et al.,* 2002) and thus provides a major potential source of local microbial organisms (Whipps *et al.,* 2008; Lighthart *et al.,* 2009). In addition to its contribution to the urban microbiome, the canopy of urban trees provides a variety of services such as reducing local temperature, limiting water runoff and increasing air quality (Pataki *et al.,* 2011). Recent research on the phyllosphere has found host species identity to be the key driver of leaf microbial community structure both in tropical (Kim *et al.,* 2012; Kembel *et al.*, 2014; Kembel & Mueller; 2014; Lambais *et al.,* 2014) and temperate ecosystems (Redford & Fierer, 2009; Redford *et al.*, 2010; Laforest-Lapointe *et al.,* 2016a). However, to our knowledge few studies have described the changes in plant-associated microbiota from the natural to urban environments (Smets *et al.,* 2016 for bacterial communities on *Hedera* sp. and Jumpponen & Jones, 2010 for fungal communities on *Quercus macroarpa*), leaving much to be learned on how the plant microbiome changes with increasing anthropogenic pressures. In this study, we will focus on tree phyllosphere bacterial communities of natural and urban environments to quantify the similarities and differences in both microbiomes.

The urban environment differs strikingly from the natural forest environment mainly through an increase in biotic and abiotic stresses caused directly and indirectly by anthropogenic activities. The increase in anthropogenic pressures in urban areas reduces tree fitness and longevity (Nowak & McBride, 1991). Numerous studies have shown that anthropogenic activities increase leaf macronutrients (nitrogen, potassium, sulfur), micronutrients (boron, manganese, selenium) and trace elements (cadmium, lead, zinc) for urban trees (Pouyat & McDonnell, 1991; Kaye *et al.,* 2006; Jumponnen & Jones, 2010). Higher temperatures in the urban environment influence vegetation phenology (Roetzer *et al.,* 2000; White *et al.,* 2002; Zhang *et al.,* 2004) and will be intensified by city growth and the progress of global warming (Kalnay & Cai, 2003). The urban heat island phenomenon (Oke, 1973) results from the increase of non-

penetrating surfaces (Hart & Sailor, 2009) and the decrease of vegetation cover (Jenerette *et al.,* 2011) in cities. Thermal accumulation could drive enzymatic processes, affecting microbial communities directly, and also provoke increased presence of insect ectotherms (Briere *et al.,* 1999), which are known disease vectors (Lounibos, 2002). This increase in insect pest abundance in urban areas (Bennett & Gratton, 2012; y Gomez & Van Dyck, 2012) could also be intensified by changes in host plant quality and natural enemy efficiency (Raupp *et al.,* 2010). In addition to these stresses, urban trees frequently suffer from limited access to water and nutrients (Wiersum & Harmanny, 1983; Fluckiger & Braun, 1999), root development limitation (see Day *et al.,* 2010 for a review), photosynthetic biomass loss and tree lesions (Sieghardt *et al.,* 2005). These stresses have been shown to affect plant survival (Mittler, 2006; Niinemets 2010a, 2010b) and induce numerous physiological responses, a phenomenon that could cause profound changes in urban tree leaf microbial communities. Therefore, urban biotic and abiotic conditions could provoke changes in the tree phyllosphere microbial community, potentially impacting host fitness and modifying the local pool of urban microbial organisms.

To improve our understanding of the urban tree microbiome, we aimed (1) to characterize and compare the bacterial communities present in tree phyllosphere bacterial communities of natural forests and the urban environment; and (2) to describe the changes in tree phyllosphere bacterial community structure and diversity along a gradient of increasing urban intensity and degree of tree isolation. While urban microbiome studies have focused on air and built environment microorganisms (but see Afshinnekoo *et al.,* 2015; Mhuireach *et al.,* 2016; Tischer *et al.,* 2016), our study provides new key information on the urban plant-associated microbiota at different levels of urban intensity and offers new explanatory paths to better understand the gap between natural and urban environments' microbiome.

4.3 Methods

4.3.1 Study Sites

The seven study sites are located in natural forest and urban settings. Four natural stands were selected across Quebec's temperate forest: Sutton (45°6'46"N; 72°32'28"W), Abitibi (48°9'45"N; 79°24'4"W), Gatineau (45°44'50"N; 75°17'57"W) and Bic (48°20'1"N; 68°49'3"W). This region is characterized by a cold and humid continental climate with temperate summer. Three urban locations were selected on the Island of Montreal (Canada) along a gradient of increasing urban intensity: Pierrefonds (45°27'26"N; 73°53'14"W) for low urban intensity, Ahuntsic (45°33'22"N; 73°39'49"W) for medium intensity, and Mont-Royal (45°31'32"N; 73°34'00"W) for high intensity (Figure 4.1). We assessed the urban intensity of sampled trees' location based on a composite index of human influence (IHI) as described by Nock *et al.* (2013). This index incorporates information on human infrastructures and presence, movements, landscape use and electric infrastructure (Sanderson *et al.,* 2002) to estimate humans' footprint. The IHI of the trees sampled ranged from 38 to 60 in function of both the site identity and tree isolation.

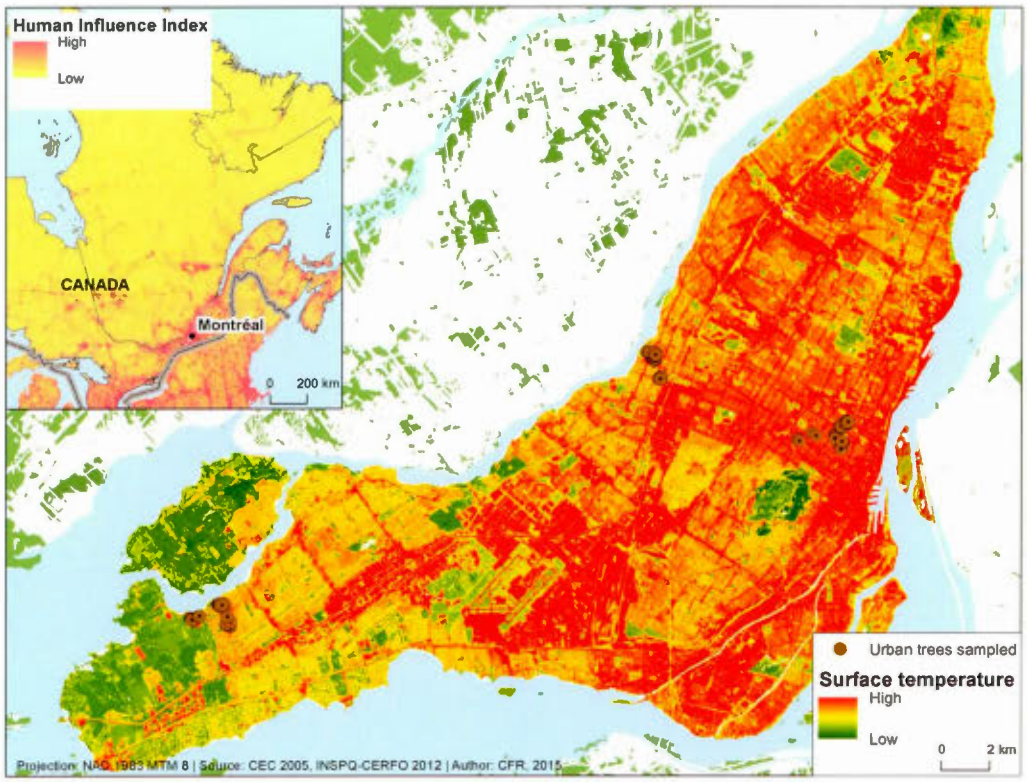


Figure 4.1 Location of trees sampled along an urban gradient (three intensities: low, medium and high) on Montreal island, Canada. An index of Human influence (IHI) on terrestrial ecosystem is overlaid.

4.3.2 Bacterial community collection

To compare natural and urban sites, we sampled three tree species (*Acer rubrum*, *Acer saccharum* and *Picea glauca*) commonly found in both environments. At each of the natural sites we randomly selected and sampled three individuals per species during July 2013. At each of the urban sites, six individuals per tree species were randomly

selected from the public district database to be sampled: three in parks (with tree neighbors and close plant community) and three in streets (no close tree neighbors and no close plant community). This summed up to 90 samples to compare natural and urban sites (Table 4.1). In order to better characterize the tree phyllosphere microbiome found in urban settings, we sampled seven tree species at urban sites (*Acer platanoides*, *Acer rubrum*, *Acer saccharum*, *Celtis occidentalis*, *Fraxinus americana*, *Fraxinus pensylvanica*, *Picea glauca*). This summed up to 126 samples to compare changes along the gradient of urban intensity (Table 4.1). All urban samples were acquired on July 31, 2014. For each randomly chosen tree, we clipped 50–100 g of shade leaves at mid-canopy height (1-2 m above the bottom of the tree's canopy) into sterile roll bags with surface-sterilized shears. For bacterial community collection and amplification, we used the protocols described by Kembel *et al.* (2014). We collected microbial communities from the leaf surface by agitating the samples in a diluted Redford buffer solution and then resuspended cells in 500 µL of PowerSoil bead solution (MoBio, Carlsbad, California). We extracted DNA from isolated cells using the PowerSoil kit according to the manufacturer's instructions and stored at -80 °C.

Table 4.1 Description of the seven sites sampled during the summers of 2013-14.

Environment	Site	Urban gradient	Tree isolation	Natural vs. Urban		Urban gradient	
				#species	# samples	#species	# samples
Natural forest	Abitibi	NA	Forest	3	9	NA	
	Bic			3	9		
	Gatineau			3	9		
	Sutton			3	9		
Urban	Pierrefonds	Low 38-42	Street	3	9	7	21
			Park		9		21
	Ahuntsic	Mid 50-60	Street	3	9	7	21
			Park		9		21
	Mont-Royal	High 50-60	Street	3	9	7	21
			Park		9		21

4.3.3 DNA library preparation and sequencing

Natural samples were amplified using a two-stage PCR approach and normalized with primers designed to attach an 8 base pair barcode. Urban samples were amplified using a one-step PCR step and normalized with primers designed to attach a 12 base pair barcode and Illumina adaptor sequence to the fragments during PCR (Fadrosh, 2014). For all samples, we used chloroplast-excluding primers targeting the V5–V6 region [799F and 1115R (Redford *et al.,* 2010)] of the 16S rRNA gene. These primers contained a heterogeneity spacer along with the Illumina linker sequence (Forward (799F): 5' - CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCT TCCGATCT xxxxxxxxxxxx HS AACMGGATTAGATACCCKG – 3', Reverse (1115R): 5' - AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCT TCCGATCT xxxxxxxxxxxx HS – AGGGTTGCGCTCGTTG - 3') where x represents barcode nucleotides and HS represents a 0-7 base pairs heterogeneity spacer. Each sample was submitted to either a double (natural forest) or single (urban) 25µL PCR reaction containing 5 µL 5xHF buffer (Thermo Scientific), 0.5 µL dNTPs (10 µM), 0.5 µL forward primer (10 µM), 0.5 µL reverse primer (10 µM), 0.75 µL DMSO, 0.25 µL Phusion HotStart II polymerase (Thermo Scientific), 1 µL DNA, and 16.5 µL molecular-grade water. The reaction was performed using: 30 s initial denaturation at 98 °C, 35 cycles of 15 s at 98 °C, 30 s at 60 °C, and 30 s at 72 °C, with a final 10-minute elongation at 72 °C. The resulting product of natural forest samples were cleaned using MoBio UltraClean PCR cleanup kit. We isolated a ~445 bp fragment by electrophoresis in a 2 % agarose gel, and recovered DNA with the MoBio GelSpin kit. We prepared multiplexed 16S libraries by mixing equimolar concentrations of DNA, and sequenced the DNA library using Illumina MiSeq 250 bp paired-end sequencing

at Genome Quebec. The urban samples were processed with an Invitrogen Sequalprep PCR Cleanup and Normalization Kit (Frederick, MD) to be then pooled with equal concentration and sequenced on an Illumina MiSeq platform at the University of Montreal. To avoid any bias that could have come from a protocol or sequencing run effect, we re-extracted and re-sequenced 27 samples of the urban and natural environment (from all species) with the one-step PCR protocol.

We processed the raw sequence data with PEAR (Zhang *et al.,* 2014) and QIIME (Caporaso *et al.,* 2010) pipelines to merge paired-end sequences to a single sequence of length of approximately 350 bp, eliminate low quality sequences (mean quality score <30 or with any series of five bases with a quality score <30), and de-multiplex sequences into samples. We eliminated chimeric sequences using the Uclust and Usearch algorithms (Edgar 2010). Then, we binned the remaining sequences into operational taxonomic units (OTUs) at a 97 % sequence similarity cutoff. We determined the taxonomic identity of each OTU using the BLAST algorithm and Greengenes database (DeSantis *et al.,* 2006) as implemented in QIIME (Caporaso *et al.,* 2010).

4.3.4 Statistical analyses

To exclude the spurious OTUs that could have been created from PCR or sequencing errors, we filtered OTUs that were represented by less than 5 sequences. The natural vs. urban microbiome dataset contained 8,129 OTUs with 3,630 to 47,570 sequences per sample summing up to 1,262,881 quality sequences. The gradient of urban intensity contained 8,752 OTUs with 3,634 to 33,041 sequences per sample summing up to 1,866,943 quality sequences. We rarefied the samples to 3,000 sequences each, with

21 samples excluded due to insufficient sequence reads as a result of extraction or sequencing errors. Rarefaction and analyses were repeated 100 times and showed no qualitative differences across iterations. Therefore, we present the result of a single random iteration. We performed the analyses in R (R Development Core Team 2013).

We performed an analysis of variance (ANOVA) and a subsequent post-hoc Tukey's test to compare the relative abundance of the most abundant bacterial classes and the changes in alpha-diversity in natural forest vs. urban settings. The abundances were transformed a priori to account for non-normal distribution and heterogeneity of variance. To detect patterns of differential relative abundances in specific OTUs, we calculated the average relative abundance of all OTUs in each environment and for those that had a relative abundance of >0.5 % we plotted the respective relative environment-specific abundances. We also tested for the significant associations between bacterial taxa and environment type using the linear discriminant analysis effect size (LEfSe) algorithm (Segata *et al.,* 2011). The LEfSe algorithm aims to discover biomarkers (genes, pathways, or taxa) of different sample groups employing the linear discriminant analysis to approximate the effect size of each biomarker identified. A significant association between bacterial clades and a specific group will be detected when there is consistently higher relative abundance of the clade in the group's samples. Among the bacterial clades detected as statistically and biologically relevant, the strongest scores identify which clades have the greatest explanatory power for differences between communities (Segata *et al.,* 2011). We quantified the relative influence multiple drivers on leaf bacterial community structure by conducting a permutational multivariate analysis of variance (PERMANOVA, Anderson, 2001) on Bray-Curtis dissimilarities among samples. For the comparison between natural and urban leaf microbiomes, the tested drivers were host species identity (*Acer rubrum*, *Acer saccharum* and *Picea glauca*), environment (natural vs. urban) and site identity (Abitibi, Ahuntsic, Bic, Gatineau, Mont-Royal, Pierrefonds and Sutton). To test if our

results were the product of protocol differences, we ran the same PERMANOVA model on the subset of samples that we re-sequenced. Both models yielded similar results (see *Annex I-J*) thus confirming that our results were not due to protocol differences and therefore the following discussion is based on the full model.

Regarding the gradient of urban intensity dataset, we performed an analysis of variance (ANOVA) and a subsequent post-hoc Tukey's test to compare the relative abundance of each the most common bacterial classes across the urban intensity gradient. We measured a sample alpha-bacterial diversity using Shannon diversity index calculated from OTU relative abundances for each community. Then we employed an ANOVA and post-hoc Tukey's test to compare the changes in leaf alpha-bacterial diversity across the urban intensity gradient. We compared the relative influence of multiple drivers of leaf bacterial community composition along the gradient of urban intensity: host species identity (*Acer platanoides*, *Acer rubrum*, *Acer saccharum*, *Celtis occidentalis*, *Fraxinus americana*, *Fraxinus pensylvanica* and *Picea glauca*), tree isolation (street or park) and site identity (Ahuntsic, Mont-Royal, Pierrefonds). We illustrated bacterial community structure patterns by performing a nonmetric multidimensional scaling (NMDS) ordination of Bray-Curtis dissimilarities.

4.4 Results

4.4.1 Natural Forest vs. Urban Environments

Sequencing identified an average of 902 ± 63 OTUs and 877 ± 63 (mean ± SE) per tree sampled for natural forest and urban sites respectively. Of the total 8129 OTUs identified, 3124 OTUs were present in both environments while 2107 OTUs were

present only in natural forest and 2898 OTUs only in the urban environment (Figure 4.2a). Among natural forest samples, the ten most abundant bacterial classes were in order the *Alphaproteobacteria* (59.5 % of sequences), *Betaproteobacteria* (8.0 %), *Actinobacteria* (6.4 %), *Gammaproteobacteria* (6.3 %), *Cytophagia* (5.7 %), *Acidobacteria* (4.8 %), *Deltaproteobacteria* (2.6 %), *Saprospirae* (1.8 %), *Sphingobacteria* (1.3 %) and *Deinococci* (0.9 %). In comparison, the ten most abundant bacterial classes of the urban samples were the *Alphaproteobacteria* (42.7 % of sequences), *Betaproteobacteria* (12.1 %), *Cytophagia* (12.1 %), *Gammaproteobacteria* (10.1 %), *Actinobacteria* (7.0 %), *Deinococci* (5.2 %), *Deltaproteobacteria* (2.2 %), *Saprospirae* (1.7 %), *Sphingobacteria* (1.4 %) and TM7-3 (1.0 %). Five OTUs were present on 99 % or more of all trees sampled. These OTUs belong to 2 phyla (*Proteobacteria* and *Bacteroidetes*), 3 classes (*Alpha-* and *Beta-proteobacteria*, and *Cytophagia*), and 4 orders (*Burkholderiales*, *Cytophagales*, *Sphingomonadales* and *Rhizobiales*).

Across the different host species, both natural and urban phyllosphere bacterial communities were dominated by *Alphaproteobacteria*, ranging from an average 52.7 % of total community for *P. glauca*, 58.3 % for *A. saccharum* and 72.6 % for *A.rubrum* in natural forests in comparison to 43.5 %, 44.0 % and 39.7 % for the same host species respectively in urban settings (Figure 4.2b). Among the five most abundant bacterial classes on tree species, the significant changes in communities from natural forest to urban environment (Post-hoc Tukey's tests on ANOVA) were a decrease in the relative abundance of *Alphaproteobacteria* (8.4 %; $F = 27.23$, $P < 0.001$), and increases in *Betaproteobacteria* (2.0 %; $F = 13.68$, $P < 0.001$), in *Gammaproteobacteria* (1.5 %; $F = 13.36$, $P < 0.001$), and in *Cytophagia* (2.0 %; $F = 15.06$, $P < 0.001$). Leaf bacterial alpha diversity was not statistically different from natural to urban environment (Post-hoc Tukey's test on ANOVA; $P = 0.86$).

