UNIVERSITÉ DU QUÉBEC À MONTRÉAL

LIENS ENTRE LA STRUCTURE ET LA PERFORMANCE MÉTABOLIQUE DES COMMUNAUTÉS BACTÉRIENNES AQUATIQUES EN RÉPONSE AUX GRADIENTS DE L'ENVIRONNEMENT

THÈSE
PRÉSENTÉE
COMME EXIGENCE PARTIELLE
DU DOCTORAT EN BIOLOGIE

PAR
JÉRÔME COMTE

SEPTEMBRE 2010

REMERCIEMENTS

Je désire remercier les différentes personnes qui ont contribué au bon déroulement et aboutissement de ce doctorat :

Paul del Giorgio, mon directeur, pour avoir partagé avec moi sa passion et vision pour l'écologie microbienne aquatique. J'ai énormément apprécié chacune de nos rencontres au cours desquelles son grand talent de dessinateur conceptuel réussissait à faire ressortir une histoire dans un graphique de prime à bord chaotique. Je le remercie pour sa patience, sa confiance en moi (qui souvent dépassait la mienne), son aide dans le développement du projet, son implication dans l'écriture des articles et en particulier ses efforts à canaliser mon « côté Molière ». Son excellent encadrement basé sur l'éthique et le soucis du bien être de chacun restera pour moi une source d'inspiration tout au long de ma carrière. Je le remercie en particulier de m'avoir présenté « ses amis » qui composent la grande majorité des sommités en écologie microbienne ainsi que de m'avoir permis d'assister aux congrès internationaux majeurs dans ce domaine.

Les membres de comité de la thèse, Beatrix Beisner (UQÀM), Isabelle Laurion (INRS-ETE) et Emilio Casamayor (CEAB-CISC) pour avoir accepté d'évaluer cette thèse dans un délais exceptionnellement court. Je les remercie de leurs précieux commentaires, suggestions et leur suis très reconnaissant de leur intérêt pour mon travail.

Yves Prairie de l'Université du Québec à Montréal; Leigh McCallister de Virginia Commonwealth University (USA); Stuart Findlay de Institute of Ecosystem Studies (USA); David Kirchman de University of Delaware (USA) pour leur participation dans les comités du projet de thèse et examen de synthèse. Leurs suggestions et commentaires ont contribué à améliorer ce projet de thèse tant au niveau conceptuel que technique.

Merci aux membres du groupe de recherche interuniversitaire (GRIL) pour proposer un environnement exceptionnel à leurs étudiants. En particulier, je remercie les membres du GRIL-UQÀM (Dolors Planas, Beatrix Beisner, David Bird, Yves Prairie et Pedro Peres-

Neto) pour l'ensemble des conversations échangées tout au long de ce doctorat. J'exprime ma gratitude à Pedro pour sa patience et disponibilité pour les analyses Procrustes.

Les membres du département des sciences biologiques de l'UQÀM qui ont fait de mon passage à l'université, une période agréable et sans souci. Je remercie plus particulièrement François Dragon et Louise Brissette (directrion du programme de doctorat), Ginette Lozeau-Lacroix (secrétaire de direction) et Nathalie Schad (assistante administrative). Merci également au corps enseignant et plus spécialement à Denis Jacques, Daniel Rivest et Madeleine Chagnon et l'ensemble des démonstrateurs, moniteurs et étudiants au baccalauréat pour ces merveilleuses années à Montréal et Saint-Michel-des-Saints.

Mes collègues et amis : Marie-Ève Ferland, Véronique Ducharme-Riel, Delphine Marchand, Annick Saint-Pierre, Jean-François Lapierre, Dominic Vachon, Soren Brothers, Jean-Baptiste Leguet. Nous avons passé plusieurs années ensemble à partager joie, succès mais aussi doutes et désespoir. Votre support m'a été d'une grande aide.

Un merci plus spécial à Lisa « Poulain » Fauteux pour son sourire, son intarissable enthousiasme et son implication lors de mes 2 étés de terrains plus qu'intensif. Je lui suis très reconnaissant d'avoir voulu lié son fabuleux destin au mien ces dernières années.

Je suis également très reconnaissant à l'unique et inspirant Rich Vogt pour ses nombreux commentaires et suggestions dans certains de mes chapitres.

Merci à ma chère compatriote Maud pour sa bienveillance en mon égard, ses conseils et aide dans les derniers jours de l'écriture du manuscrit m'ont été d'une grande aide. Je suis heureux d'avoir pu partager mon aventure québécoise avec elle.

Catherine Beauchemin, Alice Parkes, Pascal Tremblay-Dauphinais, Alexandre Guindon et Marilou Bergevin pour leur aide sur le terrain et au laboratoire. Votre implication a grandement contribué au bon déroulement de ce projet.

François Guillemette *alias* « ma blonde estivale »; je n'aurais pu imaginer meilleur partenaire pour effectuer mon doctorat. Ce merveilleux été 2005 restera comme l'une des plus belles périodes de ma vie. Je te remercie pour ton support, tes conseils et ta grande

générosité. Merci pour tous ces moments merveilleux passés chez vous ou Anny, à Lake Placid, en Arizona et ailleurs.

La Société des établissements de plein air du Québec (SEPAQ) du Mont-Orford, secteur du lac Fraser, pour leur collaboration. Merci à Mme et M. Beaulieu ainsi que Mme et M. Potvin qui ont permis d'utiliser leurs accès privés pour certains lacs et marais.

Bill Shipley de l'université de Sherbrooke pour sa merveilleuse formation en analyses des pistes, mais aussi pour ses conseils concernant les analyses présentées dans le chapitre III.

L'équipe de limnologie du département d'écologie et évolution de l'université d'Uppsala (Suède). En particulier, Eva Lindström pour son aide et ses conseils avec la technique DGGE après avoir ouvert cette boîte de Pandore au laboratoire. Merci À Silke Langenheder d'avoir été une inépuisable source d'inspiration pendant tout le long de mon doctorat. J'ai fait de sa thèse ma bible! Je suis heureux de pouvoir maintenant les rejoindre en Suède et travailler ensemble. Merci également à Lars Tranvik pour son implication dans la procédure de demande de la bourse Wenner-Gren. Je tiens à remercier Jürg pour les bons moments passé ensemble à Montréal et à Piran (SAME 11) et tous les autres à venir à Uppsala.

Pep Gasol de Institut de Ciences del Mar (Barcelone) pour ses chaleureux accueils au sein de son équipe lors de mes visites en Catalogne. Merci également pour avoir eu continuellement un œil sur mes travaux, et les nombreux commentaires constructifs sur les manuscrits. Merci également à Ramon Massana et Carlos Pedrós-Alió membres de l'institut, pour leurs commentaires sur mon projet de thèse et plus particulièrement à Carlos pour ses commentaires pour l'article soumis à *Ecology*. Merci également à Vanessa Balague pour son sourire et sa bonne humeur. Je lui suis très reconnaissant d'avoir partagé ses petits secrets pour rendre la DGGE plus « amicale ». Merci aussi à Laura Alonso-Sáez, Itziar Lekunberri, Fernando Unrein et Thomas Lefort, étudiants et postdoc de l'époque, pour leur accueil à l'institut et pour les bons moments passés ensemble à Barcelone et Piran.

Emilio Casamayor de Centre d'Estudis Avançats de Blanes (Espagne) pour ses commentaires sur mon projet de thèse et son grand intérêt pour mes travaux. Merci à Jean-Christophe Auguet, Albert Barberan et Pierre Galand pour les bons moments passés à Piran.

Les copains français et plus particulièrement à Gilles pour avoir partagé avec moi son expérience de fin de thèse. Notre séjour à Marseille, et les balades dans les calanques reste un moment inoubliable : Allez l'OM!. À Corinne, pour son passage à Montréal, notre collaboration fut plus qu'enrichissante. En attendant de visiter ton île…

Les amis québécois d'avoir fait une place au maudit français et d'avoir su partager cette magnifique culture québécoise dont j'ai fait mienne aujourd'hui: la musique, la bière, le CH et la poutine! Merci donc à Anne-Marie, Sébastien, Hugues, Agnès, Sylvain et le très précieux Philippe. Un merci spécial au merveilleux couple franco-québécois Maud et Charles pour leur soutien et aide et surtout aux bons moments passés ensemble.

Denis Laplante pour son aide et son écoute lors de cette dernière année.

Les familles Comte et Gauthier, pour leur soutien et amour tout au long de cette aventure. Un merci particulier pour mes parents qui ont toujours su m'encourager et me supporter tout au long de mes études. Un gros merci à Joseph pour les agréables moments passés ensemble et surtout tous les privilèges culinaires dont j'ai pu bénéficier!

Je n'aurai pu traverser ces six intenses années sans le support et l'affection de Valérie, mon épouse. Quelle merveilleuse muse elle a été. Sa force, son éternel sourire et intarissable, parfois incontrôlable, énergie m'a grandement encouragé lors de mes périodes de doutes et, le plus souvent empli le cœur de joie. Je n'aurais pu espérer meilleure compagne tant sa patience et sa compréhension ont été mises à rude épreuve. Merci pour les moments merveilleux que nous avons passé ensemble au Québec ou en voyage, et surtout tous les autres qui suivent. La grandeur de mon amour pour elle ainsi que l'importance de sa présence à mes côtés ne pourrait être résumées en ces quelques lignes. Je lui dédie cette thèse.

Ce travail a été soutenu par des subventions octroyées par le Centre de Recherches en Sciences Naturelles et en Génie du Canada (CRSNG) à Paul del Giorgio.

AVANT-PROPOS

Depuis leur découverte par Antonie van Leeuwenhoek (1632--1723), les micro-organismes n'ont eu de cesse d'alimenter la curiosité et la passion chez de nombreux de scientifiques dont les théories et découvertes ont façonné la biologie contemporaine tant fondamentale qu'appliquée. Trois cents ans plus tard, nous continuons encore à découvrir l'étendue de la diversité et des services de ces organismes aux écosystèmes mais surtout à notre propre existence sur Terre.

Cette thèse étudie la réponse des communautés bactériennes aquatiques aux gradients de l'environnement ainsi que les mécanismes impliqués dans cette réponse. Cette thématique est aujourd'hui d'une importance particulière à l'heure de mesurer les effets des changements climatiques sur le fonctionnement des écosystèmes. Les communautés bactériennes constituent en ce sens, les meilleures modèles à étudier tant le fonctionnement des écosystèmes repose sur leur diversité tant génétique que fonctionnelle.

Mon superviseur, Pr. Paul del Giorgio, a assuré les moyens financiers et logistiques pour mener à bien ces recherches, a participé au développement des idées, donné des conseils sur les expériences, les analyses et ses commentaires sur les articles et l'ensemble du manuscrit. Son nom apparaît donc comme co-auteur à chacune des différentes publications.

Ce travail contribue grandement à l'avancement des connaissances en écologie microbienne aquatique :

1- Cette thèse décrit les patrons de métabolisme et de différents aspects de la structure des communautés bactériennes à l'échelle d'un bassin versant en utilisant une approche multiparamétrique. La performance métabolique des communautés bactériennes apparaît clairement suivre les conditions environnementales. Cependant cette réponse métabolique n'apparaît pas être médiée par une réponse similaire au niveau des composants de la structure des communautés dont certains présentent un degré de spécificité écosystémique, d'autres suivent les conditions environnementales et d'autres ne présentent aucun patron (Chapitre I).

2- Cette thèse est également le premier rapport d'une connexion forte entre la composition phylogénétique et la capacité métaboliques des communautés bactériennes naturelles. Alors

que l'approche traditionnelle montre des patrons, souvent peu significatifs, où la fonction des communautés peut être prédite à partir de la diversité, les résultats ici montrent d'une part, que le lien en fait existe plus clairement en terme de leur magnitude de changements face à des gradients dans l'environnement. D'autre part, il apparaît que ce la force de cette relation varie selon le type et l'intensité de gradient vécu par les bactéries (Chapitre II).

3- C'est la première étude qui adoptant une approche multivariée pour décrire chaque niveau d'organisation des communautés bactériennes, montre dans un premier temps que la réponse des communautés bactériennes face à des changements dans les ressources s'effectue principalement par des ajustements des caractéristiques individuelles et de la physiologie des bactéries et non par un remplacement des phylotypes existants. La composition n'apparaît donc pas principalement jouer un rôle direct dans cette réponse, mais plutôt indirect en déterminant le niveau de plasticité métabolique de la communauté, ce qui suggère également un haut niveau de redondance fonctionnelle au sein de ces communautés bactériennes (Chapitre III). Dans un deuxième temps, que le niveau de redondance fonctionnelle ou de plasticité métabolique des bactéries face à un changement dans les ressources varie selon le niveau d'organisation dans la structure des communautés ainsi que du type d'habitat dont les communautés bactériennes sont originaires (Chapitre IV).

J'ai trouvé une source d'inspiration dans cette citation de l'un des plus influents chercheurs de l'histoire:

"Ayez le culte de l'esprit critique. Réduit à l'échec, il n'est ni éveilleur d'idée, ni un stimulant de grandes choses. Sans lui, tout est caduc. Il a toujours le dernier mot. Ce que je vous demande là, et ce que vous demanderez à votre tour aux disciples que vous formerez, c'est ce qu'il y a de plus difficile à l'inventeur. Croire que l'on a trouvé un fait scientifique important, avoir la fièvre de l'annoncer, et se combattre soi-même, à s'efforcer de ruiner ses propres expériences, et ne proclamer sa découverte que lorsqu'on a épuisé toutes les hypothèses contraires, oui, c'est une tâche ardue. Mais quand, après tant d'efforts, on est enfin arrivé à la certitude, on éprouve une des plus grandes joies que puisse ressentir l'âme humaine..." - Louis Pasteur.