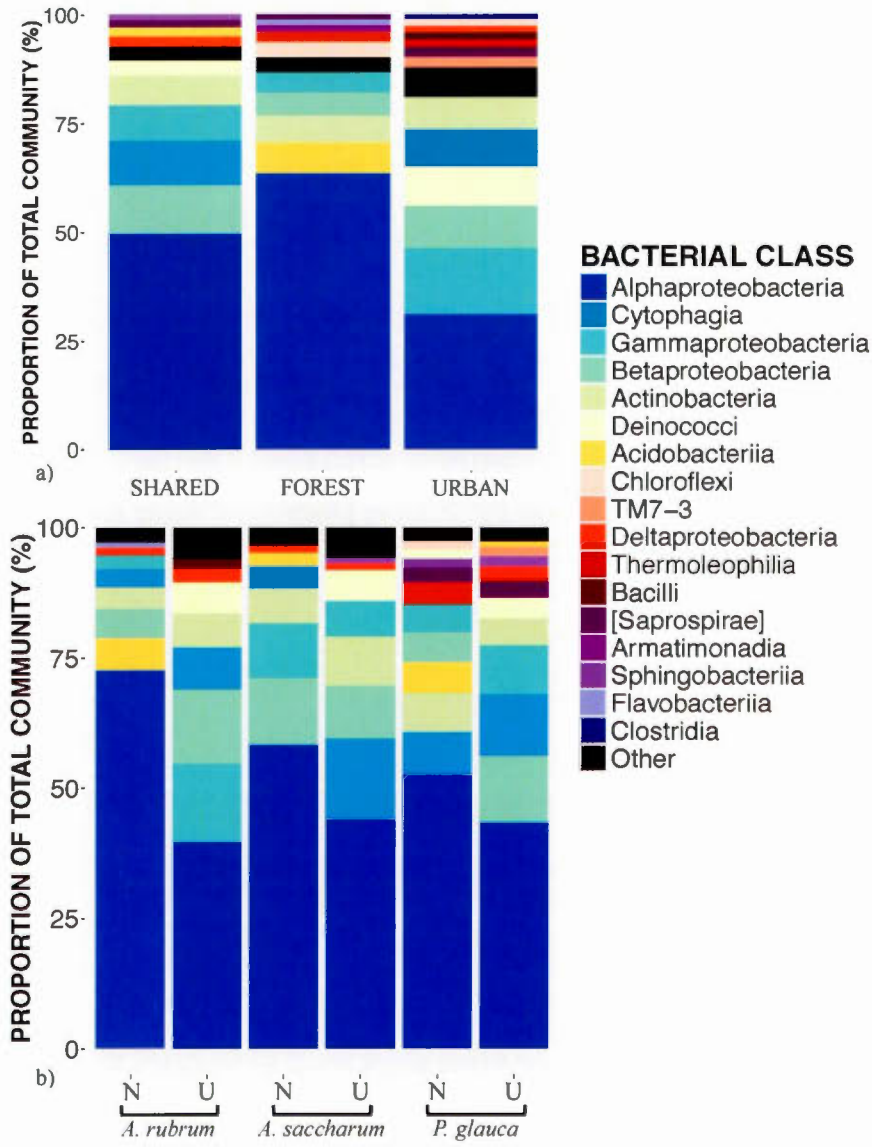


Figure 4.2 Relative abundance of sequences from bacterial classes in the phyllosphere microbiome. Class community composition of a) three tree species in natural forest (N) and urban (U) environments; b) shared and unique OTUs of both environments.

So as to better characterize the phyllosphere bacterial communities of the natural forest and the urban environment, we identified the most abundant OTUs (OTUs having a total relative abundance >0.5 %) and compared their respective average relative abundance in each environment (Figure 4.3 and *Annex K*). Among the OTUs that were more abundant in the natural forest environment, the most represented order was the *Rhizobiales* (including the families *Methycystaceae* and *Beijerinckiaceae*). In comparison, in the urban environment, many orders were almost equally represented including the *Rhodospiralles* (*Acetobacteraceae*), followed by the *Burkholderiales* (*Burkholderiaceae*, *Comamonadaceae*, *Oxalobacteraceae*), the *Cytophagales* (*Cytophagaceae*), the *Rhizobiales* (*Methylobacteriaceae*, *Rhizobiaceae*) and the *Sphingomonadales* (*Sphingomonadaceae*). The biomarker analysis (LEfSe; Segata *et al.*, 2011) indicated that 130 bacterial taxa are biomarkers of the natural forest environment including the phyla *Acidobacteria*, *Chlamydia*, Kazan-3B-28 and *Proteobacteria* (Figure 4.4). For the urban environment, 253 taxa were biomarkers including the phyla *Bacteroidetes*, *Chloroflexi*, FBP, *Firmicutes*, *Fusobacteria*, *Gemmatimonadetes*, *Nitrospirae* and *Thermi* (Figure 4.4; Table 4.2). In addition, several OTUs were also identified as bio-indicators of environment type (Figure 4.5).

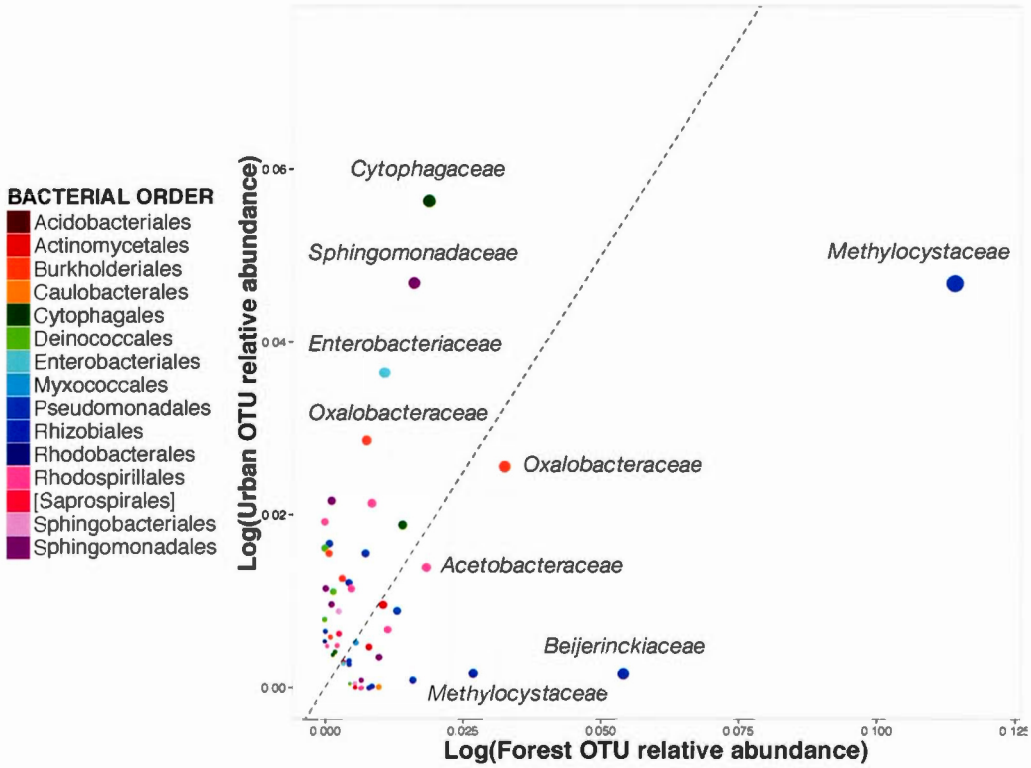


Figure 4.3 Mean relative abundance of the most abundant OTUs natural forest (*x-axis*) and urban (*y-axis*) environments. Only the OTUs with a sum of mean relative abundance in both environments of >0.5 % are shown. The axes are both $\log_{10}$-transformed. The size of the points is relative to the abundance of the OTU while the color represents the bacterial order. The dashed line indicates an isocline of equal average relative abundance in natural forest and urban environments. OTUs above the line are more frequent in the urban environment while OTUs below the line are more abundant in the natural forest environment. Labels indicate the bacterial family of the 5 most abundant OTUs in both environments.

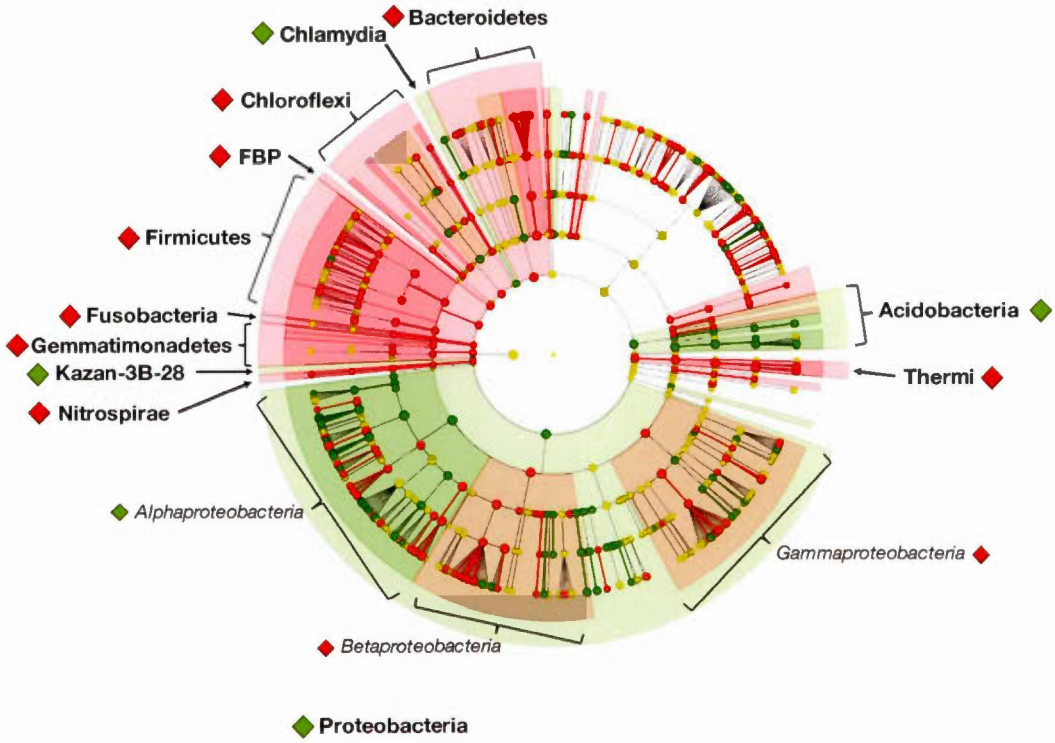


Figure 4.4 Cladogram of significant associations between phyllosphere bacterial taxon and environment type. Color indicate environment type (red for urban environment and green for natural forest). The circles, parentheses and shading indicate with which environment type the bacterial taxonomic group is associated.

Table 4.2 The five strongest biomarkers of associations between bacterial taxonomic groups and environment type (LEfSe analyses). Scores identify which clades have the greatest explanatory power on differences between communities.

Environment	Taxonomical level	Biomarker	Score
Natural forest	Genus	*Beijerinckia* sp.	5.08
	Order	*Rhizobiales*	5.07
	Family	*Methylocystaceae*	5.04
	Phylum	*Acidobacteria*	5.00
	Class	*Alphaproteobacteria*	4.91
Urban	Species	*Methylobacterium adhesivium*	4.83
	Species	*Deinococcus aquatilis*	4.77
	Family	*Sphingomonadaceae*	4.64
	Order	*Sphingomonadales*	4.64
	Genus	*Sphingomonas* sp.	4.55

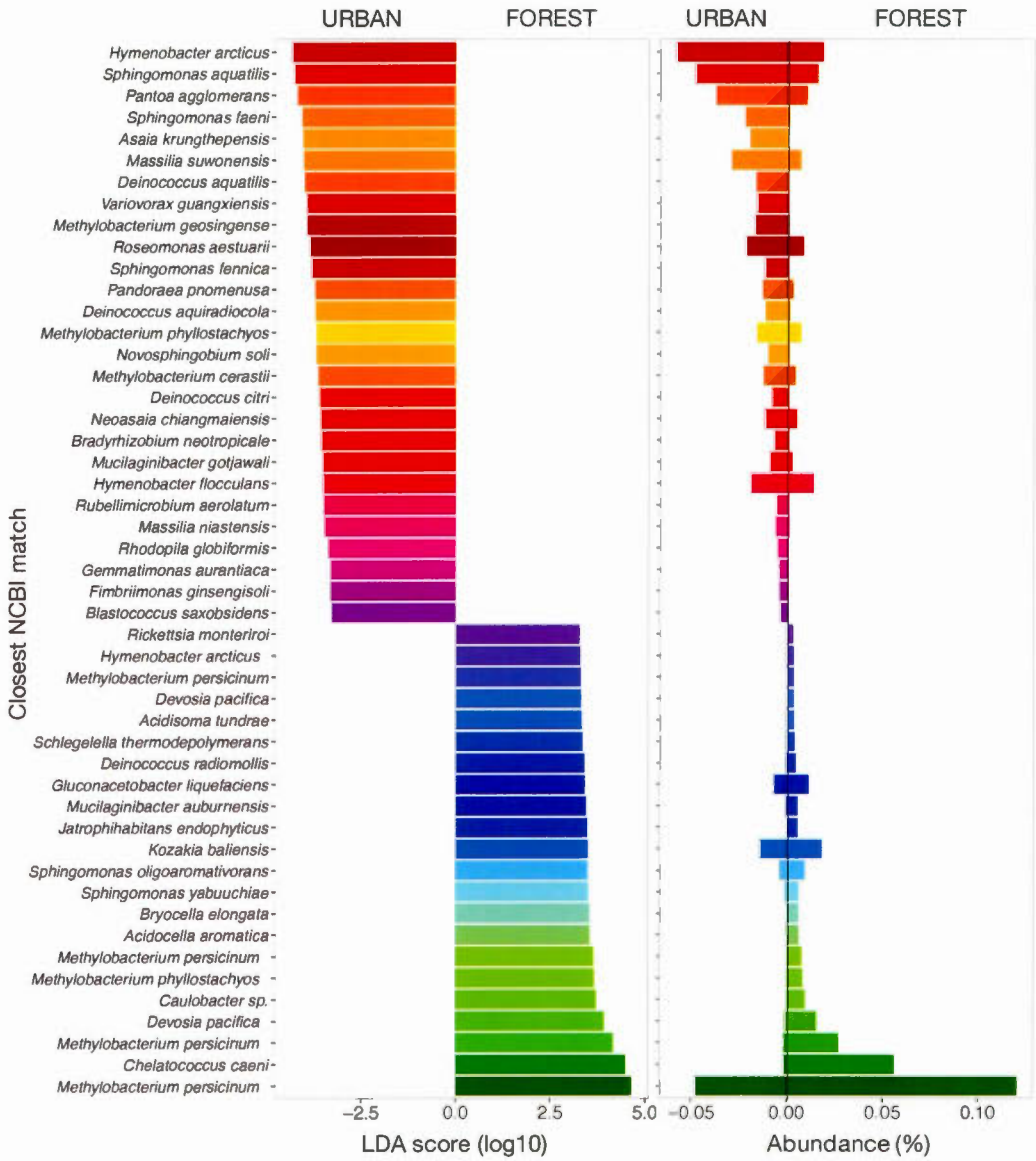


Figure 4.5 50 strongest OTU bio-indicators in urban and natural forest environments identified by closest match in NCBI database. Left panel shows the score given by the LEfSe analysis and right panel indicates the mean relative abundance of each OTU.

When comparing the relative influence of host species identity, site and environment type (Table 4.3), environment type was the strongest driver of community structure (R^2 = 17.4 %, P = 0.001; PERMANOVA on Bray-Curtis distances). Site nested in environment type (R^2 = 10.9 %, P = 0.001), host species identity (R^2 = 7.9 %, P = 0.001) and the triple interaction between environment, site and host species (R^2 = 10.6 %, P = 0.001) also made a significant contribution to the model bringing the total variance explained to 52 % (Table 4.3 and Figure 4.6).

Table 4.3 Bacterial community structure explained by host species identity, environment, site nested in environment, and their interaction with host species identity (PERMANOVA on Bray-Curtis dissimilarities). The model explained a total of 52 % of the variation in bacterial community structure. [Complete set of 76 samples from 3 species and 7 sites].

Variables		F-value	R^2	Pr(>F)
1st level	Host species identity	4.58	7.90	0.001
	Environment	20.17	17.42	0.001
2nd level	Environment X Site	2.53	10.94	0.001
	Environment * Species	2.75	4.75	0.001
3rd level	Environment * Site * Species	1.37	10.64	0.001

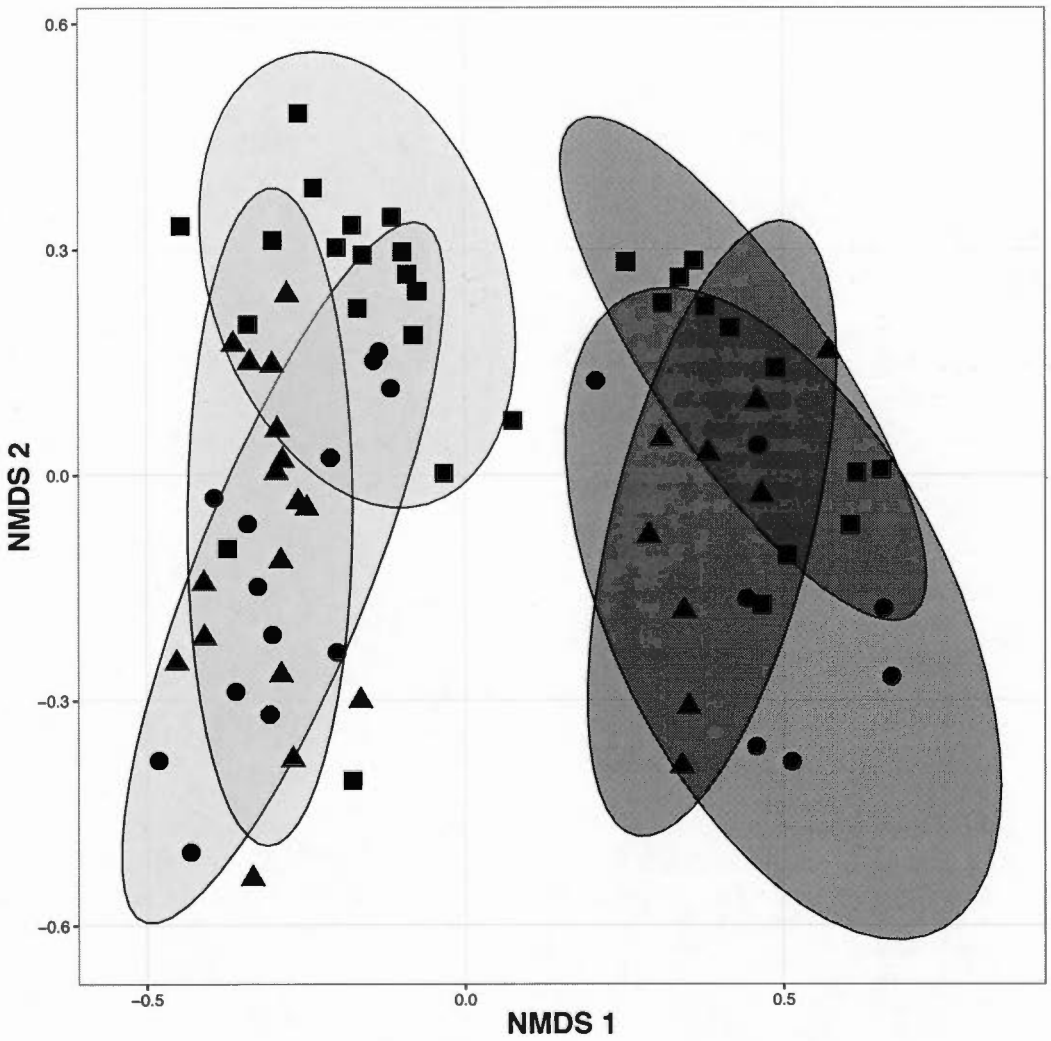


Figure 4.6 Non-metric multidimensional scaling (NMDS) ordination of variation in bacterial community structure of natural forest and urban tree phyllosphere. Ordination based on Bray-Curtis distances among 76 samples. Ellipses are shaded based on environment (light grey for urban trees and dark grey for natural forest) and shaped based on host species identity (circles for *Acer rubrum*; triangles for *Acer saccharum*; and squares for *Picea glauca*). Ellipses indicate 1 standard deviation confidence intervals around samples from species in different environments.

4.4.2 Urban intensity gradient

Along the urban gradient, phyllosphere bacterial communities were dominated by *Alphaproteobacteria* averaging 40.8 %, 39.4 %, and 31.9 % of sequences in communities from lower to higher urban intensity respectively. Among the five most abundant bacterial classes in the urban phyllosphere, the only significant change in community composition along the urban gradient (Post-hoc Tukey's tests on ANOVA) was a decrease in the relative abundance of *Alphaproteobacteria* (3.4 % and 2.0 % when comparing high to the low and medium sites respectively; $F = 6.7$, $P < 0.005$). While no significant changes were detected in the relative abundance of specific taxonomic classes at different urban intensity levels, the highest level of urban intensity exhibited a higher leaf bacterial alpha-diversity (4.6; Shannon index) than the low (4.2) and medium (4.2) intensities ($P = 0.002$ and $P = 0.004$ respectively; Post-hoc Tukey's test on ANOVA).