TABLE DES MATIÈRES

LISTE DES FIGURES

Figure Page

LISTE DES TABLEAUX

LISTE DES ABRÉVIATIONS

ATP	« Adenosine Tri-Phosphate »
AWCD	« Average well color development of BIOLOG Ecoplates »
BA	« Bacterial abundance »
BCC	« Bacterial community composition »
BCD	« Bacterial carbon demand »
BCM	« Bacterial community metabolism »
BGE	« Bacterial growth efficiency »
BP	« Bacterial production »
BR	« Bacterial respiration »
DAG	« Directed acyclic graph »
DGGE	« Denaturing Gradient Gel Electrophoresis »
DOC	« Dissolved organic carbon »
ET	« Environmental transitions »
FC	« Bacterial functional capacities »
FCM	« Flow Cytometry Method »
HNA	« High DNA content cells »
LNA	« Low DNA content cells »
MC	« Bacterial metabolic capacities »
MDS	« Multidimensional scaling »
PCR	« Polymerase chain electrophoresis »
PS	« Bacterial physiological structure »
SCC	« Bacterial single-cell characteristics »
SEM	« Structural equation modeling »
TP	« Total phosphorus concentration »
TN	« Total nitrogen concentration »
TT	« Transition water transit time »

LISTE DES SYMBOLES

Δ	« Rate of change estimate »
m^2	« Metric of association between dissimilarity matrices in Procrustes analyses »
r^2	« Coefficient of determination in least-square regression models »
χ^2	« Maximum likelihood chi-squared statistic in SEM analyses »

RÉSUMÉ GÉNÉRAL

Les communautés bactériennes aquatiques sont extrêmement sensibles et réactives aux gradients environnementaux. Il a été proposé que leur réponse résultent de changements dans leur structure, tels que la composition, la fonction et la structure physiologique. L'objectif de cette thèse est de décrire les processus qui déterminent la réponse métabolique des communautés bactériennes face à des gradients dans les principales ressources, avec un intérêt particulier pour le rôle de la composition dans cette réponse. Les chapitres de cette thèse explorent dans un contexte de métacommunauté: Dans quelle mesure les ressources déterminent le métabolisme bactérien et leur structure, comment les composantes de la structure sont liées les unes aux autres, avec les ressources et la performance de la communauté, comment la composition des commuautés est liée à la fonction, comment la plasticité métabolique et la redondance fonctionnelle influencent le rôle de la composition dans la réponse de la communauté. Ces différents aspects ont été explorés *in situ* dans divers écotones dans un bassin versant et par des expériences de transplantations en laboratoire. Spécifiquement, les objectifs sont (1) d'examiner si les patrons en termes du métabolisme et des composantes de la structure de la communauté présentent une certaine spécificité écosystémique (2) d'étudier le lien entre la composition et la fonction des communautés, (3) de décrire la séquence des relations entre les composantes de la structure des communautés qui médient la réponse de la communauté aux changements des ressources, (4) d'évaluer l'influence de la plasticité métabolique et redondance fonctionnelle sur le métabolisme en réponse aux changements environnementaux. Les résultats indiquent que la régulation du métabolisme bactérien par les ressources est médiée par des changements dans les composantes de la structure qui peuvent être soit directionnels, spécifiques aux écosystèmes, ou aléatoire. En fait, les résultats montrent que la réponse peut être médiée d'une part, par des ajustements physiologiques des phylotypes dominants ou par le remplacement même des phylotypes dominants. Le type de réponse n'apparaît pas être déterminé par le type, ni l'intensité des gradients, mais par la plasticité métabolique de la communauté, qui à son tour semble être déterminée par des facteurs indépendants des gradients eux-mêmes. Les résultats montrent que la composition et la fonction des communautés sont liées l'une à l'autre d'une manière très dynamique, tel que leurs patrons absolus ne sont pas corrélés. La force et la forme de la relation varient en fonction du type et de l'intensité des gradients, suggérant un haut niveau de redondance fonctionnelle tant au sein de la communauté, qu'au sein de la métacommunauté, à partir de laquelle les phylotypes sont sélectionnés pour occuper les nouvelles niches créées le long des écotones. Les résultats des expériences de transplantations indiquent : L'existence d'un seuil environnemental qui détermine le niveau de redondance fonctionnelle, et une spécificité écosystémique dans la plasticité métabolique. Collectivement, les résultats de cette thèse montrent que les conditions environnementales locales ont une plus grande influence sur la structure de la communauté que la dispersion, et que la composition des communautés bactériennes joue toujours un rôle dans cette réponse en déterminant le niveau de plasticité de la communauté, mais que ce rôle n'est qu'apparent lorsque la réponse implique un remplacement des phylotypes dans les communautés qui sont intrinsèquement moins plastiques.

Mots clés: bactérioplancton, gradients environnementaux, métabolisme du carbone, structure des communautés, métacommunauté.

GENERAL ABSTRACT

After decades of research on microbial processes in aquatic systems, it has been hypothesized that the extreme sensitivity and reactivity of bacterioplankton communities to environmental gradients results from complex changes within community structure, such as in their composition, functional capacities and physiological structure. The overall aim of this thesis is to describe the processes that drive the carbon (C) metabolic response of bacterioplankton communities to gradients in main resources with a special emphasis on the role of community composition in this response. The chapters of this thesis explore: The extent to which the environment shapes bacterial community C metabolism and structure in a metacommunity context, how the components of structure are linked to each other and with both resources and community performance, how the composition of communities relate to their functional capacities, how bacterial plasticity and redundancy influence the role of the composition in the overall community response to resources gradients. These different issues have been addressed ro freshwater bacterioplankton communities, using both field studies focusing on ecotones in a watershed and transplant laboratory experiments. Specific objectives are to (1) investigate whether patterns in community metabolism and components of community structure are ecosystem-specific (2) explore the nature of the connection that exists between community composition and function, (3) describe the sequence of pathways that occur among components of community structure and mediate the overall community response to changes in resources, (4) assess the influence of metabolic plasticity and functional redundancy on community C metabolism along environmental gradients. Results indicate that the regulation of bacterial communities performance by resources is mediated by complex shifts within components of community structure that can be directional, ecosystem-specific, or apparently random.. In fact, the results show that the response can be mediated by either adjustments in the physiology of the dominant phylotypes or by replacement of the dominant phylotypes. The type of response is not driven by either the type nor the intensity of the gradients involved, but rather by metabolic plasticity at the community level, which in turn appeared to be determined by factors independent of the gradients themselves. The results further show that community composition and function are strongly related to each other but in a very dynamic manner, such that their absolute patterns do not appear to be connected and that the strength and shape of the relationship varies relative to the type and intensity of gradient considered. This suggests a high level of functional redundancy that occurs both within the existing community, and in the metacommunity, from which phylotypes are selected to occupy the new niches that are created along the transitions. Results from transplant experiments show: The existence of an environmental threshold that determines the overall level of functional redundancy, and an ecosystem-specificity in metabolic plasticity. Collectively, the results of this thesis show in a metacommunity context, that local environmental conditions have a stronger influence on the overall community metabolism than dispersal, and that bacterial community composition always plays a role in this response by determining the level of community plasticity, but that this role is only apparent when the response implies a replacement of phylotypes in communities that are intrinsically less plastic.

Key words: bacterioplankton, environmental forcing, C metabolism, community structure, metacommunity.

INTRODUCTION GÉNÉRALE

ÉTAT DES CONNAISSANCES

Historiquement, la chaîne trophique des écosystèmes aquatiques a été vue selon un modèle linéaire où la production primaire phytoplanctonique était transférée directement aux niveaux supérieurs, ignorant ainsi le rôle des microorganismes (Sherr et Sherr, 2008). Notre connaissance des microbes, et des bactéries en particulier, a longtemps été limitée aux organismes capables de croître sur milieux de culture. Or, la majorité des bactéries dans l'environnement ne peuvent être cultivées encore aujourd'hui (DeLong et Pace, 2001; Kirchman et Pedros-Alio, 2007). Ce n'est qu'à partir du milieu des années 1970 grâce aux articles séminaux de Pomeroy (1974) et plus tard de Azam (1983) que la structure trophique et le fonctionnement des écosystèmes aquatiques ont profondément été révisés avec la description de la boucle microbienne. Cette avancée conceptuelle couplée à plusieurs avancées techniques (Hobbie, Dalley et Jasper, 1977; Hagström *et al.,* 1979; Porter et Feig, 1980; Fuhrman et Azam, 1982), a tout d'abord montré que les bactéries dominent l'abondance et la biomasse des écosystèmes aquatiques (Whitman, Coleman et Wiebe, 1998). Puis, le récent développement de techniques moléculaires a révélé que cette biomasse bactérienne est composée d'un niveau insoupçonné de diversité génétique (Zwart *et al.*, 2002 ; Venter *et al.*, 2004 ; Lozupone et Knight, 2007) et métabolique (King, 2005) démontrant ainsi que les communautés bactériennes jouent un rôle clé dans le flux de carbone et d'énergie des écosystèmes aquatiques (Azam, 1998; Pomeroy *et al.,* 2007; Ducklow, 2008). Cependant, le lien qui existe entre la diversité et fonction des communautés bactériennes demeure encore aujourd'hui un défi majeur en écologie microbienne (Horner-Devine *et al.*, 2006 ; Smith, 2007 ; Allison et Martiny, 2008).

Durant la dernière décennie, la relation entre la diversité des communautés et le fonctionnement des écosystèmes a reçu une attention accrue en écologie (Loreau, 2000; Norberg, 2004; Hooper *et al.,* 2005). Cet intérêt grandissant s'explique en grande partie par le constat de l'érosion de la diversité due aux activités anthropiques et leurs conséquences potentielles en termes de structure et de fonctionnement des écosystèmes (Vitousek *et al.,* 1997). La plupart des travaux menés dans cette discipline ont été réalisés sur des

communautés terrestres (Schwartz *et al.,* 2000), et ce n'est que récemment que cette problématique a été adressée aux milieux aquatiques (Giller *et al.,* 2004). Des expériences en laboratoire ont été largement utilisées pour explorer ces liaisons (Horner-Devine *et al.,* 2003; Bell *et al.,* 2005) mais les résultats sont difficiles à extrapoler aux systèmes naturels (Cottingham, Brown et Lennon, 2001). Bien que l'utilisation de nouveaux outils moléculaires a permis d'identifier des activités métaboliques spécifiques de groupes phylogénétiques définis au sein de communautés naturelles (Gray et Head, 2001), de nombreuses études rapportent des résultats contradictoires concernant le rôle de la diversité pour la performance métabolique des communautés bactériennes (Langenheder, Lindström et Tranvik, 2005 ; Findlay et Sinsabaugh, 2006 ; Bertilsson *et al.*, 2007). Ces résultats contradictoires indiquent que le lien entre la diversité et la fonction des communautés n'est pas déterministe, dû en grande partie à la grande diversité ainsi que la versatilité métabolique des communautés bactériennes. Ceci suggère donc l'existence d'un seuil environnemental qui déterminerait le rôle direct de la composition dans la performance des communautés. Identifier les processus qui régissent la structure des communautés bactériennes est donc d'une grande importance pour pouvoir prédire la réponse métabolique de ces communautés face à des changements dans l'environnement et par conséquent, les conséquences en termes de fonctionnement de l'écosystème.

Métabolisme des communautés bactériennes hétérotrophes aquatiques

L'affranchissement des cultures bactériennes pour l'estimation d'abondance des bactéries planctoniques a ouvert la voie à une série d'outils de mesure spécifique permettant de caractériser des aspects clés du métabolisme bactérien. Ces méthodes tout d'abord développées en milieux marins, ont été utilisées par la suite en milieux d'eaux douces et ont permis de démontrer le rôle déterminant des communautés bactériennes dans le fonctionnement des écosystèmes aquatiques (Azam, 1998). Ainsi les bactéries représentent les principaux assimilateurs de matière organique et de carbone dissous, mais également participent à la reminéralisation de la matière inorganique, assimilent certains composés inorganiques qui seront redistribués aux autres niveaux du réseau trophique, et métabolisent une grande variété de molécules (Azam et Worden, 2004 ; Pomeroy *et al.,* 2007). Les bactéries hétérotrophes assument deux fonctions importantes dans la transformation de la matière organique: elles produisent de la nouvelle biomasse bactérienne (production

secondaire ou BP), et respirent le carbone organique en carbone inorganique (la respiration bactérienne ou BR).

La production bactérienne (BP) est traditionnellement mesurée comme le taux d'incorporation de substrats radioactifs. Fuhrman et Azam (1982) ont tout d'abord suggéré l'utilisation de la thymidine tritiée, qui en s'incorporant dans l'ADN représente une mesure du taux de croissance des communautés bactériennes. Peu de temps après, Kirchman, K'Nees et Hodson (1985) développèrent une méthode basée sur l'incorporation de la leucine tritiée qui permet de quantifier le taux de production de protéines par les bactéries. Les protéines constituant en grande partie la biomasse bactérienne, le taux d'incorporation de leucine dans les protéines des bactéries peut alors être considéré comme une estimation du taux de production de la biomasse bactérienne. Aujourd'hui ces méthodes constituent la base des mesures de la BP. Depuis les trente dernières années, la quantification et la compréhension des patrons de BP entre différents types d'écosystèmes aquatiques ainsi qu'au sein d'un même système ont été l'un des objectifs en microbiologie aquatique (Ducklow, 2000 ; Lennon et Cottingham, 2008).

La respiration bactérienne constitue également un élément clé dans le fonctionnement des écosystèmes aquatiques (del Giorgio et Williams, 2005; Lennon et Cottingham, 2008). Elle correspond au processus catabolique au cours duquel les bactéries utilisent le carbone organique dissous pour générer de l'énergie. Au cours de ce processus s'établit un transfert de protons et d'électrons qui à partir de l'oxidation de substrats organiques réduits, vers un récepteur (l'oxygène), produit du dioxyde carbone. Au plan écosystémique, la respiration représente un indice de dégradation de la matière organique et par conséquent des flux de carbone organique (del Giorgio et Williams, 2005). Malgré le fait que Williams (1981) trouva que la majeure partie de la respiration planctonique de la colonne d'eau était assurée par les organismes dont la taille était inférieure à 3µm, donc majoritairement les bactéries, les mesures de respiration bactériennes et les processus la régulant ont reçu moins d'attention que les mesures de production primaire et secondaire. Ce n'est que récemment avec la découverte de l'hétérotrophie des milieux aquatiques peu productifs, là où la respiration tend à surpasser la production primaire, que l'intérêt vers les mesures de respiration bactérienne a repris (del Giorgio, Cole et Cimbleris, 1997). De fait, la prise en considération de la respiration bactérienne et plus particulièrement le ratio entre la photosynthèse et la respiration

permet un meilleur estimé de la fonction de l'écosystème (Robinson et Williams, 2005).

La mesure combinée de la BP et la BR permet de déterminer l'efficacité de croissance des bactéries (BGE). La BGE qui se définit comme la proportion de nouvelle biomasse produite par unite de carbone assimilé par les bactéries, indique la proportion de carbone qui sera allouée à BR et BP (del Giorgio et Cole, 1998). Elle présente de grandes variations entre types d'écosystèmes avec des valeurs allant de moins de 5% à 60% (del Giorgio et Cole, 1998).

Collectivement, les innombrables mesures métaboliques effectuées depuis la deuxièmc moitié du XXe siècle ont montré que les communautés de bactérioplancton sont extrêmement sensibles aux changements dans l'environnement. Par exemple, de faibles changements dans les ressources (concentrations en nutriment et carbone organique dissous), et d'autres conditions comme la salinité ou la température se traduisent souvent par de fortes réponses en termes de métabolisme de la communauté (Carrero-Colón, Nakatsu et Konopka, 2006; Lennon et Cottingham, 2008). La direction et amplitude de ces réponses métaboliques sont à ce jour relativement bien décrites ce qui contraste avec notre compréhension limitée des processus impliqués dans cette réponse. À cet égard, une caractéristique intéressante des communautés de bactérioplancton est que l'abondance totale (et la biomasse) a tendance à varier beaucoup moins, à la fois spatialement et temporellement, que ne le fait soit la performance métabolique de la communauté (BP, BGE), ou les facteurs environnementaux qui influencent les bactéries. Par exemple, l'abondance des bactéries dans les lacs tempérés est généralement comprise entre 1 et 6.10^6 cellules par millilitre, et pourtant les taux de croissance ainsi que la production bactérienne peut varier de plusieurs ordres de grandeur (White *et al.*, 1991; Cotner et Biddanda, 2002). Si les changements dans le métabolisme de la communauté ne sont pas principalement attribuables à des changements dans l'abondance ou la biomasse, cela suggère qu'il doit nécessairement y avoir des changements profonds dans d'autres aspects de la structure des communautés, tels que la structure physiologique et l'activité individuelle des cellules (Smith et del Giorgio, 2003; del Giorgio et Gasol, 2008).

Structure des communautés bactériennes

La structure des communautés bactériennes se définit par une série complexe de propriétés qui vont de la cellule individuelle à la performance globale de l'assemblage

bactérien. Des exemples de ces différents éléments sont la distribution de taille des cellules, leur morphométrie et propriétés physiologiques, la distribution des états physiologiques au sein de la communauté, la répartition des capacités métaboliques, et le nombre, l'identité et la distribution des différents phylotypes. Les changements dans le métabolisme global de la communauté peuvent ainsi résulter de changements dans le nombre total ou la taille des cellules, dans le niveau intrinsèque de l'activité des cellules, dans la proportion de cellules avec différents niveaux d'activité (del Giorgio et Gasol, 2008), mais également de changements dans la composition de la communauté (Fisher *et al.*, 2000) ou, plus probablement, par une combinaison de tous ces aspects.

Historiquement, les différents niveaux de la structure de la communauté n'ont pas été étudiés avec le même degré d'intensité à cause de certaines limitations techniques. Après plusieurs décennies de mesures de taux de production et de respiration bactérienne dans divers types d'écosystèmes aquatiques, explorer si l'activité métabolique de la communauté est distribuée de façon uniforme parmi les bactéries ou selon un contiuum d'activité avec des bactéries actives et peu actives s'est imposé comme une problématique centrale en microbiologie aquatique. La simple distinction entre bactéries actives et inactives est rapidement devenue inadéquate suite à l'utilisation massive des fluorochromes en cytométrie en flux et en microscopie à épifluorescence (Joux et Lebaron, 2000) qui a montré qu'il existait en fait, une très grande hétérogénéité dans l'activité individuelle (del Giorgio et Gasol, 2008) et les caractéristiques intrinsèques (Bouvier, del Giorgio et Gasol, 2007) des bactéries. De plus, les faibles taux d'activité observés dans les écosystèmes peu productifs, comme les systèmes marins et de nombreux lacs, venaient appuyer l'idée que la plupart des bactéries aquatiques sont soit peu ou pas actives (latence, dormance) ou mortes (Morita, 1997). À cet égard, Smith et del Giorgio (2003) ont proposé que la simple distinction entre cellules actives et inactives ne permettait pas une description efficace de l'activité individuelle car la majorité des méthodes utilisées ne mesurent qu'un aspect particulier de l'activité ou de l'intégrité des cellules et non toute la gamme des états physiologiques. Les auteurs suggèrent de considérer la structure physiologique des bactéries comme une hiérarchie d'états physiologiques qui pourrait expliquer pourquoi, selon la méthode utilisée, le pourcentage de cellules actives varie entre moins de 5 et plus de 90%.

Cette dynamique mesurée au sein de la structure physiologique s'observe également à d'autres niveaux de la structure de la communauté comme les capacités fonctionnelles (Garland, Mills et Young, 2001), les activités enzymatiques (Findlay et Sinsabaugh, 2006) ou la composition (Crump *et al.*, 2007). Parmi les forces abiotiques et biotiques reconnues pour configurer tant la structure physiologique, fonctionnelle que compositionnelle des communautés, la qualité et disponibilité des ressources (Fisher *et al.*, 2000 ; Judd *et al.*, 2006 ; Kritzberg, Langenheder et Lindström, 2006), la prédation protozoaire (Gasol *et al.*, 2002 ; Corno et Jürgens, 2008) ainsi que la lyse virale (Weinbauer, 2004 ; Bouvier et del Giorgio, 2007) ont suscité un grand intérêt. Tous ces aspects allant de la structure de taille des cellules, d'activités et capacités fonctionnelles, de composition sont certainement liés les uns aux autres, mais la nature des liens entre eux est extrêmement complexe et encore à ce jour mal comprise. En ce sens, les résultats trouvés dans la littérature sont contradictoires avec d'une part, certains liens hiérarchiques entre les différentes composantes (del Giorgio, Prairie et Bird, 1997; Bertilsson *et al.*, 2007), et d'autre part, peu ou pas de liens (Langenheder, Lindström et Tranvik, 2005; Findlay et Sinsabaugh, 2006).

Collectivement les avancées techniques de ces dernières décennies ont permis d'ouvrir la boîte noire des communautés bactériennes. Décrire les mécanismes qui déterminent la structure des communautés est de grande importance pour une meilleure compréhension de leur réponse aux gradients de l'environnement. En particulier, le rôle que la diversité et la composition de la communauté tiennent dans cette réponse apparaît particulièrement critique dans le contexte des changements climatiques et de l'érosion de la biodiversité.

Composition phylogénétique des communautés bactériennes

Quantifier la diversité biologique demeure l'un des plus grand défi en écologie. En écologie microbienne aquatique, la description de la diversité et composition des communautés bactériennes est déterminante pour une meilleure compréhension de la distribution des taxa bactériens, et ultimement, de leur contribution relative au fonctionnement des écosystèmes (Pedrós-Alio, 2006b). L'étude de la diversité bactérienne a connu un essor incroyable depuis les débuts du séquençage de l'ADN, révolutionnant du même coup notre compréhension de la phylogénie microbienne (Woese, 1987). Le

développement de nouvelles approches moléculaires s'affranchissent dc l'incapacité de la majorité des bactéries naturelles de croître sur milieux de culture et permettent une exploration de la vaste diversité et richesse spécifique des communautés bactériennes à un haut niveau de résolution (DeLong et Pace, 2001).

Différentes approches sont disponibles pour explorer la diversité bactérienne à différentes échelles de résolution (Dahllöf, 2002 ; Logue *et al.*, 2008). Par exemple, Cottrell et Kirchman (2000a) ont trouvé des résultats sensiblement différents concernant l'importance relative des groupes de bactéries selon qu'ils étudiaient la diversité bactérienne en utilisant l'hybridation fluorescente *in situ* (FISH) qui permet d'identifier des groupes spéciaux de bactéries et une analyse de clones des gènes d'ARNr 16S qui donne un aperçu global de la diversité bactérienne.

De nombreuses études ont décrit la diversité bactérienne sur une vaste gamme d'écosystèmes aquatiques (Glöckner, Fuchs et Amann, 1999; Zwart *et al.*, 2002 ; Lozupone et Knight, 2007). Il est maintenant reconnu que les systèmes d'eau douce sont dominés par la sous-classe bêta du groupe des protéobactéries. Les bactéries appartenant aux groupes des Actinobacteria, Bacteroides ainsi que les Cyanobactéries, sont aussi généralement observées mais dans une moindre proportion (Zwart *et al.*, 2002 ; Barberan et Casamayor, 2010). De plus, l'existence de certains groupes bactériens propres (*Polynucleobacter*, *GKS98* groups of *Betaproteobacteria*) au sein des écosystèmes d'eau douce a été décrite (Hahn, 2006). Certains de ces groupes bactériens dulçaquicoles présentent une distribution cosmopolite alors que d'autres présentent des niches écologiques différentes bien que proches phylogénétiquement (Hahn, 2006).

Plusieurs études ont montré que la composition des communautés bactériennes (BCC) des lacs, en particulier, varie temporairement et spatialement au sein des habitats ainsi qu'entre habitats (Lindström, 2000 ; Yannarell et Triplett, 2004). Des exemples de synchronisme et saisonnalité dans la composition des communautés bactériennes ont en outre été observés (Crump et Hobbie, 2005 ; Kent *et al.*, 2007 ; Nelson, 2009). Plusieurs facteurs écologiques influencent la BCC, comme par exemple, la chimie (Methé et Zehr, 1999 ; Lindström, Kamst-Van Agterveld et Zwart, 2005) et la température de l'eau (Lindström, Kamst-Van Agterveld et Zwart, 2005), l'apport en matière organique (Crump *et al.*, 2003), la prédation métazoaire (Langenheder et Jürgens, 2001) et protozoaire (Šimek *et al.*, 1997;

Jezbera, Hornák et Šimek, 2005), la lyse virale (Bouvier et del Giorgio, 2007), la composition du phytoplancton (Höfle *et al.*, 1999), la taille de l'habitat (Reche *et al.*, 2005) et le temps de rétention de l'eau (Lindström *et al.*, 2006). Bien que ces études fournissent de l'information sur les liens entre la BCC et les facteurs physico-chimiques des écosystèmes aquatiques, les observations rapportées sont souvent limitées d'une part, à l'étude de systèmes individuels et isolés, et d'autre part, à des variables particulières de l'environnement (ex.: température, matièrc organique, prédation). Ainsi, peu d'études ont considéré les successions compositionnelles qui s'établisse le long de gradients environnementaux. La majorité des études conduites dans ce domaine l'ont été sur des gradients de salinité dans des systèmes estuairiens (Bouvier et del Giorgio 2002; Crump *et al.* 2004; Castle et Kirchman 2004; Crump et al., 2004; Bernhard *et al.* 2005).

Récemment, plusieurs études ont montré que la BCC à l'échelle locale pouvait être influencée par des processus régionaux tels que les conditions climatiques, la végétation, la géologie (Lindström et Leskinen, 2002 ; Curtis et Sloan, 2004 ; Crump et Hobbie, 2005 ; Yannarell et Triplett, 2005 ; Nelson, 2009). Par ailleurs, il a été démontré que des apports externes à la fois de matière organique et de bactéries (Levine et Crump, 2002; Crump *et al.*, 2003; Stepanauskas *et al.*, 2003; Lindström et Bergström 2004, 2005; Hervàs *et al.*, 2009) pouvait influencer la composition des systèmes récepteurs. Par exemple, Lindström et Bergström (2004) ont observé que 70% de la production secondaire d'un lac pouvaient provenir de l'activité des bactéries allochtones provenant de ses affluents.