The strongest driver of urban phyllosphere community structure (PERMANOVA on Bray-Curtis distances; Table 4.4 and Figure 4.7) was host species identity ($R^2 = 19.4$ %, $P = 0.001$). Urban intensity ($R^2 = 6.1$ %, $P = 0.001$) and tree isolation ($R^2 = 1.8$ %, $P = 0.001$) were both weaker but significant drivers of leaf community structure. All 2nd level interactions were significant the strongest being the interaction between urban intensity and host species identity ($R^2 = 12.1$ %, $P = 0.001$) and the 3rd level interaction was also significant ($R^2 = 8.0$ %, $P = 0.017$).

Table 4.4 Bacterial community structure explained by host species identity, urban intensity, tree isolation (street or park), and their interactions (PERMANOVA on Bray-Curtis dissimilarities). The model explained a total of 56 % of the variation in bacterial community structure. [Urban subset of 108 samples from 7 species]

Variables		F-value	R^2	Pr(>F)
1st level	Host species identity	4.97	19.38	0.001
	Urban intensity	4.66	6.05	0.001
	Tree isolation	2.78	1.81	0.001
2nd level	Urban intensity * Tree isolation	1.33	1.73	0.056
	Species * Tree isolation	1.74	6.77	0.001
	Urban intensity * Species	1.55	12.10	0.001
3rd level	Urban intensity * Species * Tree isolation	1.23	7.98	0.017

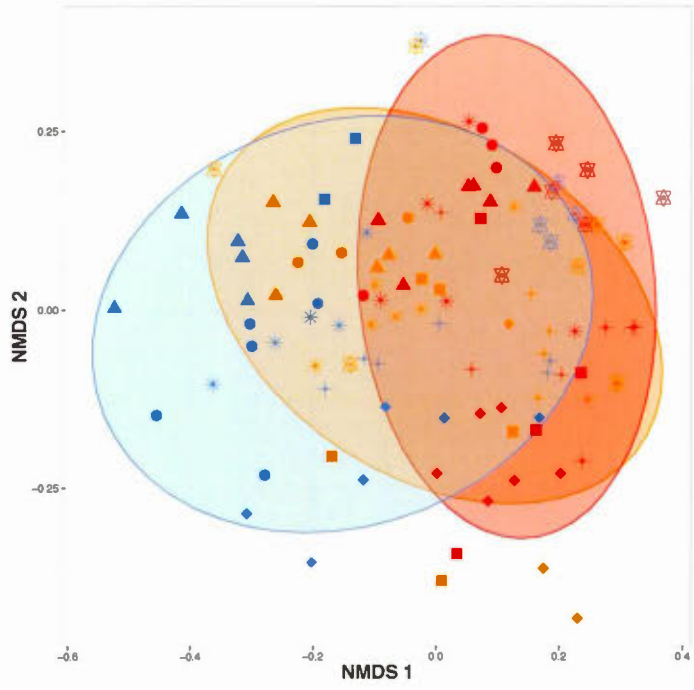


Figure 4.7 Non-metric multidimensional scaling (NMDS) ordination of variation in bacterial community structure of tree phyllosphere along a gradient of urban intensity. Ordination based on Bray-Curtis distances among 108 samples. Samples (points) are colored based on the urban gradient (blue for low intensity, orange for medium intensity and red for high intensity) and shaped based on host species identity (squares for *Acer platanoides*, circles for *Acer rubrum*; triangles for *Acer saccharum*; diamonds for *Celtis occidentalis*, asterix for *Fraxinus Americana*, crosses for *Fraxinus pensylvanica* and stars for *Picea glauca*); ellipses indicate 1 standard deviation confidence intervals around samples from urban gradient intensity.

4.5 Discussion

In this study, we compared the tree phyllosphere bacterial communities in natural forest and urban environments among several different tree species, along a gradient of urban intensity and degree of tree isolation. Our results show that leaf bacterial communities of the natural forest and urban environments are clearly distinct in structure but not in diversity (Tables 4.3-4.4 and Figures 4.4-4.6). In the context of the urban microbiome, this work provides an unprecedented comparison of urban and natural plant-associated microbiomes, providing key information to understand the impact of urban conditions on leaf microbial communities. In addition, by identifying the changes in phyllosphere bacterial community structure along a gradient of increasing anthropogenic pressures for a multitude of tree host species, our study offers a unique input into the plant and the urban microbiome research. Studying the potential sources of the air and built-environment microbiome offers great insights for the eventual management of the urban microbiome.

In previous studies of tree phyllosphere microbial communities in natural environments, host species identity had often been found to be the strongest determinant of community structure (Redford *et al.,* 2010; Kembel *et al.,* 2014; Laforest-Lapointe *et al.,* 2016a). Here, we show that the environment type (natural vs. urban environment) is a stronger driver of leaf bacterial community structure than host species identity (Table 4.3 and Figure 4.6) when comparing both environments. In accordance with previous descriptions of tree phyllospheres (Delmotte *et al.,* 2009; Kembel *et al.,* 2014; Laforest-Lapointe *et al.,* 2016a), the urban phyllosphere bacterial communities are dominated by the *Alphaproteobacteria* (Figure 4.2). However, our results demonstrate that the abiotic and biotic changes in the urban environment reduced the relative abundance of the most abundant bacterial taxonomic groups (the

class *Alphaproteobacteria* and the order *Rhizobiales*; Figures 4.2-4.4) and enriched or depleted the relative abundance of many specific OTUs (Figure 4.5). The distinction between urban and natural forest phyllosphere can also be detected by the significant increase in the abundance of bacterial taxonomic groups such as the *Bacteroidetes* and the *Firmicutes* (Figures 4.3-4.5). This finding suggests that the local pool of microorganisms is changed by the abiotic and biotic conditions in the urban environment. Similar results have been found on the phyllosphere of ivy (*Hedera* sp.) by Smets *et al.* (2016) who showed shifts in leaf bacterial communities between non-urban and urban sites in relation to atmospheric contamination. Jumpponen & Jones (2010) also showed that tree phyllosphere fungal communities differed from nonurban to urban environments, in parallel with a general enrichment of foliar macronutrients in urban trees. In addition to changes in air quality and leaf composition, the differences we observed in tree leaf bacterial community composition could also be driven by the increased stress level experienced by urban trees.

Increasing levels of anthropogenic pressures including land use changes, biogeochemical changes, global warming and exotic species invasion cause an augmentation in plant stress and correspondent diminution in longevity and productivity (Mittler, 2006; Niinemets, 2010a, 2010b). Here, we show that the level of urban intensity influences leaf bacterial community structure (Table 4.4 and Figure 4.7), though the strongest determinant of community structure was host species identity when comparing different urban sites. Although we did not observe a change in the relative abundance of common bacterial taxonomic groups, we found that leaf bacterial diversity increases at the highest level of urban intensity. Therefore, although phyllosphere community structure changes from natural forest to urban environments, the host species retain a certain capacity to select for their associated microbiota. Our results also show that the degree of tree isolation in street vs. park interact with both host species identity and urban intensity to drive leaf bacterial communities (Table 4.4).

Urban trees are submitted to multiple anthropogenic stresses of different length and intensity leading to photosynthetic biomass loss and tree lesions (Sieghardt *et al.*, 2005) which could impact retroactively their interactions with leaf microbiota. Further experiments are needed to follow the changes in tree-associated microbiota when transplanted from natural to urban environments and through time as the host tree adapts to its new abiotic and biotic conditions.

Our results support previous findings showing that rural and urban microbial communities differ in composition (Pakarinen *et al.*, 2008; Burrows *et al.*, 2009; Bowers *et al.*, 2011). The proportion of green spaces and species diversity have been suggested as potential drivers of these natural-urban differences in community composition (Mhuireach *et al.*, 2016), but our work shows that the plant-associated microbiota per se is different from what is usually found in the natural environment. Urban abiotic and biotic conditions linked directly and indirectly to human actions are potential drivers of the changes in leaf microbial community structure. Therefore, future studies comparing the relative influence of the increased stress, the sources of microbial input and the host capacity to select their microbiota in urban settings on the plant-associated microbiome, are required to identify clearly the causes of this shift in the urban plant microbiome. These studies will provide key information to enable an effective management of the urban microbiome, and eventually identify which are most effective interventions (i.e. increasing plant diversity, increasing plant cover, reducing heat islands, reducing air contamination, introduce specific plant species).

4.6 Acknowledgments

We thank T. Dawson and R. Fréchon for support in the laboratory and in the field.

CONCLUSION

Although the number of researches investigating tree phyllosphere bacterial communities is on the rise, there are still very few studies that offer a dual characterization of both the natural and urban tree phyllosphere bacterial community structure across multiple host species and drivers. The work presented here therefore offers an original assessment of the dynamics at play in the tree phyllosphere, combining a strong ecological framework, advanced sequencing techniques and sophisticated bioinformatics analyses, consequently and hopefully making a noteworthy contribution to the field.

The main purpose of the work presented here was to make a significant contribution to the knowledge of tree phyllosphere bacterial community structure and dynamics in a diversity of stand types including natural forest, controlled experiments and urban stands. To reach this goal, the first objective was to characterize the phyllosphere bacterial communities of natural temperate tree species and to quantify the relative influence of host species identity, site, and time in driving leaf bacterial community assembly. The first chapter thus presented an unprecedented evaluation of the identity and dynamics of natural temperate tree phyllosphere bacterial communities across multiple host species, site and time. Then, the second objective was to compare both the intra- and inter-individual variation in the phyllosphere bacterial community

structure for multiple tree species. In the second chapter, we employed a simple design to test if the actual sampling techniques of tree phyllosphere were adequate to characterize the variation in a tree's canopy, thus providing significant methodological information for future studies. The objectives of the third chapter were (1) to describe the influence of host species identity to local tree functional diversity, species richness and functional identity on phyllosphere bacterial community structure in a tree biodiversity-ecosystem functioning experiment as well as (2) to test if phyllosphere bacterial diversity drives plant community productivity. This chapter, touching on a major field of ecological research, applied an innovative multi-trophic approach to explain the mechanisms behind the influence of plant community diversity on productivity. Finally, the fourth chapter aimed to compare the natural and urban tree phyllosphere bacterial community structure and observe the changes in phyllosphere bacterial communities along a gradient of increasing anthropogenic pressures. This chapter presented crucial results for both the domains of urban plant ecology and urban microbiome, as well as holding potential impacts for public health.

5.1 Natural Temperate Forest

Despite the increasing scientific interest for the tree phyllosphere microbiome, few studies have compared the relative influence of multiple drivers across various host species (but see Kim *et al.*, 2012; Kembel *et al.,* 2014; Kembel & Mueller, 2014). Our first chapter, characterizing the changes in natural temperate tree phyllosphere bacterial communities across multiple tree species, site, and time provided a unique perspective of the dynamics at play in the epiphytic forest ecosystem. Our study design offered an original assessment of leaf bacterial community dynamics because of its concurrent evaluation of the importance of key dispersal-related and niche-based drivers such as

host species identity (phylogeny, co-evolution, functional traits), geographical location (dispersal history and abiotic conditions) and time of sampling (abiotic conditions). The central results of the first chapter include: (1) the existence of a "core microbiome" in the temperate tree phyllosphere even when study sites were hundreds of kilometers apart; (2) the significant associations between bacterial taxonomic groups and host species; and finally (3) a greater part of the variation in phyllosphere bacterial community assembly being explained by host species identity rather than by site or time.

Individual trees showed unique communities that varied predictably across species, sites and time, suggesting a role for selection- or niche-based mechanisms during community assembly. However, the existence of a core microbiome suggests that bacteria from a similar metacommunity colonize tree leaves across Quebec's temperate forests possibly through a variety of vectors (i.e. air, rain, soil) (Bulgarelli *et al.,* 2012). Our results also provided support for the hypothesis that host ecological life strategies shape phyllosphere bacterial communities and these communities go through a succession from June to August. However, the much higher relative importance of host species and site on phyllosphere bacterial community structure suggest that once a community of bacteria successfully colonizes a leaf, temporal changes are not enough to overcome the influence of host species identity and site on community assembly.

5.2 Intra-individual vs. Inter-individual Variation

In the second chapter, we tested if one sample is enough to characterize the variation in a tree's canopy microbial community. We demonstrated for multiple host species that there is a significant amount of intra-individual variation in phyllosphere bacterial

community structure, and that the magnitude of this variation is smaller but not statistically different from the magnitude of inter-individual variation. When considering the various methodology employed in tree phyllosphere studies, efforts should be made to homogenize the sampling protocol in order to minimize the potential effect of location of sampling in the study's results. Therefore, our work reveals that tree phyllosphere bacterial community studies aiming to quantify interspecific variation should sample from a consistent location within the tree canopy for individual trees.

5.3 Biodiversity Experiment with Trees

The third chapter allowed for an unprecedented test of the relative influence of host species identity, which many studies including ours (Laforest-Lapointe *et al.,* 2016a, 2016b) have reported to be the main driver of leaf bacterial community structure, and multiple variables describing a tree's vicinity, on leaf bacterial community structure and diversity. These characteristics, including plant species richness, plant functional identity, and plant functional diversity, were found to be significant drivers of phyllosphere bacterial community structure, but could not compare to the strength of host species identity. These results confirmed that host species identity plays a dominant role in determining leaf microbial community structure even in environments of different plant functional diversity, identity and species richness. However, the most notable and novel result of the third chapter is the significant contribution of tree phyllosphere bacterial diversity to plant community productivity. This finding provides the first empirical evidence that leaf bacterial diversity is positively related to terrestrial ecosystem productivity, even after accounting for the effects of other explanatory factors. Thus, plant biodiversity-ecosystem functioning relationships could in part be

driven by positive interactions involving other trophic levels such as bacteria or fungi. Therefore, this chapter gives unique support to the hypothesis that host growth and productivity could be influenced by plant-associated microbial communities. Adding multi-trophic components to biodiversity-ecosystem functioning studies is a promising avenue aimed to improve our understanding of complementarity mechanisms structuring plant communities, by ameliorating plant ecosystem productivity models.

5.4 The Urban Environment

The fourth chapter offers a unique input into the plant and the urban microbiome research by comparing the urban and natural plant-associated microbiomes among several different tree species, along a gradient of urban intensity and degree of tree isolation. Here, we show for the first time that the environment type (natural vs. urban environment) is a stronger driver of leaf bacterial community structure than host species identity when comparing both environments. Our results show that leaf bacterial communities of the natural forest and urban environments are clearly distinct in structure, but not in diversity. Our findings suggest that the local pool of microorganisms is changed by the abiotic and biotic conditions in the urban environment potentially through alterations in air quality, leaf composition, and due to the increased stress level experienced by urban trees. In this chapter, we also aimed to improve the understanding of urban conditions' effect on leaf microbial communities by identifying the changes in phyllosphere bacterial community structure along a gradient of increasing anthropogenic pressures. While environment type was the strongest determinant of leaf bacterial community structure in the comparison between natural forest to urban environment, when looking at different levels of urban intensity (low, medium, high) our results showed the strongest determinant of community

structure was host species identity. However, the level of urban intensity also influenced leaf bacterial community structure. Our results also revealed that the influence of tree isolation in street vs. park on leaf bacterial communities varies in function of both the host species identity and urban intensity.

Although previous studies had already shown that the rural and urban air microbiomes differ in composition (Pakarinen *et al.*, 2008; Burrows *et al.*, 2009; Bowers *et al.*, 2011) and that the green spaces proportion and species diversity are potential drivers of these natural-urban differences (Mhuireach *et al.*, 2016), our study makes a unique contribution to the current literature by explicitly demonstrating that the urban plant-associated microbiota per se is different from what is found in the natural environment. Because the built-environment microbiome is influenced by the urban air microbiome, studying the potential sources of contribution to these communities holds great potential for managing the urban microbiome.

5.5 Limits

Although high-throughput sequencing techniques have allowed for unprecedented coverage of non-cultirable microbial communities (Hibbett *et al.*, 20011), sequencing the 16S bacterial gene doesn't distinguish between dormant or inactive versus active bacterial cells. Therefore, the structure of microbial communities obtained through this technology reflects the past (inactive), actual (active) and potential future (dormant) members of a habitat's community altogether. In addition, 16S sequences provide no information on the functional profiles of bacterial communities. Metagenomics and metaproteomics analyses of bacterial communities have been proposed to improve our understanding of what these bacterial communities are actually actively synthesizing

and who is truly expressing genes (Darzi *et al.*, 2015). In addition, the diversity of the phyllosphere microbiome increases the difficulty of accurately identifying the taxonomy of its member since most of the microbiota is poorly classifiable through the commonly used databases such as Greengenes (DeSantis *et al.*, 2006) and Silva (Quast *et al.*, 2013). Therefore, most of our taxonomic knowledge of phyllosphere bacterial communities is still very limited and more culture-based studies of the phyllosphere microbiome are crucial to reduce the amount of 16S sequences that are unidentified. Leaves have been shown to be hot spots for horizontal gene transfer (Lilley *et al.*, 1996; Normander *et al.*, 1998; Bjorklof *et al.*, 2000), which can lead to potential misidentification of the host cell taxonomy. In relation to the host plant, the effect of host genotype is an important factor driving leaf community structure, as it has been shown for the model plant *Arabidopsis thaliana* (Reisberg *et al.*, 2013; Horton *et al.*, 2014), that could have provided additional information to our project. Working with tree model organisms such as the poplar for which the genome has already be fully sequenced (Brunner *et al.*, 2004; Tuskan *et al.*, 2004) opens the way to characterizing how specific genes of the tree genotype can influence its phyllosphere microbiome.