Ces résultats s'intègrent dans un nouveau cadre théorique de la structure des communautés qui vise en autres à évaluer dans quelle mesure une communauté locale est structurée par des processus locaux et/ou régionaux. Le concept de métacommunauté a récemment été introduit en écologie microbienne (Leibold et Norberg, 2004 ; Logue et Lindström, 2008 ; Logue *et al.*, 2008). Une métacommunauté est définie comme un ensemble de communautés locales qui sont reliées entre elles par la dispersion (Leibold *et al.*, 2004). Dans ce concept, deux grands paradigmes font l'objet d'une attention particulière : (i) le triage d'espèces où l'hétérogénéité environnementale est suffisamment grande pour déterminer la structure des communautés. De ce concept, la dispersion bien que présente, apparaît moins influente ; (ii) L'effet de masse où la dispersion est si forte qu'elle influence directement la BCC en minimisant l'influence des variables environnementales (Logue et

Lindström, 2008). Certaines études ont trouvé une dominance de la dispersion dans la structure des communautés bactériennes (Lindström *et al.*, 2006 ; Nelson *et al.*, 2009 ; Crump *et al.*, 2007) ; une dominance des conditions environnementales locales (Beisner *et al.*, 2006 ; Van der Gucht *et al.*, 2007; Logue et Lindström, 2010) et une combinaison des deux (Langenheder et Ragnarsson, 2007).

Collectivement les résultats acquis depuis les vingt dernières années ont grandement contribué à nos connaissances de l'étendue de la diversité bactérienne et des facteurs qui la contrôle. Cependant, si la pertinence écologique de la structure compositionnelle des communautés bactériennes pour la fonction des écosystèmes constitue l'essence même des études de la diversité microbienne (Reed et Martiny, 2007; Allison et Martiny, 2008), la connexion entre la diversité et composition des communautés et leur fonction demeure un défi d'envergure en écologie microbienne.

Lien entre la composition et la fonction des communautés bactériennes

Les relations entre la diversité, la fonction et la stabilité des écosystèmes constituent un des thèmes centraux de l'écologie depuis plusieurs années (ex: Loreau, 2000). Le fonctionnement des écosystèmes se définit comme un ensemble de plusieurs processus, comme la production primaire et secondaire, les cycles biogéochimiques du carbone et des éléments nutritifs, la stabilité des communautés, ainsi que le flux d'énergie et de matières via le réseau trophique (Azam, 1998 ; Pomeroy *et al.*, 2007). Bien que la nature de la relation ne soit pas toujours cohérente et universelle, les résultats montrent en général que la diversité a un impact sur des processus fonctionnels des écosystèmes, comme la production primaire, et leur stabilité (Yachi et Loreau, 1999 ; Schwartz *et al.*, 2000). La plupart des recherches sur le rôle de la biodiversité pour le fonctionnement des écosystèmes ont utilisé la richesse en espèces (le nombre total d'espèces recensées) ainsi que des indices de richesse (indice de Shannon) comme mesure de la diversité. Cependant, la mesure de la diversité ne se résume pas seulement à la richesse spécifique, mais comprend de nombreux autres aspects (Bengtson, 1998). Parmi ceux-ci, la diversité fonctionnelle s'avère désormais comme une approche plus pertinente pour adresser la question de la connexion entre la diversité et le fonctionnement des écosystèmes (Norberg. 2004 ; Hooper *et al.*, 2005 ; Petchey et Gaston, 2006 ; Green *et al.*, 2008). En effet, les mécanismes par lesquels la diversité peut influer sur

le fonctionnement des écosystèmes semblent davantage liés à certains attributs fonctionnels des espèces (ex: qualités morphologiques, physiologiques), plutôt que la richesse des espèces en soi.

La relation entre la biodiversité et le fonctionnement des écosystèmes a été principalement testée chez les communautés de plantes et animaux (ex: Cardinale *et al.*, 2007) et a abouti à l'établissement de paradigmes écologiques sur cette question (Naeem, Loreau et Inchausti, 2002). De manière générale, la relation entre diversité et fonction est représentée selon trois hypothèses: (i) redondance, impliquant que les espèces sont au moins partiellement substituables ; (ii) rôle clé, impliquant que les espèces font des contributions uniques au fonctionnement de l'écosystème et (iii) idiosyncrasique, impliquant que les espèces font des contributions différentes au fonctionnement de l'écosystème dépendant de facteurs externes ou internes. L'application de telles théories aux systèmes aquatiques ne fait l'objet d'un intérêt que depuis récemment (Cardinale *et al.*, 2006 ; Woodward, 2009). En effet, même si les communautés microbiennes ont été grandement étudiées dans des expériences en microcosmes (Horner-Devine *et al.*, 2003; Bell *et al.*, 2005 ; Salles *et al.*, 2009), ces dernières ne constituaient pas des tests directs visant à savoir si la diversité ou composition microbienne influençait le fonctionnement des écosystèmes, mais plutôt à utiliser les microbes comme modèles de communautés de macroorganismes (ex: plantes).

Ce décallage dans les connaissances acquises sur ce type de relation entre macro et microorganismes s'explique en partie par les considérations passées quant à l'importance de la contribution des bactéries au fonctionnement des écosystèmes. Jusqu'à récemment, les communautés bactériennes ont été réduites à une seule unité (ou boîte noire) dans les modélisations des processus biogéochimiques. En conséquence, la dynamique et la régulation des communautés bactériennes n'étaient pas considérées et le rôle de la diversité bactérienne dans le fonctionnement des écosystèmes ignoré. D'autre part, les mesures de biodiversité reposent traditionnellement sur la notion d'espèce, notion qui n'a pas encore abouti à un consensus pour les communautés bactériennes (Rossello-Mora et Amann, 2001; Cohan, 2002; Gevers *et al.*, 2005). Il existe deux principaux obstacles à la notion d'espèce bactérienne: le premier obstacle est la reproduction des bactéries. Alors que la plupart des macroorganismes se reproduisent sexuellement à l'exception de certaines plantes, les bactéries peuvent se reproduire de façon asexuée, mais elles sont également capables

d'obtenir des gènes provenant d'autres organismes, provenant même d'embranchements différents (Ochman *et al.*, 2005). La conséquence en est que les délimitations entre les groupes bactériens ne sont pas claires. Deuxièmement, Cohan (2002) a proposé que le concept d'espèces parmi les bactéries devrait être plutôt considéré en termes d'écotypes. Les écotypes se définissent comme des populations génétiquement cohérentes mais écologiquement distinctes. Une façon de surmonter ce problème a été le développement d'unités de conception de la diversité comme les OTU (unité opérationnelle taxonomique).

Ce n'est donc que récemment que les écologistes microbiens ont commencé à explorer la relation entre la diversité et les fonctions des communautés microbiennes dans divers types d'environnements, comme les sols (Torsvik *et al.*, 2002), et les milieux marins (Fuhrman, 2002) et d'eau douce (Cardinale *et al.*, 2009). Cependant, l'existence et la signification de ces liens sont encore aujourd'hui, un sujet d'intenses discussions (Horner-Devine *et al.*, 2006; Smith, 2007; Allison et Martiny, 2008). En effet, bien que des expériences de cultures en laboratoire ont montré des relations significatives entre la diversité bactérienne et quelques aspects fonctionnels de la communauté (Horner-Devine *et al.*, 2003; Bell *et al.*, 2005), ces résultats sont difficiles à extrapoler aux systèmes naturels. De plus, les résultats provenant d'études effectuées sur des communautés naturelles, sont souvent contradictoires avec d'une part des exemples de connexions significatives entre des mesures de la diversité et des mesures fonctionnelles de l'écosystème (Alonso-Sáez *et al.*, 2007 ; Bertilsson *et al.*, 2007) ou des caractéristiques spécifiques des bactéries tels que les capacités métaboliques individuelles des cellules (Bernard *et al.*, 2000; Longnecker, Sherr et Sherr, 2005). D'autre part, d'autres études montrent de faibles ou l'absence de liens entre diversité et fonction (Langenheder, Lindström et Tranvik, 2005, 2006; Findlay et Sinsabaugh, 2006).

La grande diversité génétique des bactéries et leur versatilité métabolique et fonctionnelle ont été suggérées comme étant les causes du manque de corrélation entre la diversité et la fonction au sein des communautés bactériennes (Curtis et Sloan, 2004 ; Franklin et Mills, 2006). À cet égard, les bactéries sont reconnues comme hautement redondantes d'un point de vue fonctionnel (Naeem et Li, 1997 ; Wohl, Arora, et Gladstone, 2004). Cette redondance fonctionnelle, peut permettre à des communautés microbiennes de maintenir la performance globale de la communauté à une certaine stabilité, en dépit du changement rapide de l'environnement ou de la diversité, car les différentes « espèces »

peuvent assurer les mêmes fonctions ou des fonctions similaires (Fernàndez *et al.*, 1999; Mills *et al.*, 2003). De plus, les bactéries sont reconnues pour présenter un haut niveau de plasticité métabolique, c'est à dire la capacité pour une même « espèce » d'assurer plusieurs fonctions différentes. À cet égard, les bactéries présentent une grande variété de modes de conversion de l'énergie (Buchan, Gonzalez et Moran, 2005) ainsi que de larges gammes d'utilisation de substrats (Meyer *et al.*, 2004). Il a par ailleurs été montré que la nature des relations entre la diversité microbienne et la fonction serait majoritairement de type redondant et idiosyncrasique dans les systèmes aquatiques (Cardinale *et al.*, 2006) suggérant que de nombreuses espèces sont fonctionnellement similaires, alors qu'une faible proportion peut exercer de façon disproportionnée des effets importants (Woodward *et al.*, 2009).

Déterminer les rôles métaboliques de taxons individuels représente désormais l'un des nouveaux enjeux actuels en écologie microbienne. Cependant, la grande diversité génétique et métabolique évoquée plus haut ne permet pas d'établir des liens directs entre ces deux aspects. Néanmoins, le progrès continuel dans de nouvelles technologies, notamment en métagénomique, permet désormais d'aborder cette question avec un très haut niveau de résolution (Gray et Head, 2001; Schmidt, 2006; Kuypers et Jørgensen, 2007; Logue *et al.*, 2008) et les premiers exemples de spécificité fonctionnelle ont alors été observés chez les bactéries (Cottrell et Kirchman, 2000b; Covert et Moran, 2001; Kirchamn *et al.*, 2004 ; Alonso-Sáez et Gasol, 2007).

Collectivement, ces résultats parfois contradictoires concernant le lien entre la diversité et la fonction des communautés (et des écosystèmes), suggèrent qu'il est peu probable que pour un ensemble de conditions environnementales données, toutes les combinaisons possibles de phylotypes disponibles à partir d'un pool génétique régional commun, puissent aboutir à une réponse métabolique et fonctionnelle identiques. Ainsi, la question ne devrait pas être seulement si la diversité est importante pour le fonctionnement de l'écosystème mais plutôt quand et comment ces deux aspects sont connectés (Cottingham, Brown et Lennon, 2001).

Approches utilisées pour adresser ces questions

Les écologistes ont depuis longtemps été intéressés par la relation entre la composition, l'identité, la diversité des organismes et les processus qui régissent les flux de la

matière et d'énergie dans un écosystème. Au cours de l'histoire, différentes approches ont été proposées pour adresser cette question (ex : Morris *et al.*, 2002).

Une hypothèse commune en écologie est qu'une diversité ou une richesse élevée aboutit à une plus grande performance fonctionnelle ou à une plus grande stabilité d'un écosystème. Cette hypothèse a été largement testée *in situ* pour les communautés de plantes à l'aide de corrélations entre le nombre d'espèces d'une communauté et certaines mesures clés du fonctionnement de l'écosystème comme la production primaire (ex : Tilman et Downing, 1994). Cette hypothèse a également été testée expérimentalement à l'aide de systèmes microbiens qui servaient alors de modèles pour adresser de grandes questions écologiques (Bohannan et Lenski, 2000). L'utilisation d'une telle approche pour les communautés microbiennes naturelles est difficile compte tenu qu'il est impossible de clairement définir une espèce bactérienne, mais surtout parce que la plasticité métabolique et redondance fonctionnelle au sein des bactéries ne permet pas de lier le nombres de taxons observés à leur fonction (voir section 0.1.4).

Une hypothèse complémentaire est que ce n'est pas combien de taxons présents mais plutôt leur identité et ce qu'ils font, qui comptent en termes de fonction. Dans ce cas, les patrons fonctionnels des communautés microbiennes devraient êtres corrélés avec la présence ou l'absence de certaines espèces ou phylotypes, de telle sorte que la fonction devrait changer en fonction de la composition. Une façon d'adresser cette question est de mesurer la réponse tant compositionnelle que fonctionnelle des bactéries face à des gradients dans l'environnement et de tester la puissance de cette relation en utilisant des modèles simples de régression. Les gradients de salinité en milieu estuarien ont à ce titre reçu une attention particulière cette dernière décennie (del Giorgio et Bouvier, 2002 ; Castle et Kirchman, 2004 ; Kirchman *et al.*, 2004 ; Bernhard *et al.*, 2005). Par exemple, Kirchman *et al.* (2004) observent une corrélation positive et significative entre le pourcentage des principaux groupes bactériens (alpha, béta-protébactéries) et certaines mesures métaboliques et fonctionnelles telles que la production secondaire et l'activité phosphatase. Or, il est difficile de concevoir que les performances métaboliques voire le fonctionnement d'un écosystème puissent être décrit avec seulement une variable. Plus tard, des approches multivariées donnaient une perspective plus intégrative de la relation entre la diversité et la fonction des communautés, car la composition n'était plus comparée à seulement un aspect métabolique

mais à plusieurs mesures qui ciblaient chacune un aspect clé du métabolisme de la communauté. Ainsi, des tests de corrélations de matrices de similarité (Mantel, Procrustes) permettent de tester l'association entre deux composants multivariés (Findlay et Sinsabaugh, 2006 ; Alonso-Sáez *et al.*, 2007 ; Kent *et al.*, 2007). Les principales limitations de telles approches (uni ou multivariées) sont qu'elles abordent la question du lien entre la diversité et la fonction des communautés d'un point de vue déterministe, c'est à dire qu'à un certain niveau de diversité correspond, de manière systématique, un certain niveau fonctionnel. Or, la nature des communautés bactériennes est loin d'être si déterministe et prévisible notamment en raison du fort taux de redondance fonctionnelle. D'autres approches alternatives en modélisation ont également été utilisées pour déterminer les rôles de la diversité des bactéries dans le fonctionnement de leur environnement (Dumont *et al.*, 2009). Bien que toutes ces approches constituent un bon point de départ pour étudier le lien entre la diversité et la composition des communautés bactériennes, elles ne démontrent pas nécessairement une relation causale mais une corrélation entre la composition microbienne et le fonctionnement des écosystèmes, en raison du lien étroit entre la composition microbienne, les variables environnementales, et les processus écosystémiques. L'un des principaux défis en écologie microbienne demeure encore de pouvoir tester l'effet direct de la composition microbienne sur le fonctionnement des écosystèmes tout en contrôlant pour certains autres paramètres environnementaux.