5.6 Recommendations for the future

In light of the great potential for applications in the domains of agriculture, viticulture, forestry and even human health, research on the phyllosphere microbiome is crucial to the development of new techniques involving leaf microbial communities to improve plant health and productivity. Experimental studies are required for the betterment of our understanding of the dynamics at play. In view of our results, future studies should aim to (1) identify the vectors of dissemination contributing to phyllosphere communities; (2) design and establish leaf microbial communities with specific

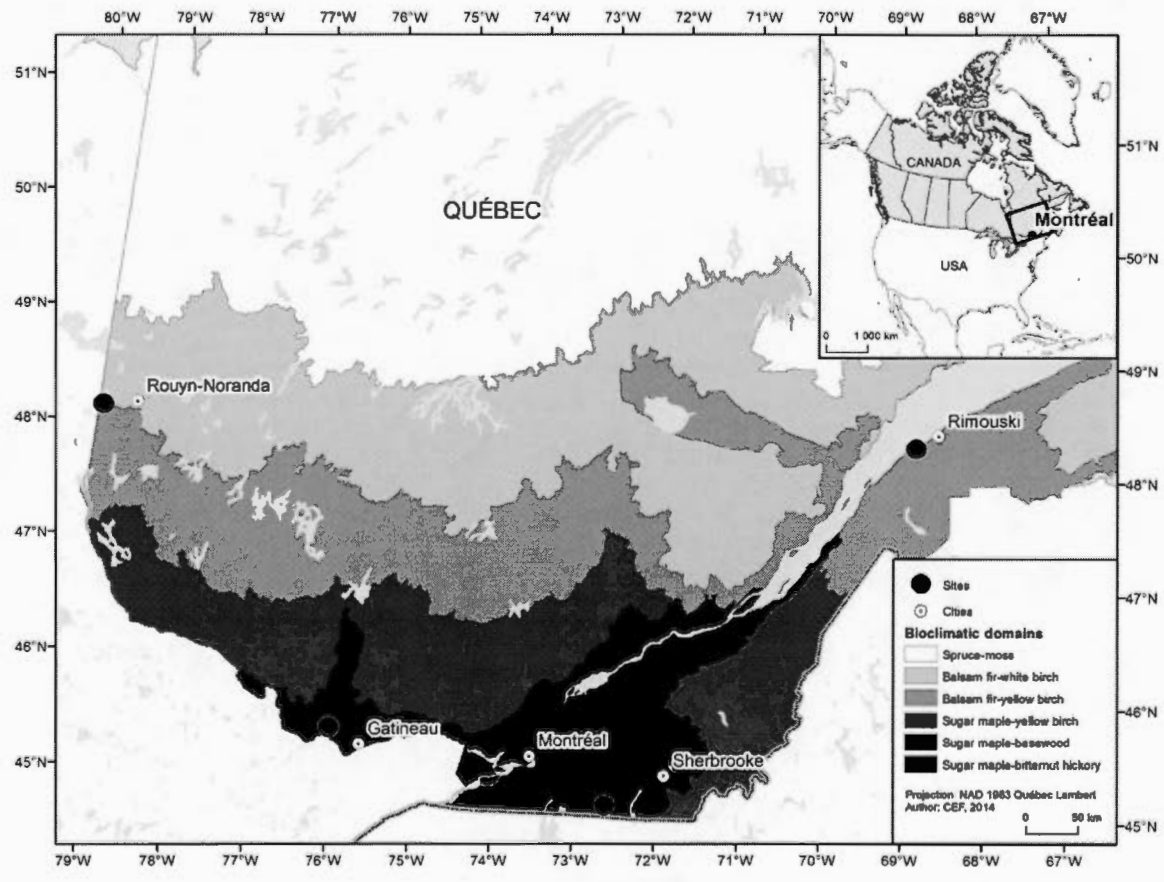
targeted effects on their host health and productivity; (3) identify the functional trade-offs in bacterial life strategies; (4) cultivate leaf microbial organisms and sequence their genomes; (5) evaluate the role of phages in controlling leaf bacterial communities; and (6) measure how global warming will modify the microbiome dynamics both in natural and urban plant ecosystems. In addition, future research on the phyllosphere looking at different members of the microbiome (bacteria, fungi, phages, etc.) will also provide depth to our understanding of the ways the phyllosphere microbiome can influence host health and productivity. Given the capacity of microbes to respond rapidly to environmental changes (Lau & Lennon, 2012), studying how the effect of microbial communities on plant productivity interacts with global change and intensified anthropogenic pressures will be crucial to optimize or maintain primary production. Characterizing the complete tree "holobiome", characterizing simultaneously multiple microbiomes (rhizosphere, dermosphere, phyllosphere, etc.), will definitely improve our understanding of the multiple mechanisms through which microbial organisms influence host health and productivity. Finally, future experiments identifying the key determinants causing the shifts in leaf microbial communities in the urban environment will provide crucial information to enable an effective management of the urban microbiome and help urban planners to employ the most effective interventions to retain the natural plant microbiome (i.e. increasing plant diversity, increasing plant cover, reducing heat islands, reducing air contamination, introduce specific plant species).

ANNEX A

Supplementary Table 1.1 Description of the four study sites during the summer of 2013 (Canadian historical climate data, http://climate.weather.gc.ca/).

Site	Elevation (m)	Month	Mean monthly temperature (°C)	Monthly precipitation (mm)
Sutton	650	June	16.0	146.2
		July	20.0	100.4
		August	17.5	124.6
Abitibi	321	June	14.8	71.2
		July	18.5	52.8
		August	16.7	41.8
Gatineau	100	June	17.8	107.4
		July	21.3	54.7
		August	19.1	68.6
Bic	254	June	14.1	180.8
		July	20.1	25.0
		August	17.7	41.8

ANNEX B



Supplementary Figure S1.1 Location of the four sites sampled during summer 2013 across the temperate forest of Quebec's province.

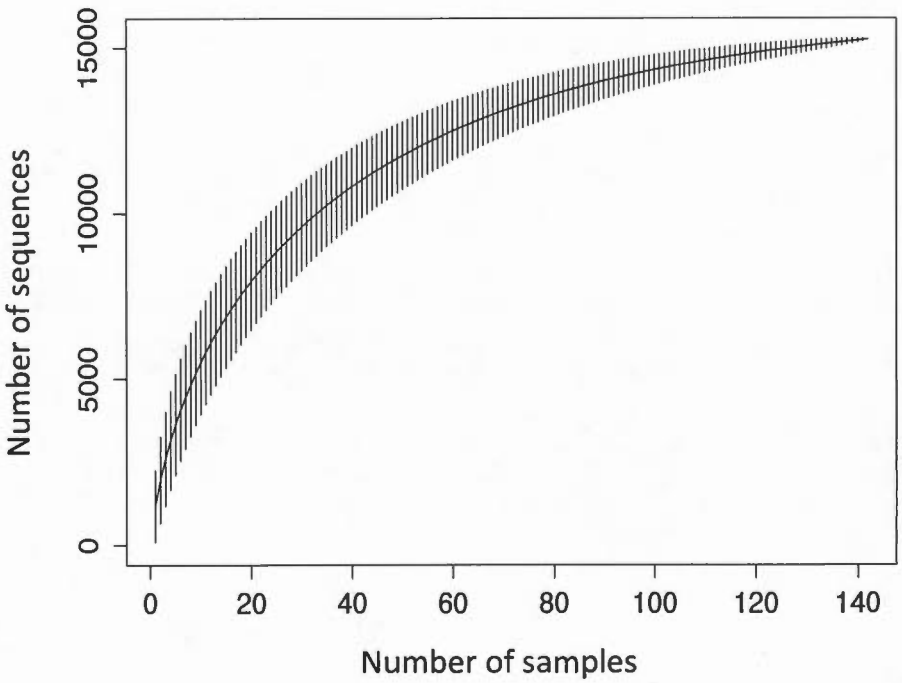
ANNEX C

Supplementary Table 1.2 Taxonomic and functional trait information of the five tree species used in this study. Sources for functional trait information are described in the main text.

Division	Family	Species	Drought tolerance*	Hmax (m)	Nmass (%)	Seed mass (mg)	Shade tolerance*	SLA (m^2/g)	WD (g/cm^3)
Angiosperm	*Aceraceae*	*Acer rubrum*	1,8	25	1,91	20	3,4	0,0141	0,49
		Acer saccharum	2,3	35	1,83	65	4,8	0,0142	0,56
	Betulaceae	*Betula papyrifera*	2	25	2,31	0,33	1,5	0,0128	0,48
Gymnosperm	*Pinaceae*	*Abies balsamea*	1	25	1,66	7,6	5	0,0066	0,34
		Picea glauca	2,9	25	1,28	2,15	4,2	0,0033	0,35

*Drought tolerance and shade tolerance are two indexes going from one (non-tolerance) to 5 (max-tolerance).

ANNEX D



Supplementary Figure S1.2 Collector's curve (mean 95 % confidence interval) of bacterial phyllosphere operational taxonomic units (OTUs; 97 % sequence similarity cut-off) richness versus number of trees sampled in the temperate forest in 2013.

ANNEX E

Supplementary Table 1.3 Taxonomic identity of the 19 core microbiome OTUs across the 142 trees sampled. Taxonomic identification was based on a BLAST against the Greengenes database with a minimum cutoff of 50 % confidence required for assignment to a given taxonomic group.

OTU ID	DOMAIN	PHYLUM	CLASS	ORDER	FAMILY	SEQUENCES	PROPORTION (%)
denovo38758	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae	691235	17,9
denovo43328	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	201341	5,2
denovo6292	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae	154426	4,0
denovo11233	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	93018	2,4
denovo37541	Bacteria	Acidobacteria	Acidobacteriia	Acidobacteriales	Acidobacteriaceae	89142	2,3
denovo26524	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	88783	2,3
denovo20227	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	47281	1,2
denovo30571	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	45477	1,2
denovo20300	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	34051	0,9
denovo7913	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	32548	0,8
denovo42054	Bacteria	Acidobacteria	Acidobacteriia	Acidobacteriales	Acidobacteriaceae	20341	0,5
denovo33295	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae	20294	0,5
denovo45353	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	Cystobacterineae	20001	0,5
denovo34795	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	19796	0,5
denovo3293	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	17600	0,5
denovo4366	Bacteria	Acidobacteria	Acidobacteriia	Acidobacteriales	Acidobacteriaceae	16530	0,4
denovo17267	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	15780	0,4
denovo45264	Bacteria	Acidobacteria	Acidobacteriia	Acidobacteriales	Acidobacteriaceae	12961	0,3
denovo30762	Bacteria	Acidobacteria	Acidobacteriia	Acidobacteriales	Acidobacteriaceae	10934	0,3

ANNEX F

Supplementary Table 1.4 Significant associations between bacterial taxonomic groups (a-Class, b-Order, c-Family, d-Species and e-OTUs) and tree species (LEfSe analyses). Scores identify which clades have the greatest explanatory power on differences between communities.

a)

Bacterial Taxonomic Level		Tree Species					Score
		ABBA	ACRU	ACSA	BEPA	PIGL	
Class	Actinobacteria					X	>4.0
	Gammaproteobacteria					X	>4.0
	Saprospirae					X	>4.0
	Sphingobacteriia					X	>3.0
	Deinococci					X	>3.0
	C0119					X	>3.0
	Thermoleophilia					X	>3.0
	ML635J_21					X	>3.0
	Fimbriimonadia					X	>3.0
	Acidimicrobiia					X	>2.0
	Alphaproteobacteria				X		>5.0
	Anaerolineae				X		>3.0
	Chlamydiia				X		>3.0
	Betaproteobacteria			X			>4.0
	Cytophagia			X			>4.0
	TM7_3		X				>3.0
	Acidobacteriia	X					>4.0
	Armanimonadia	X					>3.0
	Solibacteres	X					>3.0
	SC3	X					>3.0
	Bacilli			X			>3.0

b)

Bacterial Taxonomic Level		Tree Species					Score
		ABBA	ACRU	ACSA	BEPA	PIGL	
Order	Actinomycetales					X	>5.0
	Sphingobacteriales					X	>4.0
	Saprospirales					X	>4.0
	Deinococcales					X	>3.0
	Tremblayales					X	>3.0
	Chloroflexales					X	>3.0
	Soliubrobacterales					X	>3.0
	Fimbriimonadales					X	>3.0
	Rhodobacterales					X	>3.0
	Rhodospirillales				X		>5.0
	Caulobacterales				X		>3.0
	Pseudomonales			X			>5.0
	Burkholderiales			X			>4.0
	Cytophagales			X			>4.0
	Acidimicrobiales			X			>3.0
	Lactobacillales			X			>3.0
	Xanthomonadales		X				>3.0
	Alteromonadales		X				>3.0
	Sphingomonadales	X					>5.0
	Acidobacteriales	X					>4.0
	AKIW874	X					>3.0
	Solibacterales	X					>3.0

c)

Bacterial Taxonomic Level		Tree Species					Score
		ABBA	ACRU	ACSA	BEPA	PIGL	
Family	Chitinophagaceae					X	>4.0
	Deinococcaceae					X	>3.0
	Sphingobacteriaceae					X	>3.0
	Micromonosporaceae					X	>3.0
	FFCH7168					X	>3.0
	AK1AB1_02E					X	>3.0
	Dermacoccaceae					X	>3.0
	Myxococcaceae					X	>3.0
	Xanthobacteraceae					X	>3.0
	Haliangiaceae					X	>3.0
	Tremblayaceae					X	>3.0
	Bradyrhizobiaceae					X	>3.0
	Nocardioidaceae					X	>3.0
	Fimbriimonadaceae					X	>3.0
	0319_6G20					X	>3.0
	Patulibacteraceae					X	>2.0
	Alcaligenacea					X	>2.0
	Mycobacteriaceae					X	>2.0
	Sporichthyaceae					X	>2.0
	Acetobacteraceae				X		>5.0
	Rhodospirillaceae				X		>3.0
	Parachlamydiaceae				X		>3.0
	Legionellaceae				X		>2.0
	Erythrobacteraceae				X		>2.0
	Oxalobacteraceae			X			>4.0
	Cytophagaceae			X			>4.0
	Microbacteriaceae			X			>4.0
	Pseudomonadaceae			X			>4.0
	C111			X			>3.0
	Corynebacteriaceae			X			>3.0
	Leuconostocaceae			X			>3.0
	Bacillaceae			X			>3.0
	Kineosporiaceae			X			>3.0
	Nocardiaceae			X			>3.0
	Beutenbergiaceae			X			>3.0
	Intrasporangiaceae			X			>3.0
	Halomonadaceae			X			>3.0
	Micrococcaceae			X			>3.0
	Aurantimonadaceae			X			>3.0
	Methylocystaceae		X				>5.0
	Beijerinckiaceae		X				>4.0
	Aerococcaceae		X				>4.0
	Ruminococcaceae		X				>3.0
	Geodermatophilaceae		X				>3.0
	Conexibacteraceae		X				>2.0
	Acidobacteriaceae	X					>4.0
	Frankiaceae	X					>4.0
	Gordoniaceae	X					>3.0
	Ellin122	X					>3.0
	Bartonellaceae	X					>3.0
	Pseudocardiaceae	X					>3.0
	Streptomycetaceae	X					>3.0
	Solibacteraceae	X					>3.0
	Phyllobacteriaceae	X					>3.0

d)

Bacterial Taxonomic Level		Tree Species					Score
		ABBA	ACRU	ACSA	BEPA	PIGL	
Species	*Phytohabitans suffuscus*					X	>4.0
	Mucilaginibacter daejeonensis					X	>4.0
	Tremblaya phenacola					X	>3.0
	Rhodospirillum rubrum					X	>3.0
	Chitinilyticum aquatile					X	>2.0
	Tanticharoenia sakaeratensis				X		<4.0
	Neoasaia chiangmaiensis				X		<4.0
	Gluconacetobacter diazotrophicus				X		>3.0
	Inquilinus limosus				X		>3.0
	Liberibacter crescens BT-1				X		>2.0
	Pseudomonadas fragi			X			>5.0
	Methylobacterium adhaesivum			X			>4.0
	Syntrichia ruralis			X			>3.0
	Methylobacterium organophilum			X			>3.0
	Sphingomonas echinoides			X			>3.0
	Pseudomonadas stutzeri			X			>3.0
	Mycobacterium vaccae			X			>3.0
	Acinetobacter rhizosphaerae			X			>3.0
	Pseudoclavibacter helvolus			X			>3.0
	Salana mutivorans			X			>3.0
	Janthinobacterium lividum			X			>3.0
	Methylobacterium mesophilicum			X			>2.0
	Lysobacter brunescens		X				>3.0
	Sphingobacterium mizutaii		X				>3.0
	Sphingomonas changbaiensis		X				>2.0
	Sphingomonas wittichii	X					>5.0
	Kaistibacter ginsenosidimutans	X					>4.0
	Methylovirgula ligni	X					>3.0
	Sphingomonas yabuuchiae	X					>3.0
	Novosphingobium nitrogenifigens	X					>3.0
	Rhodococcus equi	X					>3.0
	Sphingobacterium faecium	X					>3.0
	Chitinimonas koreensis	X					>2.0

e)

Bacterial Taxonomic Level		Tree Species					Score
		ABBA	ACRU	ACSA	BEPA	PIGL	
OTUs	1933					X	>2.5
	3670	X					>2.0
	8742			X			>2.5
	38943		X				>2.0

ANNEX G

Supplementary Table 1.5 Significant associations between bacterial taxonomic groups (a-Phylum, b-Class, c-Order, d-Family and e-species) with tree species classified between angiosperms and gymnosperms (LEfSe analyses). Scores identify which clades have the greatest explanatory power on differences between communities.

a)

Bacterial Taxonomic Level		Tree Species		Score
		Angiosperm	Gymnosperm	
Phylum	Bacteroidetes		X	>3.6
	Actinobacteria		X	>3.6
	Acidobacteria		X	>3.6
	OD1		X	>3.6
	Armatimonadetes		X	>2.4
	TM7		X	>2.4
	FBP		X	>2.4
	Fusobacteria		X	>2.4
	TM6		X	>1.2
	Gemmatimonadetes		X	>1.2
	Chlamydiae	X		>1.2
	Firmicutes	X		>2.4
	Proteobacteria	X		>4.8

b)

Bacterial Taxonomic Level		Tree Species		Score
		Angiosperm	Gymnosperm	
Class	Actinobacteria		X	>3.6
	Acidobacteriia		X	>3.6
	Saprospirae		X	>3.6
	Deltaproteobacteria		X	>3.6
	Sphingobacteriia		X	>3.6
	Armatomonadia		X	>2.4
	MB_A2_108		X	>2.4
	Solibacteres		X	>2.4
	TM7_3		X	>2.4
	Spartobacteria		X	>2.4
	Thermoleophilia		X	>2.4
	Fimbriimonadia		X	>2.4
	Acidimicrobiia		X	>2.4
	TM7_1		X	>2.4
	Fusobacteriia		X	>2.4
	SC3		X	>2.4
	SJA_4		X	>1.2
	Chlamydiia	X		>1.2
	TK10	X		>1.2
	DA052	X		>1.2
	Clostridia	X		>1.2
	Pedosphaerae	X		>2.4
	Anaerolineae	X		>2.4
	Ktedonobacteria	X		>2.4
	Bacilli	X		>2.4
	Gammaproteobacteria	X		>3.6
	Alphaproteobacteria	X		>3.6

c)

Bacterial Taxonomic Level		Tree Species		Score
		Angiosperm	Gymnosperm	
Order	Sphingomonadales		X	>4.8
	Actinomycetales		X	>3.6
	Acidobacteriales		X	>3.6
	Sphingobacteriales		X	>3.6
	Saprospirales		X	>3.6
	Tremblayales		X	>3.6
	Chloroflexales		X	>3.6
	Neisseriales		X	>2.4
	Bdellovibrionales		X	>2.4
	Solibacterales		X	>2.4
	Chthoniobacterales		X	>2.4
	Fimbriimonadales		X	>2.4
	Solibrobacterales		X	>2.4
	Desulfuromonadales		X	>2.4
	0319_6G20		X	>2.4
	Rhodobacterales		X	>1.2
	B07_WMSP1	X		>1.2
	Clostridiales	X		>1.2
	Moraxellaceae	X		>2.4
	A21b	X		>2.4
	Lactobacillales	X		>2.4
	Alteromonadales	X		>2.4
	Ktedonobacterales	X		>2.4
	Oceanospirillales	X		>2.4
	Bacillales	X		>2.4
	Enterobacteriales	X		>3.6
	Rickettsiales	X		>3.6
	Rhizobiales	X		>3.6
	Pseudomonadales	X		>4.8

d)

Bacterial Taxonomic Level		Tree Species		Score
		Angiosperm	Gymnosperm	
Family	Sphingomonadaceae		X	>3.6
	Acidobacteriaceae		X	>3.6
	Frankiaceae		X	>3.6
	Chitinophagaceae		X	>3.6
	Sphingobacteriaceae		X	>3.6
	Comamonadaceae		X	>3.6
	Pelobacteraceae		X	>3.6
	FFCH7168		X	>3.6
	Ignavibacteriaceae		X	>2.4
	Chloroflexaceae		X	>2.4
	Syntrophaceae		X	>2.4
	Heliobacteriaceae		X	>2.4
	Bdellovibrionaceae		X	>2.4
	Solibacteraceae		X	>2.4
	Haliangiaceae		X	>2.4
	Tremblayaceae		X	>2.4
	Bartonellaceae		X	>2.4
	Chthoniobacteraceae		X	>2.4
	Gordoniaceae		X	>2.4
	Pseudonocardiaceae		X	>2.4
	Brazyrhizobiaceae		X	>2.4
	Fimbriimonadaceae		X	>2.4
	Streptomonadaceae		X	>2.4
	Streptomycetaceae		X	>2.4
	Nakamurellaceae		X	>2.4
	Sinobacteraceae		X	>2.4
	AKIW874		X	>2.4
	Hyphomonadaceae		X	>2.4
	Hyphomicrobiaceae		X	>2.4
	Solirubrobacteraceae		X	>2.4
	Patulibacteraceae		X	>2.4
	AK1AB1_02E		X	>2.4
	Phyllobacteriaceae		X	>2.4
	Myxococcaceae		X	>2.4
	Rhodobiaceae		X	>2.4
	Rhodospirillaceae		X	>1.2
	Coxiellaceae		X	>1.2
	Xanthobacteraceae		X	>1.2
	Mycobacteriaceae		X	>1.2
	Conexibacteraceae		X	>1.2
	Parachlamydiaceae		X	>1.2
	Koribacteraceae	X		>1.2
	Gaiellaceae	X		>1.2
	Erythrobacteraceae	X		>1.2
	Aurantimonadaceae	X		>1.2
	FFCH4570	X		>1.2
	Williamsiaceae	X		>1.2
	Lactobacillaceae	X		>1.2
	Staphylococcaceae	X		>1.2
	Shewanellaceae	X		>2.4
	Halomonadaceae	X		>2.4
	Corynebacteriaceae	X		>2.4
	Peptostreptococcaceae	X		>2.4
	Clostridiaceae	X		>2.4
	EB1003	X		>2.4
	Micrococcaceae	X		>2.4
	Bacillaceae	X		>2.4
	Streptococcaceae	X		>2.4
	Alicyclobacillaceae	X		>2.4
	Planococcaceae	X		>2.4
	Leuconostocaceae	X		>2.4
	Brevibacteriaceae	X		>2.4
	Dermatophilaceae	X		>2.4
	Aerococcaceae	X		>2.4
	Ruminococcaceae	X		>2.4
	Piscirickettsiaceae	X		>2.4
	AKYG885	X		>2.4
	Dolo_23	X		>2.4
	Prevotellaceae	X		>2.4
	Burkholderiaceae	X		>2.4
	Pseudomonadaceae	X		>3.6
	Enterobacteriaceae	X		>3.6
	Oxalobacteraceae	X		>3.6
	Methylocystaceae	X		>3.6

e)

Bacterial Taxonomic Level		Tree Species		Score
		Angiosperm	Gymnosperm	
Species	wittichi		X	>4.8
	suffuscus		X	>3.6
	elongata		X	>3.6
	ginsenosidimutans		X	>3.6
	daejeonensis		X	>3.6
	phenacola		X	>3.6
	Ellin122		X	>3.6
	ligni		X	>2.4
	ochracea		X	>2.4
	terrae		X	>2.4
	yabuuchiae		X	>2.4
	nitrogenifigens		X	>2.4
	aryamiense		X	>2.4
	acidophilus		X	>2.4
	fulvum		X	>2.4
	elegans		X	>2.4
	equi		X	>2.4
	facesium		X	>2.4
	bethesdensis		X	>2.4
	moabensis		X	>2.4
	stellata		X	>2.4
	hipranthacium		X	>2.4
	gracilis		X	>2.4
	leptocrescens		X	>1.2
	borealis		X	>1.2
	aquatile		X	>1.2
	azotifigens		X	>1.2
	globiformis		X	>1.2
	ochraceum		X	>1.2
	planiterum		X	>1.2
	rubrum		X	>1.2
	koreensis		X	>1.2
	piscis		X	>1.2
	caeruleus		X	>1.2
	rosea		X	>1.2
	changbaiensis		X	>1.2
	intestinalis	X		>1.2
	gibsonii	X		>1.2
	grandifolia	X		>1.2
	bowmanii	X		>1.2
	luteus	X		>1.2
	odysseyi	X		>1.2
	mendocina	X		>1.2
	subarcticum	X		>1.2
	versatilis	X		>1.2
	mirabilis	X		>1.2
	flexus	X		>1.2
	ginsengi	X		>2.4
	jejuensis	X		>2.4
	mixta	X		>2.4
	stutzeri	X		>2.4
	mitochondria	X		>2.4
	helvolus	X		>2.4
	Wolbachia endosymb. of C. parallelus	X		>2.4
	crescentiBT_1	X		>2.4
	aureus	X		>2.4
	pycnocarpon	X		>2.4
	vaccae	X		>2.4
	aerolata	X		>2.4
	rifensis	X		>2.4
	Kiedonobacteraceae	X		>2.4
	algae	X		>2.4
	morganii	X		>2.4
	guillouiae	X		>2.4
	intestini	X		>2.4
	diazotrophicus	X		>2.4
	ruralis	X		>3.6
	adhaesivum	X		>3.6
	agglomerans	X		>3.6
	cladoniiphilus	X		>3.6
	fragi	X		>4.8

ANNEX H

Supplementary Figure S3.1 Identity of the tree host species in each of the 54 combinations at the IDENT experiment in Montreal. Ab: *Abies balsamea*; Ap: *Acer platanoides*; Ar: *Acer rubrum*; As: *Acer saccharum*; Ba: *Betula alleghaniensis*; Ld: *Larix decidua*; Ll: *Larix laricina*; Bp: *Betula papyrifera*; Pa: *Picea abies*; Pg: *Picea glauca*; Po: *Picea omorika*; Pru: *Picea rubens*; Pre: *Pinus resinosa*; Pst: *Pinus strobus*; Psy: *Pinus sylvestris*; Qro: *Quercus robur*; Qru: *Quercus rubra*; To: *Thuja occidentalis*; Tc: *Tilia cordata.*

SPECIES RICHNESS	FUNCTIONAL DIVERSITY								
	0	1	2	3	4	5	6	7	8
1	19 host species								
2		Pru*Pre	Ll*Pst Ar*Ba Pre*Pst	Ba*Qru	Bp*Qru Ll*Pg	Pg*Pst Bp*Pst	Ab*Ar	As*Ll Ab*As Ar*To	As*To
4		Ab*Pg*Pru*Pre Ar*Ba*Ap*Tc	Ar*Ba*Bp*Qru Pg*Pru*Pre*Pst Ll*Pru*Pa*Po	Ba*Pru*Pre*Pst Pre*Qru*Ld*Psy	Ab*Bp*Ll*Pg Pg*To*Pa*Psy	Ab*As*Pg*Pru Pg*Pst*Qro*Tc	Ll*Pst*Qru*To Bp*Pru*Qro*Psy	Ar*Pa*Qru*To As*Bp*Pg*Pst Bp*As*Ap*Po	As*Ba*Pg*To Bp*To*Ap*Pa
12						Ab*Ar*As*Ba*Bp*Ll* Pg*Pru*Pre*Pst*Qru*To To*Qru*Pst*Pre*Pg*Bp* Ap*Pa*Po*Psy*Ld*Tc	As*Ba*Pru*Ar*Ab*Ll* Ap*Pa*Po*Psy*Qro*Tc		

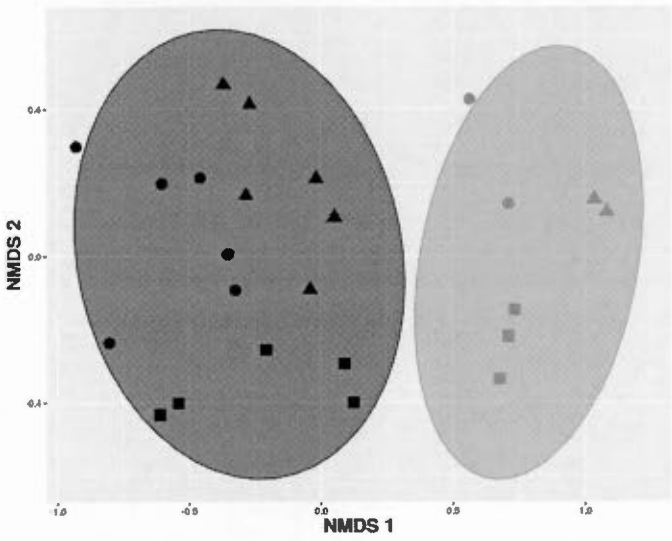
ANNEX I

Supplementary Table S4.1 Bacterial community structure explained by host species identity, environment, site nested in environment, and their interaction with host species identity (PERMANOVA on Bray-Curtis dissimilarities). The model explained a total of 73 % of the variation in bacterial community structure. [Subset of 24 samples that were reran from 3 species].

Variables	F-value	R^2	Pr(>F)
Host species identity	4.31	15.43	0.001
Environment	18.10	32.40	0.001
Environment * Site	4.08	7.30	0.001
Environment * Species	3.28	11.73	0.001
Environment * Site * Species	1.76	6.29	0.067

ANNEX J

Supplementary Figure S4.1 Non-metric multidimensional scaling (NMDS) ordination of variation in bacterial community structure of natural forest and urban tree phyllosphere. Ordination based on Bray-Curtis distances among 24 samples. Samples (points) are colored based on environment (light grey for urban trees and dark grey for natural forest) and shaped based on host species identity (circles for *Acer rubrum*; triangles for *Acer saccharum*; and squares for *Picea glauca*); ellipses indicate 1 standard deviation confidence intervals around samples from each host species.



ANNEX K

Supplementary Table S4.2 Taxonomy and mean relative abundance of the most abundant OTUs in the natural forest (*x-axis*) and urban (*y-axis*) environments shown at Figure 3.3. Only the OTUs with a sum of mean relative abundance in both environments of >0.5 % are shown. The table has been separated to improve visualization.

a)

PHYLUM	CLASS	ORDER	FAMILY	GENERA	SPECIES	MEAN RELATIVE ABUNDANCE IN FOREST SAMPLES	MEAN RELATIVE ABUNDANCE IN URBAN SAMPLES
Acidobacteria	Acidobacteriia	Acidobacteriales	Acidobacteriaceae	NA	NA	0.66	0.00
Actinobacteria	Actinobacteria	Actinomycetales	Frankiaceae	NA	NA	0.55	0.01
			Microbacteriaceae	Frondihabitans	cladoniiphilus	0.34	0.30
				NA	NA	1.05	0.97
			Micromonosporaceae	Couchioplanes	NA	0.80	0.48
Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	NA	NA	0.26	0.63
	Cytophagia	Cytophagales	Cytophagaceae	Hymenobacter	NA	1.40	1.90
						1.89	5.79
				Spirosoma	NA	0.15	0.38
						0.19	0.42
	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Mucilaginibacter	daejeonensis	0.26	0.89
				NA	NA	0.55	0.06

b)

PHYLUM	CLASS	ORDER	FAMILY	GENERA	SPECIES	MEAN RELATIVE ABUNDANCE IN FOREST SAMPLES	MEAN RELATIVE ABUNDANCE IN URBAN SAMPLES
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	NA	0.98	0.01
		Rhizobiales	Beijerinckiaceae	Beijerinckia	NA	5.56	0.17
			Methylobacteriaceae	Methylobacterium	adhaesivum	0.08	1.68
					NA	0.44	1.23
						0.74	1.57
			Methylocystaceae	NA	NA	0.44	0.27
						0.80	0.00
						12.07	4.80
						2.71	0.17
						0.86	0.02
						1.60	0.09
						0.44	0.31
			Rhizobiaceae	NA	NA	0.01	0.66
		Rhodobacterales	Rhodobacteraceae	Rubellimicrobium	NA	0.00	0.54
						0.23	0.49
						0.04	0.49
						0.00	1.94
		Rhodospirillales	Acetobacteraceae	NA	NA	1.14	0.68
						1.84	1.41
						0.85	2.16
						0.48	1.16
						0.65	0.00
		Sphingomonadales	Sphingomonadaceae	Novosphingobium	NA	0.12	0.97
				Sphingomonas	wittichii	0.02	1.16
						0.98	0.36
						0.12	2.19
					NA	0.66	0.09
						1.61	4.80
	Betaproteobacteria	Burkholderiales	Burkholderiaceae	NA	NA	0.32	1.28
			Comamonadaceae	NA	NA	0.08	1.57
			Oxalobacteraceae	NA	NA	0.11	0.60
						0.74	2.91
	Deltaproteobacteria	Myxococcales	Cystobacteraceae	Cystobacter	NA	3.31	2.59
				Erwinia	NA	0.56	0.53
	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	NA	NA	0.66	0.00
						1.07	3.71
		Pseudomonadales	Pseudomonadaceae	Pseudomonas	fragi	0.34	0.28
						1.30	0.89

c)

PHYLUM	CLASS	ORDER	FAMILY	GENERA	SPECIES	MEAN RELATIVE ABUNDANCE IN FOREST SAMPLES	MEAN RELATIVE ABUNDANCE IN URBAN SAMPLES
Thermi	Deinococci	Deinococcales	Deinococcaceae	Deinococcus	aquatilis	0.00	1.63
					NA	0.45	0.05
						0.16	1.12
						0.00	0.80

REFERENCES

Abanda-Nkpwatt, D., Müsch, M., Tschiersch, J., Boettner, M., & Schwab, W. (2006). Molecular interaction between Methylobacterium extorquens and seedlings: growth promotion, methanol consumption, and localization of the methanol emission site. *Journal of experimental botany*, *57*(15), 4025-4032.

Abrams, M. D., & Kubiske, M. E. (1990). Leaf structural characteristics of 31 hardwood and conifer tree species in central Wisconsin: influence of light regime and shade-tolerance rank. *Forest Ecology and Management*, *31*(4), 245-253.

Abril, A. B., Torres, P. A., & Bucher, E. H. (2005). The importance of phyllosphere microbial populations in nitrogen cycling in the Chaco semi-arid woodland. *Journal of tropical ecology*, *21*(01), 103-107.

Acinas, S. G., Sarma-Rupavtarm, R., Klepac-Ceraj, V., & Polz, M. F. (2005). PCR-induced sequence artifacts and bias: insights from comparison of two 16S rRNA clone libraries constructed from the same sample. *Applied and environmental microbiology*, *71*(12), 8966-8969.

Ackerly, D. D., Schwilk, D. W. & Webb, C. O. (2006). Niche evolution and adaptive radiation: testing the order of trait divergence. *Ecology* 87(sp7), S50-S61.

Afshinnekoo, E., Meydan, C., Chowdhury, S., Jaroudi, D., Boyer, C., Bernstein, N., Maritz, J. M., Reeves, D., Gandara, J., Chhangawala, S., & Ahsanuddin, S. (2015). Geospatial resolution of human and bacterial diversity with city-scale metagenomics. *Cell systems*, *1*(1), 72-87.

Agler, M. T., Ruhe, J., Kroll, S., Morhenn, C., Kim, S. T., Weigel, D., & Kemen, E. M. (2016). Microbial hub taxa link host and abiotic factors to plant microbiome variation. *PLoS Biol*, *14*(1), e1002352.

Alexander M. (1999) Biodegradation and Bioremediation. Academic Press, San Diego.

Allan, E., Manning, P., Alt, F., Binkenstein, J., Blaser, S., Blüthgen, N., Böhm, S., Grassein, F., Hölzel, N., Klaus, V. H., & Kleinebecker, T. (2015). Land use

intensification alters ecosystem multifunctionality via loss of biodiversity and changes to functional composition. *Ecology letters*, *18*(8), 834-843.

Allison, S. D., & Treseder, K. K. (2011). Climate change feedbacks to microbial decomposition in boreal soils. *Fungal Ecology*, *4*(6), 362-374.

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral ecology*, *26*(1), 32-46.

Anderson, M. J. (2006). Distance-based tests for homogeneity of multivariate dispersions. *Biometrics*, *62*(1), 245-253.

Anderson, M. J., Ellingsen, K. E., & McArdle, B. H. (2006). Multivariate dispersion as a measure of beta diversity. *Ecology letters*, *9*(6), 683-693.

Andrews, J. H., & Harris, R. F. (2000). The ecology and biogeography of microorganisms on plant surfaces. *Annual review of phytopathology*, *38*(1), 145-180.

Andrews, J. H., Spear, R. N., & Nordheim, E. V. (2002). Population biology of Aureobasidium pullulans on apple leaf surfaces. *Canadian journal of microbiology*, *48*(6), 500-513.

Arnold, A. E., & Lutzoni, F. (2007). Diversity and host range of foliar fungal endophytes: are tropical leaves biodiversity hotspots?. *Ecology*, *88*(3), 541-549.

Arnold, A. E., Mejía, L. C., Kyllo, D., Rojas, E. I., Maynard, Z., Robbins, N., & Herre, E. A. (2003). Fungal endophytes limit pathogen damage in a tropical tree. *Proceedings of the National Academy of Sciences*, *100*(26), 15649-15654.

Atamna-Ismaeel, N., Finkel, O. M., Glaser, F., Sharon, I., Schneider, R., Post, A. F., Spudich, J. L., von Mering, C., Vorholt, J. A., Iluz, D., & Béjà, O. (2012). Microbial rhodopsins on leaf surfaces of terrestrial plants. *Environmental microbiology*, *14*(1), 140-146.

Atamna-Ismaeel, N., Finkel, O. M., Glaser, F., von Mering, C., Vorholt, J. A., Koblížek, M., Belkin, S., & Béjà, O. (2012). Bacterial anoxygenic photosynthesis on plant leaf surfaces. *Environmental microbiology reports*, *4*(2), 209-216.

Barberán, A., Ladau, J., Leff, J. W., Pollard, K. S., Menninger, H. L., Dunn, R. R., & Fierer, N. (2015). Continental-scale distributions of dust-associated bacteria and fungi. *Proceedings of the National Academy of Sciences*, *112*(18), 5756-5761.

Beattie, G. A., & Marcell, L. M. (2002). Comparative dynamics of adherent and nonadherent bacterial populations on maize leaves. *Phytopathology*, *92*(9), 1015-1023.

Bennett, A. B., & Gratton, C. (2012). Local and landscape scale variables impact parasitoid assemblages across an urbanization gradient. *Landscape and Urban Planning*, *104*(1), 26-33.

Berendsen, R. L., Pieterse, C. M., & Bakker, P. A. (2012). The rhizosphere microbiome and plant health. *Trends in plant science*, *17*(8), 478-486.

Berg, G., Grube, M., Schloter, M., & Smalla, K. (2015). Unraveling the plant microbiome: looking back and future perspectives. *The plant microbiome and its importance for plant and human health*, 7.

Berg, G., Zachow, C., Müller, H., Philipps, J., & Tilcher, R. (2013). Next-generation bio-products sowing the seeds of success for sustainable agriculture. *Agronomy*, *3*(4), 648-656.

Biebl, H., Schwab-Hanisch, H., Spröer, C., & Lünsdorf, H. (2000). Propionispora vibrioides, nov. gen., nov. sp., a new gram-negative, spore-forming anaerobe that ferments sugar alcohols. *Archives of microbiology*, *174*(4), 239-247.

Björklöf, K., Nurmiaho-Lassila, E. L., Klinger, N., Haahtela, K., & Romantschuk, M. (2000). Colonization strategies and conjugal gene transfer of inoculated Pseudomonas syringae on the leaf surface. *Journal of applied microbiology*, *89*(3), 423-432.

Blair, R. B. (1999). Birds and butterflies along an urban gradient: surrogate taxa for assessing biodiversity?. *Ecological applications*, *9*(1), 164-170.

Bland, J. M., & Altman, D. G. (1995). Multiple significance tests: the Bonferroni method. *Bmj*, *310*(6973), 170.

Bodenhausen, N., Bortfeld-Miller, M., Ackermann, M., & Vorholt, J. A. (2014). A synthetic community approach reveals plant genotypes affecting the phyllosphere microbiota. *PLoS Genet*, *10*(4), e1004283.

Bodenhausen, N., Horton, M. W., & Bergelson, J. (2013). Bacterial communities associated with the leaves and the roots of Arabidopsis thaliana. *PloS one*, *8*(2), e56329.

Bowers, R. M., McLetchie, S., Knight, R., & Fierer, N. (2011). Spatial variability in airborne bacterial communities across land-use types and their relationship to the bacterial communities of potential source environments. *The ISME journal*, *5*(4), 601-612.

Bradley, D. J., Gilbert, G. S., & Martiny, J. B. (2008). Pathogens promote plant diversity through a compensatory response. *Ecology Letters*, *11*(5), 461-469.

Brandl, M. T., Quinones, B., & Lindow, S. E. (2001). Heterogeneous transcription of an indoleacetic acid biosynthetic gene in Erwinia herbicola on plant surfaces. *Proceedings of the National Academy of Sciences*, *98*(6), 3454-3459.

Brandl, M. T., & Lindow, S. E. (1998). Contribution of indole-3-acetic acid production to the epiphytic fitness of Erwinia herbicola. *Applied and environmental microbiology*, *64*(9), 3256-3263.

Briere, J. F., Pracros, P., Le Roux, A. Y., & Pierre, J. S. (1999). A novel rate model of temperature-dependent development for arthropods. *Environmental Entomology*, *28*(1), 22-29.