Les approches expérimentales permettent d'adresser plus directement le lien entre la composition et la fonction des communautés en tenant compte des effets de l'environnement. En macroécologie, les écologistes peuvent manipuler la composition et la diversité de la communauté sur le terrain et en mesurer les effets en termes fonctionnels (ex : Spehn *et al.*, 2005). En revanche, les communautés microbiennes naturelles ne permettent pas cette manipulation de la composition à l'échelle des taxons. Au lieu de cela, les études menées sur le lien entre diversité et fonction ont été réalisées à l'échelle de la communauté entière (ex : Reed et Martiny, 2007). Le lien entre la composition et la fonction des communautés peut être testé soit (i) en inoculant des communautés bactériennes issues de milieux différents dans un milieu identique à toutes (Langenheder, Lindström et Tranvik, 2006), soit (ii) en inoculant la même communauté dans plusieurs milieux qui diffèrent en termes de leur caractéristiques physico-chimiques (Horz *et al.*, 2004) ou (iii) en inoculant différentes communautés dans le

milieu original de chacune (Langenheder, Lindström et Tranvik, 2005).

Ces dernières années, de nouvelles techniques moléculaires ont permis d'explorer le lien entre la diversité et la fonction des communautés autrement qu'en testant la corrélation entre des mesures phylogénétiques et fonctionnelles. Ces méthodes alternatives permettent en autres, de décrire la composition de la fraction active des communautés bactériennes naturelles en explorant les patrons compositionnels basés sur l'ARN et non l'ADN (Logue et Lindström, 2010), ou de mesurer l'activité métabolique spécifique d'unités phylogénétiquement identifées (ex : Gray et Head, 2001). Par exemple, l'hybridation *in situ* a été couplée à la microautoradiographie (Gray *et al.*, 2000) et la spectrométrie de masse (ex : Kuypers et Jørgensen, 2007). D'autres méthodes associent l'immunofluorescence avec l'incorporation de composés radioactifs dans l'ADN (Urbach *et al.*, 1999), ou utilisent des traceurs isotopiques stables pour déterminer les composants fonctionnellement actifs des communautés microbiennes (Langenheder et Prosser, 2008). Ces dernières années, les techniques en génomique (Pedrós-Alió, 2006a) et en métagénomique (Handelsman, 2004) ont contribué à la description de la diversité microbienne à un haut niveau de résolution comme le pyroséquençage 454 et les techniques de clonage, permettant ainsi le développement d'une approche individuelle de l'étude du lien entre la diversité et la fonction des communautés bactériennes (Stepanauskas et Sieraki, 2007 ; Mou *et al.*, 2008).

Plus de trente ans après la découverte du rôle clé des communautés microbiennes dans le fonctionnement des écosystèmes aquatiques, les écologistes microbiens possèdent désormais des outils leur permettant de décrire la diversité fonctionnelle des communautés bactériennes ainsi que de mesurer les activités métaboliques de groupes ou taxons bactériens spécifiques au sein d'une communauté complexe. En outre, il est possible maintenant de déterminer les facteurs environnementaux qui façonnent certains aspects de la diversité des communautés bactériennes (Fuhrman *et al.*, 2006; Fierer *et al.*, 2007).

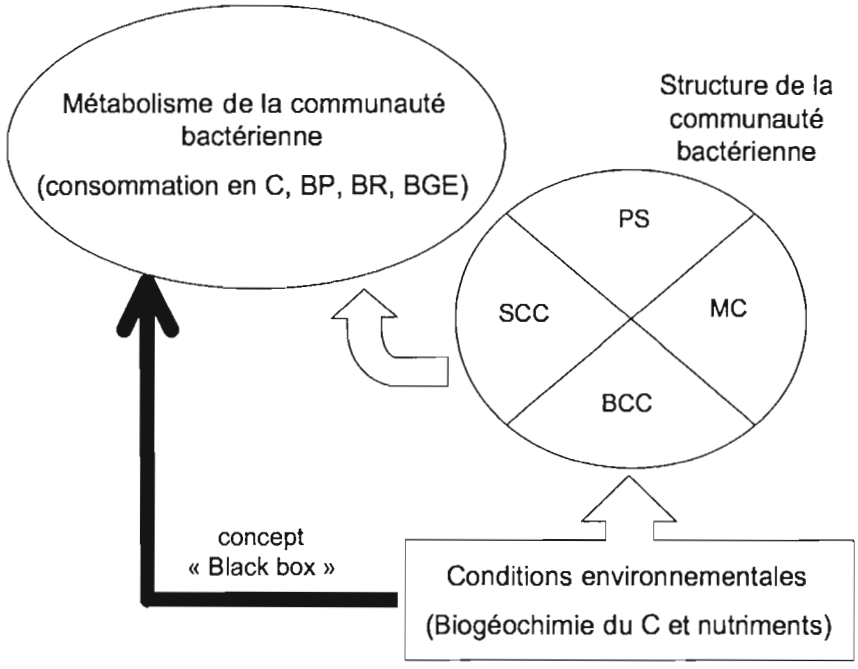


Figure 0.1 Base conceptuelle de la thèse. La réponse métabolique des communautés bactériennes aux variations dans les conditions environnementales était souvent vue selon l'approche « black box » (flèche noire) où les communautés bactériennes agissait comme un compartiment homogène fournissant énergie et matière aux réseaux trophiques. Depuis, il apparaît que la réponse de la communauté est médiée par des changements au sein des composantes de la structure telles que la composition (BCC), les capacités métaboliques (MC), les caractéristiques individuelles des cellules (SCC) et la structure physiologique (PS). Les différents aspects de la structure de la communauté sont influencés par les conditions environnementales et se combinent pour déterminer le métabolisme de la communauté (flèches blanches).

OBJECTIFS ET STRUCTURE DE LA THÈSE

La base conceptuelle de la thèse (Fig. 0.1) repose sur quatre questions fondamentales:

1- Comment la performance métabolique ainsi que les différents niveaux de la structure des communautés bactériennes (SCC, PS, BCC, MC et BA) répondent-ils aux changements environnementaux? (chapitres I, III, IV)

2- Dans quelle mesure la composition et les capacités métaboliques des communautés bactériennes sont-elles liées les unes aux autres? (chapitres I, II)

3- Quels sont les processus impliqués dans la réponse métabolique des communautés bactériennes aux changements environnementaux? (chapitres III, IV)

4- Quel est le rôle de la composition dans la réponse de la communauté ? (chapitres III, IV)

Les résultats de cette thèse sont présentés dans quatre chapitres, dont 2 ont été publiés, 1 a été soumi pour publication et 1 autre en préparation. Les quatre chapitres de cette thèse atteindront spécifiquement ces objectifs de la manière suivante:

- **Chapitre I**. Comte, J. and del Giorgio, P.A. 2009. Links between resources, C metabolism, and the major components of bacterioplankton community structure across a range of freshwater ecosystems. *Environmental Microbiology* 11(7): 1704-1716.

Le chapitre I décrit les patrons du métabolisme de communautés du bactérioplancton ainsi que de quatre composantes principales de la structure de la communauté (composition, capacités métaboliques, structure physiologique, et caractéristiques cellulaires) entre les lacs, les rivières et les marais d'un même bassin versant. L'objectif principal de ce chapitre est de savoir dans un premier temps, si le métabolisme suit de façon systématique les conditions environnementales et dans un deuxième temps, si les différents niveaux de la structure de la communauté présentent les mêmes patrons que ceux observés pour le métabolisme ou si certaines composantes présentent des caractéristiques spécifiques aux écosystèmes.

- **Chapitre II**. Comte, J. and del Giorgio, P.A. 2010. Linking the patterns of change in composition and functional capacities in bacterioplankton successions along environmental gradients. *Ecology* 91(5): 1466-1476.

Le chapitre II explore plus particulièrement le lien qui existe entre la composition et la fonction des successions bactériennes qui s'établissent le long de multiples transitions environnementales dans un bassin versant. Ces transitions sont marquées par des types et

intensités de gradients environnementaux différents. La connexion entre composition et fonction est explorée en utilisant deux approches : l'approche déterministe basée sur les patrons absolus qui suggère un lien direct et déterministe entre les deux aspects, et l'approche dynamique basée sur l'amplitude de changements des deux aspects le long des diverses transitions environnementales, qui détermine à quel niveau de changements compositionnels on peut observer une réponse fonctionnelle. Les taux de changements dans la composition et fonctions des communautés ont été tout d'abord calculés en fonction du temps de transit dans les transitions de l'environnement, et aussi par rapport aux changements dans les principales ressources.

- **Chapitre III**. Comte, J. and del Giorgio, P.A. Assessing the role of community composition in mediating the response of bacterioplankton successions to environmental gradients. Soumis pour une publication à *Ecology letters*.

Le chapitre III examine comment les taux de changements de quatre éléments clés de la structure des communautés bactériennes (SCC, PS, BCC, BA) sont liés les uns aux autres, et à la fois aux changements dans les principales ressources et le métabolisme de la communauté. L'objectif est de déterminer dans quelle mesure la réponse métabolique de la communauté aux gradients environnementaux résulte d'un ajustement métabolique et physiologique des taxons déjà établis dans la communauté ou d'un remplacement des taxons présents dans la communauté. Des modèles de corrélation ont été testés entre les différents aspects de la structure de la communauté, mais également face au métabolisme de la communauté et les ressources le long des différents écotones. Le but est d'identifier la séquence la plus probable de connexions qui est déclenchée par des changements dans les ressources, se propage au sein de la structure des communautés, et aboutit finalement à des changements dans la performance métabolique globale de la communauté. L'influence du type et de l'intensité des gradients ainsi que du niveau de redondance fonctionnelle sur le type de réponse de la part des bactéries a été évaluée.

- **Chapitre IV**. Comte, J., Fauteux, L. and del Giorgio, P.A. Relative importance of metabolic plasticity and functional redundancy in the response of bacterioplankton communities to environmental changes. En préparation pour une soumission à *Ecology Reports*.

Le chapitre IV examine l'influence des conditions environnementales dans la configuration tant métabolique, fonctionnelle et compositionnelle des communautés

bactériennes. L'approche basée sur des expériences de transplantations de bactéries impose aux communautés bactériennes un fort changement environnemental en comparaison à leur milieu d'origine. L'objectif principal de cette étude est de déterminer si les bactéries placées dans un nouvel environnement vont, au cours du temps, présenter une certaine convergence en termes de métabolisme et de composition par rapport aux communautés naturellement présentes dans ce milieu. Cette expérience est une opportunité pour déterminer dans quelle mesure, et à quelle intensité les communautés bactériennes expriment leur plasticité métabolique et redondance fonctionnelle. De plus, cette approche permet de tester si la nature du lien entre la composition et la fonction des communautés bactériennes dépend d'un certain seuil de changements dans les conditions environnementales.

APPROCHES ET MÉTHODES

Collections des données et procédures expérimentales

Dans cette étude, les échantillons ont été recueillis dans différents types d'habitats aquatiques (lacs, rivières et marais), tous originaires du même bassin versant situé dans la région des Cantons de l'Est à environ 100 kms à l'Est de Montréal. Cette stratégie d'échantillonnage limite l'influence régionale sur la structure des communautés bactériennes (Yannarell et Tripplett, 2005), permet d'adresser les questions énoncées dans la section 0.2 à l'échelle d'une métacommunauté, et de suivre la réponse des assemblages bactériens aux gradients de l'environnement sur des échelles temporelles et spatiales différentes.