Bringel, F., & Couée, I. (2015). Pivotal roles of phyllosphere microorganisms at the interface between plant functioning and atmospheric trace gas dynamics. *Frontiers in microbiology*, *6*, 486.

Brunner, A. M., Busov, V. B., & Strauss, S. H. (2004). Poplar genome sequence: functional genomics in an ecologically dominant plant species. *Trends in plant science*, *9*(1), 49-56.

Bulgarelli, D., Rott, M., Schlaeppi, K., van Themaat, E. V. L., Ahmadinejad, N., Assenza, F., Rauf, P., Huettel, B., Reinhardt, R., Schmelzer, E., & Peplies, J. (2012). Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. *Nature*, *488*(7409), 91-95.

Bulgarelli, D., Schlaeppi, K., Spaepen, S., van Themaat, E. V. L., & Schulze-Lefert, P. (2013). Structure and functions of the bacterial microbiota of plants. *Annual review of plant biology*, *64*, 807-838.

Burke, C., Thomas, T., Lewis, M., Steinberg, P., & Kjelleberg, S. (2011). Composition, uniqueness and variability of the epiphytic bacterial community of the green alga Ulva australis. *The ISME journal*, *5*(4), 590-600.

Burns, R. M., & Barbara, H. (1990). Silvics of North America: 1. Conifers; 2. Hardwoods. Agriculture Handbook 654, U.S. Dept. of Agriculture, Forest Service, Washington, D.C. vol.2, 877p.

Burrows, S. M., Elbert, W., Lawrence, M. G., & Pöschl, U. (2009). Bacteria in the global atmosphere–Part 1: Review and synthesis of literature data for different ecosystems. *Atmospheric Chemistry and Physics*, *9*(23), 9263-9280.

Canada Weather Database. (1840-). [Database]. Accessed on the 25th of March 2014 from http://climate.weather.gc.ca/

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Pena, A. G., Goodrich, J. K., Gordon, J. I., & Huttley, G. A. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature methods*, *7*(5), 335-336.

Cardinale, B. J., Duffy, J. E., Gonzalez, A., Hooper, D. U., Perrings, C., Venail, P., Narwani, A., Mace, G. M., Tilman, D., Wardle, D. A. & Kinzig, A. P. (2012). Biodiversity loss and its impact on humanity. *Nature*, *486*(7401), 59-67.

Carrell, A. A., & Frank, A. C. (2014). Pinus flexilis and Picea engelmannii share a simple and consistent needle endophyte microbiota with a potential role in nitrogen fixation. *Frontiers in microbiology*, *5*, 333.

Chaffron, S., Rehrauer, H., Pernthaler, J., & von Mering, C. (2010). A global network of coexisting microbes from environmental and whole-genome sequence data. *Genome research*, *20*(7), 947-959.

Chase, J. M., & Leibold, M. A. (2003). *Ecological niches: linking classical and contemporary approaches*. University of Chicago Press.

Chave, J., Coomes, D., Jansen, S., Lewis, S. L., Swenson, N. G., & Zanne, A. E. (2009). Towards a worldwide wood economics spectrum. *Ecology letters*, *12*(4), 351-366.

Chelius, M. K., & Triplett, E. W. (2001). The Diversity of Archaea and Bacteria in Association with the Roots of Zea mays L. *Microbial ecology*, *41*(3), 252-263.

Chesson, P. (2000a). General theory of competitive coexistence in spatially-varying environments. *Theoretical population biology*, *58*(3), 211-237.

Chesson, P. (2000b). Mechanisms of maintenance of species diversity. *Annual review of Ecology and Systematics*, *31*, 343-366.

Chesson, P. L., & Warner, R. R. (1981). Environmental variability promotes coexistence in lottery competitive systems. *American Naturalist*, 923-943.

Cincotta, R. P., & Engelman, R. (2000). Natures place: human population and the future of biological diversity. Washington, D.C., Population Action International, 79p.

Claesson, M. J., Wang, Q., O'Sullivan, O., Greene-Diniz, R., Cole, J. R., Ross, R. P., & O'Toole, P. W. (2010). Comparison of two next-generation sequencing technologies for resolving highly complex microbiota composition using tandem variable 16S rRNA gene regions. *Nucleic acids research*, gkq873.

Clay, K., & Holah, J. (1999). Fungal endophyte symbiosis and plant diversity in successional fields. *Science*, *285*(5434), 1742-1744.

Cordier, T., Robin, C., Capdevielle, X., Desprez-Loustau, M. L., & Vacher, C. (2012a). Spatial variability of phyllosphere fungal assemblages: genetic distance predominates over geographic distance in a European beech stand (Fagus sylvatica). *Fungal ecology*, *5*(5), 509-520.

Cordier, T., Robin, C., Capdevielle, X., Fabreguettes, O., Desprez-Loustau, M. L., & Vacher, C. (2012b). The composition of phyllosphere fungal assemblages of European beech (Fagus sylvatica) varies significantly along an elevation gradient. *New Phytologist*, *196*(2), 510-519.

Corpe, W. A., & Rheem, S. (1989). Ecology of the methylotrophic bacteria on living leaf surfaces. *FEMS Microbiology Ecology*, *5*(4), 243-249.

Darzi, Y., Falony, G., Vieira-Silva, S., & Raes, J. (2015). Towards biome-specific analysis of meta-omics data. *The ISME journal*, *10*, 1025-1028.

Day, S. D., Eric Wiseman, P., Dickinson, S. B., & Roger Harris, J. (2010). Tree root ecology in the urban environment and implications for a sustainable rhizosphere. *Journal of Arboriculture*, *36*(5), 193.

De Costa, D. M., Rathnayake, R. M. P. S., De Costa, W. A. J. M., Kumari, W. M. D., & Dissanayake, D. M. N. (2006). Variation of phyllosphere microflora of different rice varieties in Sri Lanka and its relationship to leaf anatomical and physiological characters. *Journal of Agronomy and Crop Science*, *192*(3), 209-220.

Delmotte, N., Knief, C., Chaffron, S., Innerebner, G., Roschitzki, B., Schlapbach, R., von Mering, C., & Vorholt, J. A. (2009). Community proteogenomics reveals insights into the physiology of phyllosphere bacteria. *Proceedings of the National Academy of Sciences*, *106*(38), 16428-16433.

DeSantis, T. Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E. L., Keller, K., Huber, T., Dalevi, D., Hu, P., & Andersen, G. L. (2006). Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and environmental microbiology*, *72*(7), 5069-5072.

De Wit, R., & Bouvier, T. (2006). 'Everything is everywhere, but, the environment selects'; what did Baas Becking and Beijerinck really say?. *Environmental microbiology*, *8*(4), 755-758.

Dickie, J. (2008). Royal Botanical Gardens KEW. Seed Information Database (SID). Version 7.1.

Dickie, I. A., Guza, R. C., Krazewski, S. E., & Reich, P. B. (2004). Shared ectomycorrhizal fungi between a herbaceous perennial (Helianthemum bicknellii) and oak (Quercus) seedlings. *New Phytologist*, *164*(2), 375-382.

Dulla, G., & Lindow, S. E. (2008). Quorum size of Pseudomonas syringae is small and dictated by water availability on the leaf surface. *Proceedings of the National Academy of Sciences*, *105*(8), 3082-3087.

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, *26*(19), 2460-2461.

Fadrosh, D. W., Ma, B., Gajer, P., Sengamalay, N., Ott, S., Brotman, R. M., & Ravel, J. (2014). An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform. *Microbiome*, *2*(1), 1.

Fargione, J., Tilman, D., Dybzinski, R., Lambers, J. H. R., Clark, C., Harpole, W. S., Knops, J. M., Reich, P. B. & Loreau, M. (2007). From selection to complementarity: shifts in the causes of biodiversity–productivity relationships in a long-term biodiversity experiment. *Proceedings of the Royal Society of London B: Biological Sciences*, *274*(1611), 871-876.

Farrar, J. L. (1996). *Les arbres du Canada*. Les Editions Fides. Service canadien des forêts, Ressources naturelles Canada, Ottawa, Canada, 488p.

Finkel, O. M., Burch, A. Y., Lindow, S. E., Post, A. F., & Belkin, S. (2011). Geographical location determines the population structure in phyllosphere microbial communities of a salt-excreting desert tree. *Applied and environmental microbiology*, *77*(21), 7647-7655.

Finkel, O. M., Burch, A. Y., Elad, T., Huse, S. M., Lindow, S. E., Post, A. F., & Belkin, S. (2012). Distance-decay relationships partially determine diversity patterns of phyllosphere bacteria on Tamrix trees across the Sonoran Desert. *Applied and environmental microbiology*, *78*(17), 6187-6193.

Flückiger, W., & Braun, S. (1997, September). Stress factors of urban trees and their relevance for vigour and predisposition for parasite attacks. In *International Symposium on Urban Tree Health 496* (pp. 325-334).

Flückiger, W., & Braun, S. (1999). Nitrogen and its effect on growth, nutrient status and parasite attacks in beech and Norway spruce. *Water, Air, and Soil Pollution*, *116*(1-2), 99-110.

Flynn, D. F., Mirotchnick, N., Jain, M., Palmer, M. I., & Naeem, S. (2011). Functional and phylogenetic diversity as predictors of biodiversity–ecosystem-function relationships. *Ecology*, *92*(8), 1573-1581.

Fravel, D. R. (1988). Role of antibiosis in the biocontrol of plant diseases*. *Annual review of phytopathology*, *26*(1), 75-91.

Friesen, M. L., Porter, S. S., Stark, S. C., von Wettberg, E. J., Sachs, J. L., & Martinez-Romero, E. (2011). Microbially mediated plant functional traits. *Annual Review of Ecology, Evolution, and Systematics*, *42*, 23-46.

Fürnkranz, M., Wanek, W., Richter, A., Abell, G., Rasche, F., & Sessitsch, A. (2008). Nitrogen fixation by phyllosphere bacteria associated with higher plants and their colonizing epiphytes of a tropical lowland rainforest of Costa Rica. *The ISME Journal*, *2*(5), 561-570.

Gause, G. F. (1934). Experimental analysis of Vito Volterra's mathematical theory of the struggle for existence. *Science*, *79*(2036), 16-17.

Gilbert, G. S. (2002). Evolutionary ecology of plant diseases in natural ecosystems. *Annual Review of Phytopathology*, *40*(1), 13-43.

Giloteaux, L., Goodrich, J. K., Walters, W. A., Levine, S. M., Ley, R. E., & Hanson, M. R. (2016). Reduced diversity and altered composition of the gut microbiome in individuals with myalgic encephalomyelitis/chronic fatigue syndrome. *Microbiome*, *4*(1), 30.

Glickmann, E., Gardan, L., Jacquet, S., Hussain, S., Elasri, M., Petit, A., & Dessaux, Y. (1998). Auxin production is a common feature of most pathovars of Pseudomonas syringae. *Molecular plant-microbe interactions*, *11*(2), 156-162.

Gloor, G. B., Hummelen, R., Macklaim, J. M., Dickson, R. J., Fernandes, A. D., MacPhee, R., & Reid, G. (2010). Microbiome profiling by illumina sequencing of combinatorial sequence-tagged PCR products. *PloS one*, *5*(10), e15406.

Goel, N. S., Maitra, S. C., & Montroll, E. W. (1971). On the Volterra and other nonlinear models of interacting populations. *Reviews of modern physics*, *43*(2), 231.

Gomes, V., Ribeiro, R., & Carretero, M. A. (2011). Effects of urban habitat fragmentation on common small mammals: species versus communities. *Biodiversity and Conservation*, *20*(14), 3577-3590.

Grace, J., Malcolm, D. C., & Bradbury, I. K. (1975). The effect of wind and humidity on leaf diffusive resistance in Sitka spruce seedlings. *Journal of Applied Ecology*, 931-940.

Gravel, D., Canard, E., Guichard, F., & Mouquet, N. (2011). Persistence increases with diversity and connectance in trophic metacommunities. *PloS one*, *6*(5), e19374.

Gravel, D., Canham, C. D., Beaudet, M., & Messier, C. (2006). Reconciling niche and neutrality: the continuum hypothesis. *Ecology letters*, *9*(4), 399-409.

Green, J. L., Holmes, A. J., Westoby, M., Oliver, I., Briscoe, D., Dangerfield, M., Gillings, M., & Beattie, A. J. (2004). Spatial scaling of microbial eukaryote diversity. *Nature*, *432*(7018), 747-750.

Gross, K., Cardinale, B. J., Fox, J. W., Gonzalez, A., Loreau, M., Polley, H. W., Reich, P. B., & Van Ruijven, J. (2014). Species richness and the temporal stability of biomass production: a new analysis of recent biodiversity experiments. *The American Naturalist*, *183*(1), 1-12.

Gunasekera, T. S., & Sundin, G. W. (2006). Role of nucleotide excision repair and photoreactivation in the solar UVB radiation survival of Pseudomonas syringae pv. syringae B728a. *Journal of applied microbiology*, *100*(5), 1073-1083.

Haas, D., & Défago, G. (2005). Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nature Reviews Microbiology*, *3*(4), 307-319.

Hallmann, J., Quadt-Hallmann, A., Mahaffee, W. F., & Kloepper, J. W. (1997). Bacterial endophytes in agricultural crops. *Canadian Journal of Microbiology*, *43*(10), 895-914.

Hanski, I., von Hertzen, L., Fyhrquist, N., Koskinen, K., Torppa, K., Laatikainen, T., Karisola, P., Auvinen, P., Paulin, L., Mäkelä, M. J., & Vartiainen, E. (2012). Environmental biodiversity, human microbiota, and allergy are interrelated. *Proceedings of the National Academy of Sciences*, *109*(21), 8334-8339.

Hantsch, L., Bien, S., Radatz, S., Braun, U., Auge, H., & Bruelheide, H. (2014). Tree diversity and the role of non-host neighbour tree species in reducing fungal pathogen infestation. *Journal of Ecology*, *102*(6), 1673-1687.

Hantsch, L., Braun, U., Scherer-Lorenzen, M., & Bruelheide, H. (2013). Species richness and species identity effects on occurrence of foliar fungal pathogens in a tree diversity experiment. *Ecosphere*, *4*(7), 1-12.

Hardin, G. (1960). The competitive exclusion principle. *Science*, *131*(3409), 1292-1297.

Hart, M. A., & Sailor, D. J. (2009). Quantifying the influence of land-use and surface characteristics on spatial variability in the urban heat island. *Theoretical and Applied Climatology*, *95*(3-4), 397-406.

Hartig, T., Mitchell, R., De Vries, S., & Frumkin, H. (2014). Nature and health. *Annual Review of Public Health*, *35*, 207-228.

Hata, K., Futai, K., & Tsuda, M. (1998). Seasonal and needle age-dependent changes of the endophytic mycobiota in Pinus thunbergii and Pinus densiflora needles. *Canadian Journal of Botany*, *76*(2), 245-250.

Hautier, Y., Tilman, D., Isbell, F., Seabloom, E. W., Borer, E. T., & Reich, P. B. (2015). Anthropogenic environmental changes affect ecosystem stability via biodiversity. *Science*, *348*(6232), 336-340.

Herre, E. A., Mejía, L. C., Kyllo, D. A., Rojas, E., Maynard, Z., Butler, A., & Van Bael, S. A. (2007). ecological implications of anti-pathogen effects of tropical fungal endophytes and mycorrhizae. *Ecology*, *88*(3), 550-558.

Hibbett, D. S., Ohman, A., Glotzer, D., Nuhn, M., Kirk, P., & Nilsson, R. H. (2011). Progress in molecular and morphological taxon discovery in Fungi and options for formal classification of environmental sequences. *Fungal Biology Reviews*, *25*(1), 38-47.

Hibbing, M. E., Fuqua, C., Parsek, M. R., & Peterson, S. B. (2010). Bacterial competition: surviving and thriving in the microbial jungle. *Nature Reviews Microbiology*, *8*(1), 15-25.

Hirano, S. S., & Upper, C. D. (2000). Bacteria in the Leaf Ecosystem with Emphasis onPseudomonas syringae—a Pathogen, Ice Nucleus, and Epiphyte. *Microbiology and molecular biology reviews*, *64*(3), 624-653.

Hochberg, Y. (1988). A sharper Bonferroni procedure for multiple tests of significance. *Biometrika*, *75*(4), 800-802.

Horton, M. W., Bodenhausen, N., Beilsmith, K., Meng, D., Muegge, B. D., Subramanian, S., Vetter, M. M., Vilhjálmsson, B. J., Nordborg, M., Gordon, J. I., & Bergelson, J. (2014). Genome-wide association study of Arabidopsis thaliana leaf microbial community. *Nature communications*, *5*.

Horton, T. R., Bruns, T. D., & Parker, V. T. (1999). Ectomycorrhizal fungi associated with Arctostaphylos contribute to Pseudotsuga menziesii establishment. *Canadian Journal of Botany*, *77*(1), 93-102.

Hubbell, S. P. (2001). *The unified neutral theory of biodiversity and biogeography (MPB-32)* (Vol. 32). Princeton University Press, ISBN 9780691021287, 392p.

Hugenholtz, P., Goebel, B. M., & Pace, N. R. (1998). Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. *Journal of bacteriology*, *180*(18), 4765-4774.

Humphrey, P. T., Nguyen, T. T., Villalobos, M. M., & Whiteman, N. K. (2014). Diversity and abundance of phyllosphere bacteria are linked to insect herbivory. *Molecular ecology*, *23*(6), 1497-1515.

Hunter, P. J., Hand, P., Pink, D., Whipps, J. M., & Bending, G. D. (2010). Both leaf properties and microbe-microbe interactions influence within-species variation in bacterial population diversity and structure in the lettuce (Lactuca species) phyllosphere. *Applied and environmental microbiology*, *76*(24), 8117-8125.

Iguchi, H., Sato, I., Sakakibara, M., Yurimoto, H., & Sakai, Y. (2012). Distribution of methanotrophs in the phyllosphere. *Bioscience, biotechnology, and biochemistry*, *76*(8), 1580-1583.

Inácio, J., Pereira, P., Carvalho, D. M., Fonseca, A., Amaral-Collaço, M. T., & Spencer-Martins, I. (2002). Estimation and diversity of phylloplane mycobiota on selected plants in a mediterranean–type ecosystem in Portugal. *Microbial Ecology*, *44*(4), 344-353.

Innerebner, G., Knief, C., & Vorholt, J. A. (2011). Protection of Arabidopsis thaliana against leaf-pathogenic Pseudomonas syringae by Sphingomonas strains in a controlled model system. *Applied and environmental microbiology*, *77*(10), 3202-3210.

Isbell, F., Calcagno, V., Hector, A., Connolly, J., Harpole, W. S., Reich, P. B., Scherer-Lorenzen, M., Schmid, B., Tilman, D., van Ruijven, J., & Weigelt, A. (2011). High plant diversity is needed to maintain ecosystem services. *Nature*, *477*(7363), 199-202.

Jackson, C. R., & Denney, W. C. (2011). Annual and seasonal variation in the phyllosphere bacterial community associated with leaves of the southern magnolia (Magnolia grandiflora). *Microbial ecology*, *61*(1), 113-122.

Jacobs, J. L., Carroll, T. L., & Sundin, G. W. (2005). The role of pigmentation, ultraviolet radiation tolerance, and leaf colonization strategies in the epiphytic survival of phyllosphere bacteria. *Microbial Ecology*, *49*(1), 104-113.

Jacobs, J. L., & Sundin, G. W. (2001). Effect of solar UV-B radiation on a phyllosphere bacterial community. *Applied and environmental microbiology*, *67*(12), 5488-5496.

Janssen, P. H. (2006). Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. *Applied and environmental microbiology*, *72*(3), 1719-1728.

Jenerette, G. D., Harlan, S. L., Stefanov, W. L., & Martin, C. A. (2011). Ecosystem services and urban heat riskscape moderation: water, green spaces, and social inequality in Phoenix, USA. *Ecological Applications*, *21*(7), 2637-2651.

Jessup, C. M., Kassen, R., Forde, S. E., Kerr, B., Buckling, A., Rainey, P. B., & Bohannan, B. J. (2004). Big questions, small worlds: microbial model systems in ecology. *Trends in Ecology & Evolution*, *19*(4), 189-197.