Dans le chapitre I, les échantillons ont été prélevés dans un ensemble de 4 lacs, 3 rivières et 3 marais, tandis que dans les chapitres II et III, un accent particulier a été consacré sur 13 transitions environnementales qui connectent tous ces différents systèmes aquatiques et qui présentent de forts changements en termes de ressources (Tableau 2.1). Les transitions environnementales ont été déterminées afin de générer un gradient environnemental entre les différentes masses d'eau. Par exemple, pour déterminer la longueur de la transition entre une rivière et un lac, nous avons utilisé le colorant rhodamine WT (Keystone). L'ajout du colorant en amont de la transition permet de suivre l'emplacement de la plume riveraine dans le lac en cartographiant la fluorescence au cours de son transport et dispersion le long du transept (Kung *et al.*, 2000). L'ensemble du bassin a été échantillonné 3 fois pendant la

saison estivale (de Juin à Août) en 2005 (chapitres I et III). Les résultats présentés dans l'article II sont basés sur des données recueillies sur Juin et Juillet seulement en raison de données manquantes en août concernant les mesures de capacités métaboliques. Le chapitre IV est basé sur deux séries expériences de croissance bactérienne en cultures dans des sacs à dialyse qui permet aux éléments nutritifs du milieu dans lequel ils sont plongés de passer au travers tout en empêchant la diffusion des bactéries. Chaque série a été réalisée en duplicat soit 4 séries d'expériences entre juin et juillet 2006. Notre approche principale, pour la première série d'expériences, a été de placer une communauté bactérienne issue d'un lac dans un nouveau type de milieu (un marais) qui présentent de grandes différences en termes des ressources. L'expérience inverse a également été réalisée. Pour la deuxième série, les transplantations ont eu lieu entre des communautés d'un lac et de son entrée (rivière) dans les deux combinaisons. Le but est de forcer les communautés à répondre à un fort changement environnemental en exprimant soit leur plasticité métabolique, redondance fonctionnelle, ou en opérant de profond changements compositionnels, métaboliques et fonctionnels au sein de la communauté. Ces expériences comprennent un milieu de culture (eau d'un site filtrée successivement sur 3 puis 0,22 μm pour enlever les organismes) et un inoculum (dilution 1%, volume/volume). Les inocula correspondent à une eau non filtrée provenant soit du même site (contrôle) ou d'habitats distincts. Les incubations ont duré 5 jours avec des échantillons prélevés aux jours 0 (données initiales), 2, 3 et 5.

Variables mesurées

Le métabolisme des communautés bactériennes, les 4 composantes de la structure de la communauté (composition, capacités métaboliques, structure physiologique et caractéristiques individuelles) ainsi que les ressources ne peuvent être décrit avec seulement une variable ou deux. Nous avons alors utilisé une approche intégrative dans les chapitres I, III et IV, pour décrire chacun de ces compartiments. Cette approche consiste à combiner différentes mesures qui ciblent plusieurs aspects écologiquement pertinents à chacun des niveaux préalablement cités (Tableau 1.1).

Nous avons ainsi généré six matrices, où chaque ligne représente un site donné à chaque date d'échantillonnage, et les colonnes correspondent aux valeurs des variables considérées de chaque catégorie pour un échantillon particulier. Ainsi, une matrice des

ressources (TP, TN, DOC et couleur de l'eau), une matrice du métabolisme total de la communauté (BCM), qui inclut la teneur en ATP (Lundin, 2000), la production bactérienne (BP) en utilisant les taux d'absorption de leucine et de thymidine tritiée (respectivement Kirchman, 1993 ; Fuhrman et Azam, 1992), la respiration (BR) en utilisant la spectrométrie de masse (Kana et al., 1994), la demande de carbone (BCD) et l'efficacité de croissance (BGE) en utilisant les mesures de BP et BR, et quatre matrices décrivant différents aspects de la structure de la communauté: une matrice de composition (BCC) issue des profils de l'ADNr 16S par gel d'électrophorèse en gradient dénaturant (DGGE, Muyer & Smalla, 1998), une matrice de capacités métaboliques (MC) reposant sur l'utilisation de substrats carbonés de type Biolog Ecoplates (Garland, Mills et Young, 2001); une matrice physiologique (PS), basée sur la distribution de plusieurs indices d'activité individuelle des bactéries en utilisant la cytométrie de flux (contenu en ADN, intégrité membranaire, respiration), et une matrice des caractéristiques individuelles des cellules (SCC) basée sur la moyenne des propriétés cytométriques (fluorescence) des cellules ont été générées. Dans le chapitre II, nous nous sommes concentrés plus particulièrement sur le lien entre la composition (DGGE) et les capacités métaboliques des communautés. Dans le chapitre IV, BCM a été déterminé en utilisant seulement les mesures de la production bactérienne (assimilation de leucine et thymidine tritiée) en raison d'un volume restreint d'eau disponible dans les sacs pour toutes les analyses. Les matrices dans le cadre de cette expérience, ont été construites selon le même format que décrit précédemment à la différence près que les lignes correspondaient aux différents temps de mesures de l'expérience.

Approches utilisées

Pour étudier les liens entre tous ces aspects des communautés bactériennes et leurs ressources, nous avons utilisé deux approches: explorer la corrélation entre les patrons absolus dans chacun de ces compartiments (Chapitres I, II) et la corrélation entre ces mêmes compartiments mais en termes de leur amplitude de changements face à des gradients de l'environnement (Chapitres II , III et IV). Dans les deux approches, nous avons généré des matrices de dissimilitude en utilisant les distances euclidiennes entre tous les sites d'échantillonnage sur la base de l'ensemble des variables considérées dans les différents niveaux. Les valeurs de distances euclidiennes renseignent sur le degré de différence entre les

sites pour chacunc dcs composantes considérées. Dans le chapitre I, l'obejctif est de décrire les liens entre les patrons des composants de la structure, des resources et du métabolisme de la communauté. Les corrélations entre les patrons absolus ont été réalisées en utilisant l'analyse Procruste (Peres-Neto et Jackson, 2001). Cette méthode compare des matrices de dissimilitude, en faisant correspondre les points par des processus de rotations des deux matrices afin de déterminer la meilleure superposition. Dans le chapitre II, nous nous sommes intéressés particulièrement à la connection entre la composition et la fonction des successions des communautés bactériennes le long de gradients en vironnmentaux, non pas en termes des patrons absolus mais en en termes de leur magnitude de changements. Nous avons donc utilisé une deuxième approche où un taux de changement dans tous les compartiments a été estimé pour chaque transition environnementale (Figure 2.2). Ces taux ont été calculés par rapport au temps de transit de l'eau (TT), c'est-à-dire le temps de transit moyen d'une masse d'eau entre deux points successifs d'échantillonnage dans une transition donnée. TT a été calculé pour les cours d'eau en utilisant la distance entre les sites, et la décharge moyenne mesurée dans le transept. Pour les lacs et les marais, la vitesse latérale moyenne des eaux de surface basée sur la vitesse moyenne du vent (données de Environnement Canada) a été utilisée selon la méthode décrite par Kalff (2002). Le premier point d'échantillonnage de chaque transition a été considéré comme la référence de la transition, c'est-à-dire les mesures de dissimilitude des différents points d'échantillonnage le long de la transition sont calculées par rapport à ce point. Ces mesures de distance euclidienne sont en suite mise dans un modèle de régression des moindres carrés en fonction de TT, et la pente de cette relation est alors considérée comme une estimation du taux de changement de BCC, MC et autres, le long de chaque transition. Nous avons fait cela pour chacune des 13 zones de transitions et pour toute la période d'échantillonnage (Figure 2.2). Dans le chapitre II, en plus d'avoir estimé les taux de changements de MC et BCC par rapport à TT, ces mêmes taux de changements ont été estimés comme une fonction d'une part des différences de concentration dans l'ensemble des principales ressources et également dans la concentration en phosphore (TP), azote (TN) et du carbone organique (DOC) entre ces mêmes stations. La pente issue de ces modèles de régressions représente ainsi l'estimation des taux de changements de BCC et MC par unité de changement dans chaque type de ces ressources.

Dans le Chapitre III, l'objectif est de décrire la séquence de relations de cause à

effetx qui partant de changement dans les ressources se propagent au sein de la structure de la communauté bactérienne et détermine la performance métabolique globale de la commuanuté. Pour se faire, nous avons utilisé les méthodes d'analyses de pistes de type équations structurelles (SEM) pour identifier la séquence des relations de causalité qui peuvent exister entre les taux de changements des ressources, des composantes de la structure des communautés (BCC, PS, le SCC et BA) et le métabolisme de la communauté (BCM). En particulier, ces analyses nous permettront de déterminer la position (c-à-d le rôle) que BCC a dans cette séquence. Les analyses SEM sont une extension des modèles de régression linéaire et permettent d'identifier et d'interpréter les relations linéaires parmis plusieurs composants, en vérifiant si les covariances entre les variables sont conformes à une hypothétique structure causale des relations qui existent entre ces variables (Shipley, 2002).

Dans le chapitre IV, l'objectif est de déterminer dans quelle mesure l'environnement détermine la configuration des communautés bactériennes. Pour se faire, on a estimé la dissimilitude, en termes des cinq compartiments considérés (SCC, BCC, PS, MC, BCM), entre les deux sources d'inocula bactériens (ex: bactéries issues du marais et du lac) placés dans le même milieu (ex : eau du lac), en observant la tendance de ces valeurs de dissimilitude aux différents temps de mesures. Le signe de la pente de la relation entre la dissimilitude et le temps nous renseigne si, au cours du temps, les communautés présentent une certaine convergence (pente négative), divergence (pente positive) ou aucun patron (pente nulle) dans les différents compartiments étudiés.

CHAPITRE I

LINKS BETWEEN RESOURCES, C METABOLISM, AND THE MAJOR COMPONENTS OF BACTERIOPLANKTON COMMUNITY STRUCTURE ACROSS A RANGE OF FRESHWATER ECOSYSTEMS.

Jérôme Comte and Paul A. del Giorgio

Published in *Environmental Microbiology* (2009), 11(7): 1704-1716.

Groupe de Recherche Interuniversitaire en Limnologie (GRIL), Dépt. des sciences biologiques, Université du Québec à Montréal, CP 8888, Succ. Centre Ville, Montréal, Québec, Canada, H3C 3P8.

AUTHOR CONTRIBUTIONS:

J. Comte designed and performed research, analyzed the data, created the figures, and wrote the paper. P.A. del Giorgio participated in developing the ideas, provided advice on experimental setup and analyses, and commented on the paper.

N.B : References cited in this chapter are presented at the end of the thesis.

1.1 RÉSUMÉ

Dans cette étude, nous avons exploré les patrons en termes du métabolisme global des communautés de bactérioplancton (BCM) ainsi que quatre composants de la structure des communautés (composition (BCC), les capacités métaboliques (MC), la structure physiologique (PS), et les caractéristiques de cellule unique (SCC)), entre les lacs, les rivières, et les marais d'un même bassin versant. L'objectif de l'étude est d'évaluer les liens qui existent entre ces différents composants et avec les principales ressources (matières organiques, nutriments). Les résultats montrent que les types d'habitats étaient bien distincts à la fois en termes des ressources et de BCM et leurs matrices de dissimilitude correspondantes étaient significativement corrélées, ce qui suggère que BCM suit les conditions de ressources de manière cohérente à travers les différents types d'écosystèmes. MC a également séparé les différents habitats et a été corrélé à BCM, mais moins avec les ressources, alors que BCC parfois abouti à une séparation claire des habitats, mais a rarement été corrélée aux ressources et jamais à BCM, ce qui suggère un degré élevé de spécificité écosystémique à ce niveau. Enfin, il n'y avait pas de séparation nette des habitats en termes de PS et SCC, et aucune corrélation n'a été observée ni avec les ressources ni avec BCM. Les patrons d'habitats en termes de ces différents composants de la structure sont rarement corrélés les uns aux autres, ce qui indique de faibles connexions déterministes entre eux. MC semble médié le lien entre les ressources et BCM plus directement et de façon uniforme à travers les différents systèmes; BCC semble être plus influencé par des facteurs spécifiques aux écosystèmes qui affaiblissent sa connexion à la fois avec les ressources et BCM, alors que PS et SCC ne montrent aucune tendance perceptible. Nos résultats suggèrent donc que la régulation de BCM par les ressources est médiée par des changements complexes dans les composantes de la structure de la communauté qui peuvent être directionnels, spécifiques aux écosystèmes ou apparemment aléatoire, mais qui néanmoins se combinent pour conduire à une réponse systématique du métabolisme du carbone à l'ensemble des ressources.

MOTS CLÉS: bacterioplancton, métabolisme du carbone, structure de la communité, patrons d'habitats, basin versant

1.2 ABSTRACT

We explored the patterns in bacterioplankton community metabolism (BCM) and four components of community structure (composition (BCC), metabolic capacities (MC), physiological structure (PS), and single-cell characteristics (SCC)), between lakes, rivers, and marshes within a watershed in Québec, to assess the connections that exist between them and with the main resources (organic matter, nutrients). Habitat types were well segregated by both resources and BCM and their corresponding dissimilarity matrices were significantly correlated, suggesting that BCM tracks resource conditions in a consistent manner across ecosystem types. MC also segregated the various habitats and was correlated to BCM but less so to resources, whereas BCC at times resulted in a clear separation of habitats, but was rarely correlated to resources and never to BCM, suggesting a higher degree of ecosystem specificity at this particular level. Finally, there was no clear separation of habitats in terms of PS and SCC, and none covaried with resources or BCM. The habitat patterns based on these different components of structure were rarely correlated to each other, indicating weak deterministic connections between them. MC appear to mediate the link between resources and BCM more directly and consistently across systems; BCC appears to be more influenced by ecosystem-specific factors that weaken its overall connection to both resources and BCM, whereas PS and SCC show no discernible patterns. Our results thus suggest that the bottom-up regulation of BCM by resources is mediated by complex shifts within components of community structure that can be directional, ecosystem-specific or apparently random, which combined nevertheless result in a systematic overall response to resources in terms of C metabolism.

KEY WORDS: bacterioplankton, carbon metabolism, community structure, habitat patterns, watershed.