Jo, Y., Cho, J. K., Choi, H., Chu, H., Lian, S., & Cho, W. K. (2015). Bacterial communities in the phylloplane of Prunus species. *Journal of basic microbiology*, *55*(4), 504-508.

Johnson, P. C. (2014). Extension of Nakagawa & Schielzeth's R2GLMM to random slopes models. *Methods in Ecology and Evolution*, *5*(9), 944-946.

Jumpponen, A., & Jones, K. L. (2009). Massively parallel 454 sequencing indicates hyperdiverse fungal communities in temperate Quercus macrocarpa phyllosphere. *New Phytologist*, *184*(2), 438-448.

Jumpponen, A., & Jones, K. L. (2010). Seasonally dynamic fungal communities in the Quercus macrocarpa phyllosphere differ between urban and nonurban environments. *New Phytologist*, *186*(2), 496-513.

Kadivar, H., & Stapleton, A. E. (2003). Ultraviolet radiation alters maize phyllosphere bacterial diversity. *Microbial ecology*, *45*(4), 353-361.

Kalnay, E., & Cai, M. (2003). Impact of urbanization and land-use change on climate. *Nature*, *423*(6939), 528-531.

Kaneko, M., Hwang, E. I., Ohnishi, Y., & Horinouchi, S. (2003). Heterologous production of flavanones in Escherichia coli: potential for combinatorial biosynthesis of flavonoids in bacteria. *Journal of Industrial Microbiology and Biotechnology*, *30*(8), 456-461.

Kaye, J. P., Groffman, P. M., Grimm, N. B., Baker, L. A., & Pouyat, R. V. (2006). A distinct urban biogeochemistry?. *Trends in Ecology & Evolution*, *21*(4), 192-199.

Kembel, S. W., Cowan, P. D., Helmus, M. R., Cornwell, W. K., Morlon, H., Ackerly, D. D., Blomberg, S. P., & Webb, C. O. (2010). Picante: R tools for integrating phylogenies and ecology. *Bioinformatics*, *26*(11), 1463-1464.

Kembel, S. W., Jones, E., Kline, J., Northcutt, D., Stenson, J., Womack, A. M., Bohannan, B. J. M., Brown, G. Z., & Green, J. L. (2012). Architectural design influences the diversity and structure of the built environment microbiome. *The ISME journal*, *6*(8), 1469-1479.

Kembel, S. W., & Mueller, R. C. (2014). Plant traits and taxonomy drive host associations in tropical phyllosphere fungal communities 1. *Botany*, *92*(4), 303-311.

Kembel, S. W., O'Connor, T. K., Arnold, H. K., Hubbell, S. P., Wright, S. J., & Green, J. L. (2014). Relationships between phyllosphere bacterial communities and plant functional traits in a neotropical forest. *Proceedings of the National Academy of Sciences*, *111*(38), 13715-13720.

Khanna, S., Pardi, D. S., Kelly, C. R., Kraft, C. S., Dhere, T., Henn, M. R., Lombardo, M. J., Vulic, M., Ohsumi, T., Winkler, J., & Pindar, C. (2016). A novel microbiome therapeutic increases gut microbial diversity and prevents recurrent Clostridium difficile infection. *Journal of Infectious Diseases*, jiv766.

Kim, M., Heo, E., Kang, H., & Adams, J. (2013). Changes in soil bacterial community structure with increasing disturbance frequency. *Microbial ecology*, *66*(1), 171-181.

Kim, M., Singh, D., Lai-Hoe, A., Go, R., Rahim, R. A., Ainuddin, A. N., Chun, J., & Adams, J. M. (2012). Distinctive phyllosphere bacterial communities in tropical trees. *Microbial ecology*, *63*(3), 674-681.

King, G. M. (2014). Urban microbiomes and urban ecology: How do microbes in the built environment affect human sustainability in cities?. *Journal of Microbiology*, *52*(9), 721-728.

Kirchman, D. L. (2012). *Processes in microbial ecology*. Oxford University Press, New York, United States of America, 311p.

Knapp, S., Kühn, I., Schweiger, O., & Klotz, S. (2008). Challenging urban species diversity: contrasting phylogenetic patterns across plant functional groups in Germany. *Ecology Letters*, *11*(10), 1054-1064.

Knief, C., Frances, L., Cantet, F., & Vorholt, J. A. (2008). Cultivation-independent characterization of Methylobacterium populations in the plant phyllosphere by automated ribosomal intergenic spacer analysis. *Applied and environmental microbiology*, *74*(7), 2218-2228.

Knief, C., Frances, L., & Vorholt, J. A. (2010a). Competitiveness of diverse Methylobacterium strains in the phyllosphere of Arabidopsis thaliana and identification of representative models, including M. extorquens PA1. *Microbial ecology*, *60*(2), 440-452.

Knief, C., Ramette, A., Frances, L., Alonso-Blanco, C., & Vorholt, J. A. (2010b). Site and plant species are important determinants of the Methylobacterium community composition in the plant phyllosphere. *The ISME journal*, *4*(6), 719-728.

Knief, C., Delmotte, N., Chaffron, S., Stark, M., Innerebner, G., Wassmann, R., von Mering, C., & Vorholt, J. A. (2012). Metaproteogenomic analysis of microbial communities in the phyllosphere and rhizosphere of rice. *The ISME journal*, *6*(7), 1378-1390.

Köberl, M., Schmidt, R., Ramadan, E. M., Bauer, R., & Berg, G. (2015). The microbiome of medicinal plants: diversity and importance for plant growth,

quality, and health. *The plant microbiome and its importance for plant and human health*, 45.

Koskella, B., & Parr, N. (2015). The evolution of bacterial resistance against bacteriophages in the horse chestnut phyllosphere is general across both space and time. *Philosophical Transactions of the Royal Society B*, *370*(1675), 20140297.

Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., & Schloss, P. D. (2013). Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Applied and environmental microbiology*, *79*(17), 5112-5120.

Krajina, V. J., Klinka, K., & Worrall, J. (1982). *Distribution and ecological characteristics of trees and shrubs of British Columbia.* University of British Columbia, Faculty of Forestry, Vancouver, Canada, 131p.

Kühn, I., Brandl, R., & Klotz, S. (2004). The flora of German cities is naturally species rich. *Evolutionary ecology research*, *6*(5), 749-764.

Laforest-Lapointe, I., Messier, C., & Kembel, S. W. (2016). Host species identity, site and time drive temperate tree phyllosphere bacterial community structure. *Microbiome*, *4*(1), 27.

Laforest-Lapointe, I., Messier, C., & Kembel, S. W. (2016). Tree phyllosphere bacterial communities: exploring the magnitude of intra-and inter-individual variation among host species. *PeerJ*, *4*, e2367.

Lambais, M. R., Crowley, D. E., Cury, J. C., Büll, R. C., & Rodrigues, R. R. (2006). Bacterial diversity in tree canopies of the Atlantic forest. *Science*, *312*(5782), 1917-1917.

Lambais, M. R., Lucheta, A. R., & Crowley, D. E. (2014). Bacterial community assemblages associated with the phyllosphere, dermosphere, and rhizosphere of tree species of the Atlantic forest are host taxon dependent. *Microbial ecology*, *68*(3), 567-574.

Laliberté, E., & Legendre, P. (2010). A distance-based framework for measuring functional diversity from multiple traits. *Ecology*, *91*(1), 299-305.

Last, F. T. (1955). Seasonal incidence of Sporobolomyces on cereal leaves. *Transactions of the British Mycological Society*, *38*(3), 221-239.

Last, F. T., & Deighton, F. C. (1965). The non-parasitic microflora on the surfaces of living leaves. *Transactions of the British Mycological Society*, *48*(1), 83-IN12.

Lau, J. A., & Lennon, J. T. (2012). Rapid responses of soil microorganisms improve plant fitness in novel environments. *Proceedings of the National Academy of Sciences*, *109*(35), 14058-14062.

Lavorel, S., Grigulis, K., McIntyre, S., Williams, N. S., Garden, D., Dorrough, J., Berman, S., Quétier, F., Thébault, A., & Bonis, A. (2008). Assessing functional diversity in the field–methodology matters!. *Functional Ecology*, *22*(1), 134-147.

Leff, J. W., Del Tredici, P., Friedman, W. E., & Fierer, N. (2015). Spatial structuring of bacterial communities within individual Ginkgo biloba trees. *Environmental microbiology*, *17*(7), 2352-2361.

Legendre, P., & Legendre, L. (1998). Numerical Ecology, Volume 24, (Developments in Environmental Modelling), Elsevier, 1006p.

Leveau, J. H., & Lindow, S. E. (2001). Appetite of an epiphyte: quantitative monitoring of bacterial sugar consumption in the phyllosphere. *Proceedings of the National Academy of Sciences*, *98*(6), 3446-3453.

Leveau, J. H. J., & Tech, J. J. (2010, October). Grapevine microbiomics: bacterial diversity on grape leaves and berries revealed by high-throughput sequence analysis of 16S rRNA amplicons. In *International Symposium on Biological Control of Postharvest Diseases: Challenges and Opportunities 905* (pp. 31-42).

Liang, J., Crowther, T. W., Picard, N., Wiser, S., Zhou, M., Alberti, G., Schulze, E. D., McGuire, A. D., Bozzato, F., Pretzsch, H., & de-Miguel, S. (2016). Positive biodiversity-productivity relationship predominant in global forests. *Science*, *354*(6309), aaf8957.

Lichtenthaler, H. K., Buschmann, C., Döll, M., Fietz, H. J., Bach, T., Kozel, U., Meier, D., & Rahmsdorf, U. (1981). Photosynthetic activity, chloroplast ultrastructure, and leaf characteristics of high-light and low-light plants and of sun and shade leaves. *Photosynthesis research*, *2*(2), 115-141.

Lighthart, B., Shaffer, B. T., Frisch, A. S., & Paterno, D. (2009). Atmospheric culturable bacteria associated with meteorological conditions at a summer-time site in the mid-Willamette Valley, Oregon. *Aerobiologia*, *25*(4), 285-295.

Lilley, A. K., Bailey, M. J., Day, M. J., & Fry, J. C. (1996). Diversity of mercury resistance plasmids obtained by exogenous isolation from the bacteria of sugar beet in three successive years. *FEMS microbiology ecology*, *20*(4), 211-227.

Lindow, S. E., Arny, D. C., & Upper, C. D. (1978). Distribution of ice nucleation-active bacteria on plants in nature. *Applied and environmental microbiology*, *36*(6), 831-838.

Lindow, S. E., & Brandl, M. T. (2003). Microbiology of the phyllosphere. *Applied and environmental microbiology*, *69*(4), 1875-1883.

Lindow, S. E., & Leveau, J. H. (2002). Phyllosphere microbiology. *Current Opinion in Biotechnology*, *13*(3), 238-243.

Lopez-Velasco, G., Welbaum, G. E., Boyer, R. R., Mane, S. P., & Ponder, M. A. (2011). Changes in spinach phylloepiphytic bacteria communities following

minimal processing and refrigerated storage described using pyrosequencing of 16S rRNA amplicons. *Journal of applied microbiology*, *110*(5), 1203-1214.

Lotka, A. J. (1910). Contribution to the theory of periodic reactions. *The Journal of Physical Chemistry*, *14*(3), 271-274.

Lounibos, L. P. (2002). Invasions by insect vectors of human disease. *Annual review of entomology*, *47*(1), 233-266.

Lozupone, C., Hamady, M., & Knight, R. (2006). UniFrac–an online tool for comparing microbial community diversity in a phylogenetic context. *BMC bioinformatics*, *7*(1), 1.

Lymperopoulou, D. S., Adams, R. I., & Lindow, S. E. (2016). Contribution of vegetation to the microbial composition of nearby outdoor air. *Applied and environmental microbiology*, AEM-00610.

Maas, J., Verheij, R. A., Groenewegen, P. P., De Vries, S., & Spreeuwenberg, P. (2006). Green space, urbanity, and health: how strong is the relation?. *Journal of epidemiology and community health*, *60*(7), 587-592.

MacArthur, R., & Levins, R. (1967). The limiting similarity, convergence, and divergence of coexisting species. *American naturalist*, 377-385.

Manulis, S., Haviv-Chesner, A., Brandl, M. T., Lindow, S. E., & Barash, I. (1998). Differential involvement of indole-3-acetic acid biosynthetic pathways in pathogenicity and epiphytic fitness of Erwinia herbicola pv. gypsophilae. *Molecular Plant-Microbe Interactions*, *11*(7), 634-642.

Martiny, J. B. H., Bohannan, B. J., Brown, J. H., Colwell, R. K., Fuhrman, J. A., Green, J. L., Horner-Devine, M. C., Kane, M., Krumins, J. A., Kuske, C. R., & Morin, P. J. (2006). Microbial biogeography: putting microorganisms on the map. *Nature Reviews Microbiology*, *4*(2), 102-112.

Mattson, W. J., & Haack, R. A. (1987). The role of drought in outbreaks of plant-eating insects. *BioScience*, *37*(2), 110-118.

McGuire, K. L., & Treseder, K. K. (2010). Microbial communities and their relevance for ecosystem models: decomposition as a case study. *Soil Biology and Biochemistry*, *42*(4), 529-535.

McKinney, M. L. (2002). Urbanization, biodiversity, and conservation the impacts of urbanization on native species are poorly studied, but educating a highly urbanized human population about these impacts can greatly improve species conservation in all ecosystems. *BioScience*, *52*(10), 883-890.

Meaden, S., Metcalf, C. J. E., & Koskella, B. (2016). The effects of host age and spatial location on bacterial community composition in the English Oak tree (Quercus robur). *Environmental microbiology reports*, *8*(5), 649-658.

Medina-Martínez, M. S., Allende, A., Barberá, G. G., & Gil, M. I. (2015). Climatic variations influence the dynamic of epiphyte bacteria of baby lettuce. *Food Research International*, *68*, 54-61.

Meadow, J. F., Altrichter, A. E., Kembel, S. W., Kline, J., Mhuireach, G., Moriyama, M., Northcutt, D., O'connor, T. K., Womack, A. M., Brown, G. Z., & Green, J. L. (2014a). Indoor airborne bacterial communities are influenced by ventilation, occupancy, and outdoor air source. *Indoor Air*, *24*(1), 41-48.

Meadow, J. F., Altrichter, A. E., Kembel, S. W., Moriyama, M., O'Connor, T. K., Womack, A. M., Brown, G. Z., Green, J. L., & Bohannan, B. J. (2014b). Bacterial communities on classroom surfaces vary with human contact. *Microbiome*, *2*(1), 1.

Medina-Martínez, M. S., Allende, A., Barberá, G. G., & Gil, M. I. (2015). Climatic variations influence the dynamic of epiphyte bacteria of baby lettuce. *Food Research International*, *68*, 54-61.

Meineke, E. K., Dunn, R. R., Sexton, J. O., & Frank, S. D. (2013). Urban warming drives insect pest abundance on street trees. *PLoS One*, *8*(3), e59687.

Mendes, R., Kruijt, M., de Bruijn, I., Dekkers, E., van der Voort, M., Schneider, J. H., Piceno, Y. M., DeSantis, T. Z., Andersen, G. L., Bakker, P. A., & Raaijmakers, J. M. (2011). Deciphering the rhizosphere microbiome for disease-suppressive bacteria. *Science*, *332*(6033), 1097-1100.

Mercier, J., & Lindow, S. E. (2000). Role of leaf surface sugars in colonization of plants by bacterial epiphytes. *Applied and Environmental Microbiology*, *66*(1), 369-374.

Mhuireach, G., Johnson, B. R., Altrichter, A. E., Ladau, J., Meadow, J. F., Pollard, K. S., & Green, J. L. (2016). Urban greenness influences airborne bacterial community composition. *Science of The Total Environment*, *571*, 680-687.

Miller, W. G., Brandl, M. T., Quiñones, B., & Lindow, S. E. (2001). Biological sensor for sucrose availability: relative sensitivities of various reporter genes. *Applied and environmental microbiology*, *67*(3), 1308-1317.

Mittler, R. (2006). Abiotic stress, the field environment and stress combination. *Trends in plant science*, *11*(1), 15-19.

Monier, J. M., & Lindow, S. E. (2003). Differential survival of solitary and aggregated bacterial cells promotes aggregate formation on leaf surfaces. *Proceedings of the National Academy of Sciences*, *100*(26), 15977-15982.

Monier, J. M., & Lindow, S. E. (2004). Frequency, size, and localization of bacterial aggregates on bean leaf surfaces. *Applied and environmental microbiology*, *70*(1), 346-355.

Monod, J. (1949). The growth of bacterial cultures. *Annual Reviews in Microbiology*, *3*(1), 371-394.

Morris, C. E., Barnes, M. B., McLean, R. J. C., Lindow, S. E., Hecht-Poinar, E. I., & Elliott, V. J. (2002). Biofilms on leaf surfaces: implications for the biology, ecology and management of populations of epiphytic bacteria. *Phyllosphere microbiology*, 139-155.

Morris, C. E., Kinkel, L. L., Lindow, S. E., Hecht-Poinar, E. I., & Elliott, V. J. (2002). Fifty years of phyllosphere microbiology: significant contributions to research in related fields. *Phyllosphere microbiology*, 365-375.

Moyes, A. B., Kueppers, L. M., Pett-Ridge, J., Carper, D. L., Vandehey, N., O'Neil, J., & Frank, A. C. (2016). Evidence for foliar endophytic nitrogen fixation in a widely distributed subalpine conifer. *New Phytologist*. 210: 657–668.

Maignien, L., DeForce, E. A., Chafee, M. E., Eren, A. M., & Simmons, S. L. (2014). Ecological succession and stochastic variation in the assembly of Arabidopsis thaliana phyllosphere communities. *MBio*, *5*(1), e00682-13.

Müller, T., & Ruppel, S. (2014). Progress in cultivation-independent phyllosphere microbiology. *FEMS microbiology ecology*, *87*(1), 2-17.

Müller, D. B., Vogel, C., Bai, Y., & Vorholt, J. A. (2016). The Plant Microbiota: Systems Biology Insights and Perspectives. *Annual Review of Genetics*, *50*(1).

Nakagawa, S., & Schielzeth, H. (2013). A general and simple method for obtaining R2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, *4*(2), 133-142.

Newton, A. C., Gravouil, C., & Fountaine, J. M. (2010). Managing the ecology of foliar pathogens: ecological tolerance in crops. *Annals of Applied Biology*, *157*(3), 343-359.

Niinemets, Ü. (2010a). Mild versus severe stress and BVOCs: thresholds, priming and consequences. *Trends in plant science*, *15*(3), 145-153.

Niinemets, Ü. (2010b). Responses of forest trees to single and multiple environmental stresses from seedlings to mature plants: past stress history, stress interactions, tolerance and acclimation. *Forest Ecology and Management*, *260*(10), 1623-1639.

Niinemets, Ü., & Valladares, F. (2006). Tolerance to shade, drought, and waterlogging of temperate Northern Hemisphere trees and shrubs. *Ecological monographs*, *76*(4), 521-547.

Nock, C. A., Paquette, A., Follett, M., Nowak, D. J., & Messier, C. (2013). Effects of urbanization on tree species functional diversity in eastern North America. *Ecosystems*, *16*(8), 1487-1497.

Normand, P. (2006). The families frankiaceae, geodermatophilaceae, acidothermaceae and sporichthyaceae. In *The Prokaryotes* (pp. 669-681). Springer New York.

Normander, B., Christensen, B. B., Molin, S., & Kroer, N. (1998). Effect of bacterial distribution and activity on conjugal gene transfer on the phylloplane of the bush

bean (Phaseolus vulgaris). *Applied and Environmental Microbiology*, *64*(5), 1902-1909.

Nowak, D. J., & McBride, J. R. (1991). Comparison of Monterey pine stress in urban and natural forests. *Journal of Environmental Management*, *32*(4), 383-395.