ACKNOWLEDGMENTS

The authors thank F. Guillemette, L. Fauteux, P. Tremblay-Dauphinais, M. Bergevin, and. C. Beauchemin for their help in the collection and analysis of samples. Stream physical data were provided by Yves Prairie and Alice Parkes. We appreciate the helpful discussions and assistance provided by P. R. Peres-Neto for the statistical analyses. We are grateful to Richard Vogt and two anonymous reviewers for critical and constructive comments on the manuscript. This work was supported by grants from the National Science and Engineering Research Council of Canada.

1.3 INTRODUCTION

The structure of bacterioplankton communities is multifaceted and extremely complex (Smith and del Giorgio, 2003). Community structure is determined by a series of properties that range from individual cell characteristics to the overall performance of the assemblage. Examples of these various components are the distribution of cell sizes, morphometry and physiological properties, the distribution of physiological states, the diversity and distribution of metabolic capacities, and the number, identity and distribution of different phylotypes. These various components of community structure together determine the overall community performance, in terms of carbon and nutrient metabolism, for example.

These different aspects of community structure have not all been explored with the same intensity, mostly due to past methodological limitations, but nevertheless it is now clear that they are all highly dynamic, both spatially and temporally. There is now evidence that certain aspects appear to be ecosystem-specific and others follow environmental gradients. For example, Crump et al. (2007) observed similar bacterial community composition (BCC) among lakes within a watershed, whereas streams had a distinct composition and connecting streams were closely related to lakes, suggesting an ecosystem-specific differentiation in BCC. Likewise, large-scale comparative studies have reported systematic differences in phylogenetic composition between marine and freshwaters (Glöckner et al., 1999). Other components of bacterial community structure, such as bacterial metabolic capacities (Garland and Mills, 1991) or single-cell cytometric characteristics (Bouvier et al., 2007), have been reported to present varying degrees of ecosystem specificity. Shifts in various aspects of community structure have been reported along productivity and salinity gradients. For example, various studies have showed changes in the distribution of physiological states (del Giorgio and Gasol, 2008) and carbon metabolism and function (del Giorgio and Cole, 1998; del Giorgio and Bouvier, 2002; Kirchman et al., 2004) along such gradients, but it is not clear to what extent these shifts are ecosystem-specific or whether they are linked to each other.

The sampling strategy may strongly influence and obscure the apparent patterns, since bacterial communities are influenced by both local and regional forces (Yannarell and Triplett, 2005), which are confounded in most studies. Ecosystem-specific patterns in

Table 1.1

Description of the variables included in each of the dissimilarity matrices considered in this study.

Dissimilarity matrices	Variables
Resource conditions (Res)	Concentrations of Total nitrogen, phosphorus and dissolved organic carbon; water absorbance at 440 and 280 nm
Bacterial community metabolism (BCM)	Rates of bacterial biomass production (BP, ^{3}H-Leucine), growth (BGR, ^{3}H-Thymidine), ratio BP:BGR, respiration, growth efficiency, carbon demand, ATP content
Bacterial community composition (BCC)	relative contribution of each band to the total intensity of the lane
Bacterial community metabolic capacities (MC)	Average absorbance values of the 31 carbon substrates for the time at which the overall plate color development was closest to the reference point of 0.5 AWCD
Bacterial physiological structure (PS)	Specific rates of BP, BGR, respiration, ATP content, proportion of high DNA content (HNA), respiring, injured, intact and dead cells
Bacterial single-cell characteristics (SCC)	Fluorescence and side scatter values of HNA, respiring cells, fluorescence values of injured, intact and dead cells

bacterial community structure may exist but be confounded by cross-regional differences that are superimposed on local patterns. In addition, most studies have focused on a particular component of bacterial community structure (for example phylogenetic composition or metabolism), and most often limited to individual properties within each component (for example, number of phylotypes or bacterial production). The limitation of current approaches is that they do not provide an integrative view of the overall response of bacterial structure to environmental forcing, and how its different components interact to shape the resulting bacterial processes at the ecosystem level. The few studies that have combined several approaches to describe bacterial community structure have yielded conflicting results, some reporting links between the different components (del Giorgio et al., 1997; Bertilsson et al., 2007), while others reporting weak or no links (Langenheder, Lindström et Tranvik, 2005; Findlay and Sinsabaugh, 2006).

As a result, there are two key questions that remain unanswered: 1) Are there components of bacterioplankton community structure that are ecosystem-specific and others that show a common response to environmental forcing regardless of the ecosystem-type? 2) Are these components of structure linked, and which tend to be more closely related and covary in the environment? While we have a wealth of published information on various aspects of bacterioplankton structure for a wide range of aquatic systems, it is presently difficult, if not impossible, to effectively address these fundamental questions.

In this paper, we explore the distribution of five distinct components of bacterial community structure (metabolism, composition, metabolic capacities, distribution of cell physiologic states, and single-cell characteristics) in different habitats (lakes, rivers, marshes) within a complex watershed in Southern Québec. We worked within a single watershed to avoid confounding regional influences. Because bacterial community structure cannot be effectively described with a single factor, we developed a more integrative approach by combining measurements that target several ecologically relevant aspects at each level. We have thus generated six matrices characterizing these systems and their respective bacterioplankton communities (Table 1): A resource matrix, a community metabolism matrix, that includes total bacterial ATP content, bacterial production, respiration, carbon demand and growth efficiency; and four matrices describing various aspects of community structure: a composition matrix based on the DGGE profiles; a metabolic capacity matrix

based on substrate utilization profiles; a physiological matrix, based on the distribution of several indices of bacterial single-cell activity, and a single-cell matrix, based on the average cytometric properties of individual cells.

We calculated dissimilarity matrices of sites based on each of the above six categories, and carried out multidimensional scaling (MDS) analyses to assess which of the components of bacterioplankton structure are ecosystem-specific, and which show little or no distinct ecosystem patterns. We further explored the links between these various aspects by analyzing the correlation (using Procrustes analysis) between the various matrices. Finally, we assessed which aspects of community structure are more strongly related to the ensemble of resources.

1.4 METHODS

1.4.1 Sampling site and environmental parameters

The study area is located in Southern Québec (Fig. 1), 100kms East of Montreal (45.508N, 73.588W). We sampled a set of 4 lakes, 3 marshes and 3 rivers in the same watershed, 3 times in 2005 during the growing season (June to August). Water was collected at the sub-surface (50 cm) to avoid contamination by sediment, and stored in acid washed bottles. All the laboratory analyses were carried out within 2-3 hours of sample collection.

Concentrations of total phosphorus (TP) and total nitrogen (TN) were measured by the persulfate digestion method. Colorimetric analyses were carried out on spectrophotometer for phosphorus and on an Alpkem RFA300 Flow Solution IV autoanalyzer (OI analytical) for nitrogen. Concentrations of DOC were determined in acidified 0.2 mm filtered samples by high temperature persulfate oxidation on a TIC TOC 1010 (OI analytical). In addition, water absorbance was measured spectrophotometrically at both 440 and 280 nm.

1.4.2 Bacterial community metabolism and activity

Bacterial production (BP) rates were estimated by the use of both ^{3}H-Leucine (Kirchman, 1993) and ^{3}H Thymidine (Fuhrman and Azam, 1982) uptake. ^{3}H-Thymidine incorporation may be used as an indication of cell division and DNA synthesis, while ^{3}H-Leucine incorporation is an estimation of protein synthesis. 1.5 ml samples incubated for 1 h in the dark in microcentrifuge tubes containing 40 nM of ^{3}H-Leucine (60 Ci/mmol, MP Biochemicals) and 20 nM of ^{3}H-Thymidine (60 Ci/mmol, MP Biochemicals) and then were fixed by adding TCA. Killed controls (with TCA) of ^{3}H-Leucine and ^{3}H-Thymidine were regularly carried out. All the incubations were performed with the same brand of tube in order to avoid variations in the measurements as observed by Pace et al. (2004). Rates of leucine incorporation were converted to BP assuming a leucine to C conversion factor of 3.1 (Kirchman, 1993). The ratio Leucine:Thymidine was calculated as an indication of whether bacteria are dividing or producing biomass.

Bacterial respiration (BR) was determined as oxygen consumption in water samples that were pre-filtered through Whatman GF-D filters to remove larger planktonic organisms, following del Giorgio and Bouvier (2002). Briefly, filtered samples were incubated in flow-

through systems consisting of a 4-L Erlenmeyer connected to a 4-L Cubitainer, which acts as a reservoir. The systems were incubated in the dark, and the water in the Erlenmeyer was sampled at 0, 2, 4, 6 hours through an outlet valve, and three 5-ml glass tubes were filled at each sampling time point, for oxygen determination; additional water was taken for leucine incorporation measurements, and the resulting average BP values were used to calculate bacterial growth efficiency (see below). The tubes were poisoned with 8µl saturated HgCl solution, and capped with a ground glass stoppers. The tubes were kept immersed in water at 10°C prior to analysis. Oxygen concentration was determined using a dual-inlet mass spectrometer (Kana et al., 1994), which determines the ratio of argon to oxygen after the gases in the sample have diffused through a permeable capillary. The rate of oxygen consumption was determined using linear regression of oxygen concentration versus incubation time, and the rates were converted to C units assuming an RQ = 1 (del Giorgio and Cole, 1998).

Bacterial growth efficiency (BGE) was calculated as BGE=BP/(BP+BR), where BR is the respiration rate, and BP was derived as the average of the different measurements of leucine incorporation made during the incubation. Bacterial carbon demand (BCD) was calculated as BP + BR.

Total ATP concentration was determined by firefly bioluminescence, using the Microbial ATP Kit from Bio Thema (Lundin, 2000). Briefly, extracellular ATP was enzymatically degraded before the release of intracellular ATP. ATP content was assessed using high sensitive ATP reagents and light emission was measured using a microplate reader (TECAN) before and after the addition of a known amount of ATP standard.

1.4.3 Bacterial community metabolic capacities

Bacterial carbon substrate utilization profiles, determined with Biolog Ecoplate, were used as a proxy of bacterial community metabolic capacities. These 96 wells microplates contain 31 different carbon sources (in triplicate wells), plus a tetrazolium salt, which is reduced to a colored compound by active bacteria (Garland and Mills, 1991) that can be measured by colorimetry. The use of Biolog Ecoplates in aquatic systems has been questioned (e.g. Konopka et al., 1998) on the basis that it creates an artificial enrichment culture that does not represent *in situ* conditions. While it is a fact that incubation under single C sources necessarily results in the selective enrichment of certain phylotypes and

therefore an alteration of community structure relative to *in situ* conditions, this selection operates over the existing, probably the dominant phylotypes, and in that respect, the resulting metabolic profile does reflect the distribution of ambient capacities.

Each well was inoculated with 125 µl of unfiltered natural samples. Immediately upon inoculation, the zero time-point absorbance of each plate was read. Changes in color development were measured using microplate reader (TECAN) at 595 nm. The time course of color development was followed for 3 to 7 days until maximum color development was reached. The overall color development of each plate was expressed as average well color development (AWCD) as suggested by Garland and Mills (1991), but adapted for the triplicate wells per substrate in Biolog Ecoplate that gives a total of 93 response wells (containing carbon substrates). AWCD was then defined as $[\Sigma(R - C)]/93$, where R is the absorbance of each response well, C is the average of the absorbance of the control wells.

Preliminary tests confirmed the good repeatability and consistency of Biolog Ecoplates results by preparing replicate plates with the same inocula, which yielded identical sigmoidal patterns (data not shown). We also compared both kinetic (rate of color development, maximum absorbance) and single point reading approaches (0.5 and 1 AWCD) as described in Garland et al. (2001) in order to maximize the discrimination of the different habitat types in terms of metabolic properties on the dataset considered here. We observed a similar ordination between kinetic and single point reading approaches. Since the kinetic approach has been reported to be more time consuming and more sensitive to the initial density of bacterial inocula (Garland et al., 2001), differences in the overall rate of color development between samples were assessed using the single point reading approach. To do this, the average well-color development for the entire plate was computed at each time that the plates were read and profiles for each plate for the time at which the AWCD was closest to the reference absorbance target were examined. Here, we used a value of 0.5 AWCD (± 0.2) that typically corresponds to the linear portion of the sigmoidal curve of the overall plate color development and gives the greatest difference in substrate utilization patterns among various types of bacterial communities (Garland, 1996; Choi and Dobbs, 1999; Schultz and Ducklow, 2000; Garland et al., 2001). The absorbance of the control well was subtracted from the absorbance of each well ($R - C$) (aka raw difference data in Garland and Mills, 1991). Garland and Mills (1991) suggested normalizing the data by dividing absorbance

values of each well by the AWCD in order to minimize the influence of both the incubation time and the bacterial density on the overall plate color development. However, we have tested the impact of initial density on the resulting MDS ordination of raw differences and normalized data originating from the same samples and we observed that in addition to obtaining similar MDS ordination of Biolog data, no significant difference was noted between the resulting dissimilarity matrices based on Euclidean distances (Primer 5.2 software) (data not shown). This result suggests that initial density had a negligible role in patterns of bacterial communities' metabolic capacities in our system, as already observed by others (Choi and Dobbs, 1999). Thus, we used raw differences data to build dissimilarity matrices. No data for Biolog Ecoplates are available for the third sampling date (August) due to technical problems.