O'Connor, M. I., Gonzalez, A., Byrnes, J. E., Cardinale, B. J., Duffy, J. E., Gamfeldt, L., Griffin, J.N., Hooper, D., Hungate, B. A., Paquette, A., & Thompson, P. L. (2016). A general biodiversity–function relationship is mediated by trophic level. *Oikos*. 10.1111/oik.03652

Oke, T. R. (1973). City size and the urban heat island. *Atmospheric Environment (1967)*, *7*(8), 769-779.

Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Stevens, M. H. H., Oksanen, M. J., & Suggests, M. A. S. S. (2007). The vegan package. *Community ecology package*, *10*.

Osono, T. (2006). Role of phyllosphere fungi of forest trees in the development of decomposer fungal communities and decomposition processes of leaf litter. *Canadian Journal of Microbiology*, *52*(8), 701-716.

Osono, T. (2008). Endophytic and epiphytic phyllosphere fungi of Camellia japonica: seasonal and leaf age-dependent variations. *Mycologia*, *100*(3), 387-391.

Ostfeld, R. S., Keesing, F., & Eviner, V. T. (Eds.). (2010). *Infectious disease ecology: effects of ecosystems on disease and of disease on ecosystems*. Princeton University Press, 520p.

Pakarinen, J., Hyvärinen, A., Salkinoja-Salonen, M., Laitinen, S., Nevalainen, A., Mäkelä, M. J., Haahtela, T., & Von Hertzen, L. (2008). Predominance of Gram-positive bacteria in house dust in the low-allergy risk Russian Karelia. *Environmental microbiology*, *10*(12), 3317-3325.

Paradis, E., Claude, J., & Strimmer, K. (2004). APE: analyses of phylogenetics and evolution in R language. *Bioinformatics*, *20*(2), 289-290.

Pataki, D. E., Carreiro, M. M., Cherrier, J., Grulke, N. E., Jennings, V., Pincetl, S., Pouyat, R. V., Whitlow, T. H., & Zipperer, W. C. (2011). Coupling biogeochemical cycles in urban environments: ecosystem services, green solutions, and misconceptions. *Frontiers in Ecology and the Environment*, *9*(1), 27-36.

Pawson, S. M., Brin, A., Brockerhoff, E. G., Lamb, D., Payn, T. W., Paquette, A., & Parrotta, J. A. (2013). Plantation forests, climate change and biodiversity. *Biodiversity and Conservation*, *22*(5), 1203-1227.

Peiffer, J. A., Spor, A., Koren, O., Jin, Z., Tringe, S. G., Dangl, J. L., Buckler, E. S., & Ley, R. E. (2013). Diversity and heritability of the maize rhizosphere microbiome

under field conditions. *Proceedings of the National Academy of Sciences*, *110*(16), 6548-6553.

Peñuelas, J., Rico, L., Ogaya, R., Jump, A. S., & Terradas, J. (2012). Summer season and long-term drought increase the richness of bacteria and fungi in the foliar phyllosphere of Quercus ilex in a mixed Mediterranean forest. *Plant Biology*, *14*(4), 565-575.

Poisot, T., Mouquet, N., & Gravel, D. (2013). Trophic complementarity drives the biodiversity–ecosystem functioning relationship in food webs. *Ecology letters*, *16*(7), 853-861.

Pouyat, R. V., & McDonnell, M. J. (1991). Heavy metal accumulations in forest soils along an urban-rural gradient in southeastern New York, USA. *Water, Air, and Soil Pollution*, *57*(1), 797-807.

Prosser, J. I., Bohannan, B. J., Curtis, T. P., Ellis, R. J., Firestone, M. K., Freckleton, R. P., Green, J. L., Green, L. E., Killham, K., Lennon, J. J., & Osborn, A. M. (2007). The role of ecological theory in microbial ecology. *Nature Reviews Microbiology*, *5*(5), 384-392.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic acids research*, *41*(D1), D590-D596.

R Development Core Team. R: A language and Environment for Statistical Computing. Vienna, Austria. 2013. URL http://www.R-project.org/.

Raghavendra, A. K., & Newcombe, G. (2013). The contribution of foliar endophytes to quantitative resistance to Melampsora rust. *New Phytologist*, *197*(3), 909-918.

Rastogi, G., Coaker, G. L., & Leveau, J. H. (2013). New insights into the structure and function of phyllosphere microbiota through high-throughput molecular approaches. *FEMS microbiology letters*, *348*(1), 1-10.

Rastogi, G., Sbodio, A., Tech, J. J., Suslow, T. V., Coaker, G. L., & Leveau, J. H. (2012). Leaf microbiota in an agroecosystem: spatiotemporal variation in bacterial community composition on field-grown lettuce. *The ISME journal*, *6*(10), 1812-1822.

Rastogi, G., Tech, J. J., Coaker, G. L., & Leveau, J. H. (2010). A PCR-based toolbox for the culture-independent quantification of total bacterial abundances in plant environments. *Journal of microbiological methods*, *83*(2), 127-132.

Raupp, M. J., Shrewsbury, P. M., & Herms, D. A. (2010). Ecology of herbivorous arthropods in urban landscapes. *Annual review of entomology*, *55*, 19-38.

Redford, A. J., Bowers, R. M., Knight, R., Linhart, Y., & Fierer, N. (2010). The ecology of the phyllosphere: geographic and phylogenetic variability in the distribution of bacteria on tree leaves. *Environmental microbiology*, *12*(11), 2885-2893.

Redford, A. J., & Fierer, N. (2009). Bacterial succession on the leaf surface: a novel system for studying successional dynamics. *Microbial ecology*, *58*(1), 189-198.

Reisberg, E. E., Hildebrandt, U., Riederer, M., & Hentschel, U. (2012). Phyllosphere bacterial communities of trichome-bearing and trichomeless Arabidopsis thaliana leaves. *Antonie Van Leeuwenhoek*, *101*(3), 551-560.

Reisberg, E. E., Hildebrandt, U., Riederer, M., & Hentschel, U. (2013). Distinct phyllosphere bacterial communities on Arabidopsis wax mutant leaves. *PLoS One*, *8*(11), e78613.

Remus-Emsermann, M. N., Lücker, S., Müller, D. B., Potthoff, E., Daims, H., & Vorholt, J. A. (2014). Spatial distribution analyses of natural phyllosphere-colonizing bacteria on Arabidopsis thaliana revealed by fluorescence in situ hybridization. *Environmental microbiology*, *16*(7), 2329-2340.

Ren, G., Zhu, C., Alam, M. S., Tokida, T., Sakai, H., Nakamura, H., Usui, Y., Zhu, J., Hasegawa, T., & Jia, Z. (2015). Response of soil, leaf endosphere and phyllosphere bacterial communities to elevated CO2 and soil temperature in a rice paddy. *Plant and Soil*, *392*(1-2), 27-44.

Richardson, E. A., & Mitchell, R. (2010). Gender differences in relationships between urban green space and health in the United Kingdom. *Social science & medicine*, *71*(3), 568-575.

Rico, L., Ogaya, R., Terradas, J., & Peñuelas, J. (2014). Community structures of N2-fixing bacteria associated with the phyllosphere of a Holm oak forest and their response to drought. *Plant Biology*, *16*(3), 586-593.

Ritpitakphong, U., Falquet, L., Vimoltust, A., Berger, A., Métraux, J. P., & L'Haridon, F. (2016). The microbiome of the leaf surface of Arabidopsis protects against a fungal pathogen. *New Phytologist*. 210, 1033-1043

Rodriguez, R., & Redman, R. (2008). More than 400 million years of evolution and some plants still can't make it on their own: plant stress tolerance via fungal symbiosis. *Journal of Experimental Botany*, *59*(5), 1109-1114.

Rodriguez, R. J., White Jr, J. F., Arnold, A. E., & Redman, R. S. (2009). Fungal endophytes: diversity and functional roles. *New phytologist*, *182*(2), 314-330.

Roetzer, T., Wittenzeller, M., Haeckel, H., & Nekovar, J. (2000). Phenology in central Europe–differences and trends of spring phenophases in urban and rural areas. *International Journal of Biometeorology*, *44*(2), 60-66.

Rosindell, J., Hubbell, S. P., & Etienne, R. S. (2011). The unified neutral theory of biodiversity and biogeography at age ten. *Trends in ecology & evolution*, *26*(7), 340-348.

Ruinen, J. (1956). Occurrence of Beijerinckia species in the 'phyllosphere'. *Nature*, *177*(4501), 220-221.

Ryffel, F., Helfrich, E. J., Kiefer, P., Peyriga, L., Portais, J. C., Piel, J., & Vorholt, J. A. (2016). Metabolic footprint of epiphytic bacteria on Arabidopsis thaliana leaves. *The ISME journal*, *10*(3), 632-643.

Sabaratnam, S., & Beattie, G. A. (2003). Differences between Pseudomonas syringae pv. syringae B728a and Pantoea agglomerans BRT98 in epiphytic and endophytic colonization of leaves. *Applied and environmental microbiology*, *69*(2), 1220-1228.

Saikkonen, K., Mikola, J., & Helander, M. (2015). Endophytic phyllosphere fungi and nutrient cycling in terrestrial ecosystems. *Current Science*, *109*, 121-126.

Sale, P. F. (1978). Coexistence of coral reef fishes—a lottery for living space. *Environmental Biology of Fishes*, *3*(1), 85-102.

Sanderson, E. W., Jaiteh, M., Levy, M. A., Redford, K. H., Wannebo, A. V., & Woolmer, G. (2002). The Human Footprint and the Last of the Wild: The human footprint is a global map of human influence on the land surface, which suggests that human beings are stewards of nature, whether we like it or not. *BioScience*, *52*(10), 891-904.

Sapijanskas, J., Paquette, A., Potvin, C., Kunert, N., & Loreau, M. (2014). Tropical tree diversity enhances light capture through crown plasticity and spatial and temporal niche differences. *Ecology*, *95*(9), 2479-2492.

Schauer, S., & Kutschera, U. (2011). A novel growth-promoting microbe, Methylobacterium funariae sp. nov., isolated from the leaf surface of a common moss. *Plant signaling & behavior*, *6*(4), 510-515.

Scheffers, B. R., Phillips, B. L., Laurance, W. F., Sodhi, N. S., Diesmos, A., & Williams, S. E. (2013). Increasing arboreality with altitude: a novel biogeographic dimension. *Proceedings of the Royal Society of London B: Biological Sciences*, *280*(1770), 20131581.

Schmidt, T. S., Rodrigues, J. F. M., & Von Mering, C. (2014). Ecological consistency of SSU rRNA-based operational taxonomic units at a global scale. *PLoS Comput Biol*, *10*(4), e1003594.

Schnitzler, F. R., Hartley, S., & Lester, P. J. (2011). Trophic-level responses differ at plant, plot, and fragment levels in urban native forest fragments: a hierarchical analysis. *Ecological Entomology*, *36*(2), 241-250.

Schreiber, L., Krimm, U., Knoll, D., Sayed, M., Auling, G., & Kroppenstedt, R. M. (2005). Plant–microbe interactions: identification of epiphytic bacteria and their ability to alter leaf surface permeability. *New Phytologist*, *166*(2), 589-594.

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., & Huttenhower, C. (2011). Metagenomic biomarker discovery and explanation. *Genome biology*, *12*(6), 1.

Shade, A., & Handelsman, J. (2012). Beyond the Venn diagram: the hunt for a core microbiome. *Environmental microbiology*, *14*(1), 4-12.

Shendure, J., & Ji, H. (2008). Next-generation DNA sequencing. *Nature biotechnology*, *26*(10), 1135-1145.

Shipley, B., & Vu, T. T. (2002). Dry matter content as a measure of dry matter concentration in plants and their parts. *New Phytologist*, *153*(2), 359-364.

Sieghardt, M., Mursch-Radlgruber, E., Paoletti, E., Couenberg, E., Dimitrakopoulus, A., Rego, F., Hatzistathis, A., & Randrup, T. B. (2005). The abiotic urban environment: impact of urban growing conditions on urban vegetation. In *Urban forests and trees* (pp. 281-323). Springer Berlin Heidelberg.

Smets, W., Wuyts, K., Oerlemans, E., Wuyts, S., Denys, S., Samson, R., & Lebeer, S. (2016). Impact of urban land use on the bacterial phyllosphere of ivy (Hedera sp.). *Atmospheric Environment*, *147*, 376-383.

Song, Y. Y., Zeng, R. S., Xu, J. F., Li, J., Shen, X., & Yihdego, W. G. (2010). Interplant communication of tomato plants through underground common mycorrhizal networks. *PLoS One*, *5*(10), e13324.

Suda, W., Nagasaki, A., & Shishido, M. (2009). Powdery mildew-infection changes bacterial community composition in the phyllosphere. *Microbes and Environments*, *24*(3), 217-223.

Tedersoo, L., May, T. W., & Smith, M. E. (2010). Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. *Mycorrhiza*, *20*(4), 217-263.

Teste, F. P., Simard, S. W., Durall, D. M., Guy, R. D., Jones, M. D., & Schoonmaker, A. L. (2009). Access to mycorrhizal networks and roots of trees: importance for seedling survival and resource transfer. *Ecology*, *90*(10), 2808-2822.

Tischer, C., Weikl, F., Probst, A. J., Standl, M., Heinrich, J., & Pritsch, K. (2016). Urban Dust Microbiome: Impact on Later Atopy and Wheezing. *Environmental health perspectives*.

Tilman, D., & Downing, J. A. (1996). Biodiversity and stability in grasslands. In *Ecosystem Management* (pp. 3-7). Springer New York.

Tilman, D., Reich, P. B., & Isbell, F. (2012). Biodiversity impacts ecosystem productivity as much as resources, disturbance, or herbivory. *Proceedings of the National Academy of Sciences*, *109*(26), 10394-10397.

Tobner, C. M., Paquette, A., Gravel, D., Reich, P. B., Williams, L. J., & Messier, C. (2016). Functional identity is the main driver of diversity effects in young tree communities. *Ecology letters*, *19*(6), 638-647.

Tobner, C. M., Paquette, A., Reich, P. B., Gravel, D., & Messier, C. (2014). Advancing biodiversity–ecosystem functioning science using high-density tree-based experiments over functional diversity gradients. *Oecologia*, *174*(3), 609-621.

Turner, T. R., James, E. K., & Poole, P. S. (2013). The plant microbiome. *Genome biology*, *14*(6), 1.

Tuskan, G. A., DiFazio, S. P., & Teichmann, T. (2004). Poplar genomics is getting popular: the impact of the poplar genome project on tree research. *Plant Biology*, *7*(01), 2-4.

United Nations Department of Economic and Social Affairs, Population Division. (2015). *World Urbanization Prospects, the 2015 Revision: Highlights*. United Nations, New York. Accessed on 15th of August 2016 from www.un.org/en/development/desa/publications/world-urbanization-prospects-the-2015-revision.html

Uroz, S., Buée, M., Deveau, A., Mieszkin, S., & Martin, F. (2016). Ecology of the forest microbiome: Highlights of temperate and boreal ecosystems. *Soil Biology and Biochemistry*, *103*, 471-488.

NRCS, U. (2014). The PLANTS Database (http://plants. usda. gov, 9 February 2014). National Plant Data Team, Greensboro.

Vandenkoornhuyse, P., Quaiser, A., Duhamel, M., Le Van, A., & Dufresne, A. (2015). The importance of the microbiome of the plant holobiont. *New Phytologist*, *206*(4), 1196-1206.

Van Der Heijden, M. G., Bardgett, R. D., & Van Straalen, N. M. (2008). The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology letters*, *11*(3), 296-310.

Verheyen, K., Vanhellemont, M., Auge, H., Baeten, L., Baraloto, C., Barsoum, N., Bilodeau-Gauthier, S., Bruelheide, H., Castagneyrol, B., Godbold, D., & Haase, J. (2016). Contributions of a global network of tree diversity experiments to sustainable forest plantations. *Ambio*, *45*(1), 29-41.

Vogel, C., Bodenhausen, N., Gruissem, W., & Vorholt, J. A. (2016). The Arabidopsis leaf transcriptome reveals distinct but also overlapping responses to colonization by phyllosphere commensals and pathogen infection with impact on plant health. *New Phytologist*, *212*(1), 192-207.

Volterra, V. (1926). Fluctuations in the abundance of a species considered mathematically. *Nature*, *118*, 558-560.

Vorholt, J. A. (2012). Microbial life in the phyllosphere. *Nature Reviews Microbiology*, *10*(12), 828-840.

Wania, A., Kühn, I., & Klotz, S. (2006). Plant richness patterns in agricultural and urban landscapes in Central Germany—spatial gradients of species richness. *Landscape and Urban Planning*, *75*(1), 97-110.

Wei, F., Hu, X., & Xu, X. (2016). Dispersal of Bacillus subtilis and its effect on strawberry phyllosphere microbiota under open field and protection conditions. *Scientific reports*, *6*.

Wellner, S., Lodders, N., & Kämpfer, P. (2011). Diversity and biogeography of selected phyllosphere bacteria with special emphasis on Methylobacterium spp. *Systematic and applied microbiology*, *34*(8), 621-630.

Whipps, J. M., Hand, P., Pink, D., & Bending, G. D. (2008). Phyllosphere microbiology with special reference to diversity and plant genotype. *Journal of Applied Microbiology*, *105*(6), 1744-1755.

White, M. A., Nemani, R. R., Thornton, P. E., & Running, S. W. (2002). Satellite evidence of phenological differences between urbanized and rural areas of the eastern United States deciduous broadleaf forest. *Ecosystems*, *5*(3), 260-273.

Wickham H. (2009) ggplot2: Elegant Graphics for Data Analysis. Springer New York, *1*(2), p.3.

Wiersum, L. K., & Harmanny, K. (1983). Changes in the water-permeability of roots of some trees during drought stress and recovery, as related to problems of growth in urban environment. *Plant and Soil*, *75*(3), 443-448.

Williams, T. R., & Marco, M. L. (2014). Phyllosphere microbiota composition and microbial community transplantation on lettuce plants grown indoors. *Mbio*, *5*(4), e01564-14.

Williams, T. R., Moyne, A. L., Harris, L. J., & Marco, M. L. (2013). Season, irrigation, leaf age, and Escherichia coli inoculation influence the bacterial diversity in the lettuce phyllosphere. *PLoS One*, *8*(7), e68642.

Wilson, D., & Carroll, G. C. (1994). Infection studies of Discula quercina, an endophyte of Quercus garryana. *Mycologia*, 635-647.

Wright, S. J., Kitajima, K., Kraft, N. J., Reich, P. B., Wright, I. J., Bunker, D. E., Condit, R., Dalling, J. W., Davies, S. J., Díaz, S., & Engelbrecht, B. M. (2010). Functional traits and the growth–mortality trade-off in tropical trees. *Ecology*, *91*(12), 3664-3674.

Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J. H., Diemer, M., & Flexas, J. (2004). The worldwide leaf economics spectrum. *Nature*, *428*(6985), 821-827.

y Gomez, G. S. M., & Van Dyck, H. (2012). Ecotypic differentiation between urban and rural populations of the grasshopper Chorthippus brunneus relative to climate and habitat fragmentation. *Oecologia*, *169*(1), 125-133.

Yachi, S., & Loreau, M. (1999). Biodiversity and ecosystem productivity in a fluctuating environment: the insurance hypothesis. *Proceedings of the National Academy of Sciences*, *96*(4), 1463-1468.

Yang, C. H., Crowley, D. E., Borneman, J., & Keen, N. T. (2001). Microbial phyllosphere populations are more complex than previously realized. *Proceedings of the National Academy of Sciences*, *98*(7), 3889-3894.

Yashiro, E., Spear, R. N., & McManus, P. S. (2011). Culture-dependent and culture-independent assessment of bacteria in the apple phyllosphere. *Journal of applied microbiology*, *110*(5), 1284-1296.

Zamioudis, C., & Pieterse, C. M. (2012). Modulation of host immunity by beneficial microbes. *Molecular Plant-Microbe Interactions*, *25*(2), 139-150.

Zhang, J., Kobert, K., Flouri, T., & Stamatakis, A. (2014). PEAR: a fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics*, *30*(5), 614-620.

Zhang, X., Friedl, M. A., Schaaf, C. B., Strahler, A. H., & Schneider, A. (2004). The footprint of urban climates on vegetation phenology. *Geophysical research letters*, *31*(12).