1.4.4 Bacterial community composition

Bacterial community composition was determined by denaturing gel gradient electrophoresis (DGGE) of 16S rDNA. 1.5 ml water samples were pipetted into sterile eppendorf tubes and centrifuged at 14,000 rpm for 30 min. After removing the supernatant, the remaining pellets were frozen at -80°C until analysis. DNA extraction from the collected cells was done by adding 1 ml of CTAB buffer (100 mM TrisHCl pH 8.0, 1.4 M NaCl, 2% (w/v) CTAB, 0.4% (v/v) beta-mercaptoethanol, 1.0% PVP (polyvinyl pyrrolidone), 20 mM EDTA) and freezing samples overnight at -80oC. Once thawed, each sample received 4 µl of 0.4% (v/v) beta-mercaptoethanol and incubated at 65oC for 15 minutes. Equal volumes of chloroform/isoamyl alcohol (24:1) were added to the vials and mixed for 20 minutes at room temperature. After centrifugation at 12,500 rpm for 15 minutes, the aqueous phase was extracted one more time with chloroform/isoamyl alcohol and centrifuged for 5 minutes at 12,500 rpm. The aqueous layer was mixed with ½ volume of 5M NaCl and 1 volume of isopropanol and incubated at -80oC for 1-2 hours. After incubation, each tube was centrifuged at 12,500 rpm for 30 minutes. The pellet containing nucleic acids was washed with 500 µl of 70% ETOH and the mixture was centrifuged for 5 minutes at 12,500 rpm. Following ETOH removal, the pellets were air-dried and finally resuspended in 25 µl of sterile water on ice during 1-2 hours with gentle vortexing.

DNA extracts were amplified in 50 µl polymerase chain reaction (PCRs). The reaction contained 1.5 mM MgCl2, 1x PCR buffer, 200 µM of each dNTPs, 1.25U of Taq

polymerase (Taq PCR core Kit, Qiagen), 0.15 µg µl-1 of BSA (Sigma Aldrich) and 0.5 µM of each primers. Primers 358 F (5'-CCT ACG GGA GGC AGC AG-3') with a GC clamp, and 907 rM (5'-CCG TCA ATT CMT TTG AGT TT-3') (both HPLC purified, Sigma Genosys) were used. Amplification was conducted using a touchdown PCR cycle as described in Schauer et al. (2003). PCR products (250 ng of DNA) were stained with 20 µl of blue stain for DGGE and analyzed in 40–80 denaturant gradient gels, which were run for 16 h at 100V and 600C with Dcode (Biorad). Bands formed were stained with SYBR Gold (Molecular probes) and visualized under UV illumination. DGGE pictures were then analyzed with the Quantity one software (Biorad). Bands in each lane were identified and the relative contribution of each band to the total band signal in the lane was estimated. To minimize bias in the estimation of band intensity, the background intensity was subtracted using a rolling disk. Bands located in the same position in the different lanes of the gel were considered as the same population.

1.4.5 Bacterial single-cell characteristics and activity

Bacterial single-cell analyses were performed using a FACScalibur flow cytometer (Becton Dickinson), equipped with an argon laser, at the lowest possible flow rate (12 µl min^{-1}), using 1 µm beads solution as internal standard. Beads concentration was systematically controlled before running samples using Truecount Absolute counting tubes (BD biosciences). Total bacterial abundance was determined using SYTO 13 staining (del Giorgio et al., 1996). Samples (500 µl) were incubated 5 min in the dark and High and Low-DNA fractions (Lebaron et al., 1998; Trousselier et al., 1999) were discriminated on the basis of their green fluorescence (FL1) and side scatter signals (SSC). Total abundance was considered as the sum of both fractions.

Respiring cells were enumerated using CTC, which provides an index of the activity of the respiratory electron transport system. Bacteria with high rates of respiration are able to reduce CTC and produce enough red fluorescence to be detected by flow cytometry (del Giorgio et al., 1997). A stock solution of 50 mM CTC (PolySciences) was freshly prepared, filtered through 0.1 µm and added to 900 µl of sample to a final CTC concentration of 5 mM and then incubated for 1 h at room temperature in the dark. At the end of the incubation, the samples were run in the cytometer and CTC+ cells were discriminated on the basis of the

orange fluorescence of CTC (FL2) and the SSC. The percentage of CTC+ cells was calculated relative to the total bacterial counts obtained by SYTO-13 staining.

Cells with compromised membranes were enumerated with the potential-sensitive dye DiBAC4 (3) (Molecular Probes). This oxonol is actively excluded from intact cells whereas it accumulates in cells that have lost their membrane potential, producing intracellular green fluorescence (Shapiro, 2000). The samples (500 µl) were vortexed and incubated for 10 min with 2 µl of a DiBAC stock solution (0.5 mg ml^{-1}). Cells stained with DiBAC were enumerated from a cytogram on the basis of their FL1 and SSC signals.

Cells with intact membrane potential were monitored by flow cytometry using carbocyanine dihexyloxacarbocyanine iodide (DiOC6 (3), Molecular Probes). DiOC6 (3) is a cell-permeant, green-fluorescent, lipophilic dye (Shapiro, 2000). Working solutions (10 µM) were prepared in DMSO and kept at room temperature in the dark up to a month. 3.5 µl of the staining solution was added to 500 µl of sample (final concentration of 70 nM), vortexed and incubated for 20 min. Green 1 µm beads were added and the samples were run in the cytometer using the green fluorescence of DiOC (FL1) and the light SSC emission.

Cells with damaged membranes were enumerated using the Live/Dead BacLight kit (Molecular Probes), which is a mixture of a cell-impermeant nucleic acid stain (propidium iodide) that only penetrates compromised cells, and SYTO 9, which is a cell permeant green nucleic acid stain. Damaged cells have higher red fluorescence due to increased uptake of propidium iodide compared to intact cells, which have greater green fluorescence (Gasol et al., 1999). Samples (500 µl) incubated with 2 µl of the mixture during 10 min in the dark at room temperature and then 1 µm beads were added prior to cytometric analysis. Discrimination of live and dead (or damaged) cells was based on the red (FL3) versus green (FL1) fluorescence signals of the stained cells.

The matrix of single-cell characteristics consisted of the average cytometric parameters obtained from the analyses described above: 1) The average FL1 and SSC of the High- DNA cells identified using SYTO 13; 2) the average FL2 and SSC of the CTC+ cells; 3) the average FL1 of cells stained with DIOC and DIBAC, and 4) the average FL3 obtained from the Live/Dead BacLight assay.

The matrix of physiologic structure consisted of the proportion of the different cellular fractions described in the previous section, relative to the total number of cells

determined using SYTO 13: % of High- DNA cells, % CTC+ cells; % of depolarized (DIBAC+) cells, % intact cells based on DIOC and % dead cells based on the PI/SYTO 9 assay. In addition, we included in this matrix the estimates of cell-specific metabolism, calculated as the bulk measurements of activity described above (BP-leucine, BP-thymidine, BR, and ATP) divided by the total bacterial abundance estimated using SYTO 13. Low-DNA cells were not included in both the single-cell characteristics and physiological structure matrices to avoid a potential autocorrelation bias within each matrix.

1.4.6 Data treatment and statistical analyses

For each of the 4 components of structure, bacterial community metabolism and for resources, we constructed a raw data matrix where each row represented a given site at each sampling date, and the columns corresponded to the values of each category measured for that particular sample. Since the different components analyzed involved a different number of variables, the raw data matrices did not necessarily have the same number of columns. In contrast, the resulting dissimilarity matrices have the same number of columns since the number of sites is the same. Table 1 summarizes the variables (columns) involved in each of the matrices. In the case of the Composition and Metabolic Capacities matrices, each column corresponds to either a band from the DGGE analysis, or a substrate from the BIOLOG analysis.

The BCC matrix was generated from arcsine-transformed data using the Bray-Curtis dissimilarity index. The physiology dissimilarity matrix was generated using Euclidean distances on arcsin-transformed data (proportion of active or intact cells) as suggested for similarity matrices based on percentage data. The four other matrices were based on Euclidean distances using log-transformed data with the exception of BGE (in the metabolism similarity matrix), which was arcsin-transformed. Ecosystem-specific patterns in resource conditions, BCM and in the four components of bacterial community structure were explored using MDS (Primer 5.2 software), on the basis of dissimilarity matrices of sites calculated for each of the 6 major categories. Each category is composed of a set of different variables, as described above. The associations between resources, BCM, MC, BCC, physiological structure and single-cell characteristics were analyzed using a Procrustes analysis (Matlab 7.5.0 software). This approach is more robust to describe and test patterns of association among multivariate data sets than the most commonly used Mantel test (Peres-

Neto and Jackson, 2001). Briefly, the method compares sets of raw data or similarity matrices, by matching corresponding points (sampling sites in the case of this study) from both multivariate datasets, determining the best superimposition that maximizes their fit trough scales and rotations processes of data matrices (Peres-Neto and Jackson, 2001). The sum of squared residuals between scaled and rotated configurations of each matrix is used as a metric of association (m^2) (Peres-Neto and Jackson, 2001). The m^2 metric varies between 0 and 1, and smaller values of m^2 indicate stronger concordance between dissimilarity matrices. Procrustes analyses were based on dissimilarity matrices of metabolic capacities, composition, metabolism, physiology, single cell characteristic and resources that were generated using the Primer 5.2 software.

Figure 1.1 Aereal photograph of the study watershed. Sampling sites are indicated with white solid circles, arrows indicate the water flow from headwaters downstreams.

1.5 RESULTS

1.5.1 Cross-ecosystem variation in resources

The study watershed is composed of sub-basins each containing one or several headwater streams, lakes and marshes (Fig. 1.1). These systems are interconnected through a network of rivers, which in turn connect the various sub-basins. For this study we chose a set of 4 lakes, 3 marshes and 3 rivers; one of the rivers is a headwater system and, the two others connect pairs of lakes. Likewise, one of the marshes is a headwater system, whereas two others lie along the water flow path between two of the sampled lakes. The systems sampled thus have different degrees of connection to each other. These systems present clear differences in DOC and nutrient concentrations (summarized in Table 1.2). Lakes had overall lower DOC and nutrient concentrations, and rivers and marshes had similar ranges in their resource characteristics.

Analysis of the resource matrix (based on TP, TN, DOC and absorbance data) using multidimensional scaling revealed a separation between habitat types although the high connectivity that exists between the different habitats generates some overlap among the different sites (Fig. 1.2). Lakes tend to cluster together, whereas the headwater rivers and marshes form a distinct group. The connecting rivers and marshes lie between these two groupings, suggesting that these transition systems present intermediate resource conditions. The overall resource conditions appear to be relatively stable within headwater systems because the three sampling dates tend to fall close to each other in the ordination plane, whereas connecting systems present an apparent convergence in resources conditions in August (Fig. 1.2).

Table 1.2

Some key characteristics of the different habitats in the watershed. Minimal-maximal concentrations (average ± standard deviation) of dissolved organic carbon (DOC, mg l^{-1}), total phosphorus (TP, µg l^{-1}), total nitrogen (TN, mg l^{-1}) and minimal-maximal measurements (average ± standard deviation) of bacterial production rates estimated by ^{3}H-Leucine uptake (BP, µgC l^{-1} h^{-1}), bacterial respiration (BR, µgC l^{-1} h^{-1}), bacterial growth efficiency (BGE, %) from June to August 2005.

Habitats	DOC	TP	TN	BP	BR	BGE
Lakes	2.2 - 6.4 (4.4 ±1.37)	0.4 - 9.7 (5.4 ±3.34)	0.1 - 0.3 (0.2 ±0.05)	0.03 - 0.4 (0.2 ±0.1)	0.8 - 4.3 (1.8 ±1)	3.7 - 49.3 (17.4 ±14.2)
Rivers	4.8 - 12.2 (5.7 ±1.9)	4.4 - 22.3 (9.3 ±2.5)	0.2 - 0.5 (0.3 ±0.07)	0.5 – 7.9 (2.8 ±2.2)	1.7 – 7.5 (3.7 ±1.9)	8.1 – 59.2 (34.2 ±14.4)
Marshes	4.4 - 13.3 (7.55 ±2.3)	6 - 20.1 (13 ±7.1)	0.2 - 0.4 (0 32 ±0 1)	0.6 – 7.4 (2.5 ±2)	1.8 – 9.8 (3.7 ±2.5).	22.4 – 65 (46.3 ±13.1)

1.5.2 Cross-system patterns in bacterial community metabolism

Lakes generally had the lowest rates of bacterial metabolic activity as well as the lowest growth efficiency compared to marshes and rivers (Table 1.2). The pattern of distribution of habitats based on community metabolism (Fig. 1.3a) was similar to that obtained based on resource conditions (Fig. 1.2): Lakes tend to cluster closely together, the headwater systems form a distinct group, and connecting systems lie between these two. As with the resource ordination, there was modest temporal variability and the three sampling dates for any given habitat tended to cluster in relative close proximity.

1.5.3 Cross-system patterns in bacterial community structure

The pattern of distribution of habitats based on carbon substrate utilization obtained using Biolog Ecoplates (here referred to as metabolic capacities) is shown in Fig. 1.3b. Lakes clearly separate from marshes and rivers, whereas the latter two generally overlap. Although substrate utilization was measured on only two sampling dates (in June and July), the ordination shown in Fig. 1.3b still suggests large temporal variations in metabolic capacities within any given system, larger than those observed for both metabolism and the resources.

The distribution of habitats based on bacterial physiological state (Fig. 1.3c), and single-cell characteristics (Fig. 1.3d) did not present any clear ecosystem-specific patterns. There was, however, a clear temporal separation of systems based on single-cell properties, with the August samples of most habitats grouping closely together, distinct from the two other sampling periods. This pattern would suggest a surprising convergence of single-cell characteristics between different habitats at the end of the summer.

Fig. 1.4 shows the distribution of habitats based on bacterial community composition. Each graph represents one sampling period, since the samples were analyzed in separate gels. No ecosystem-specific pattern was observed in BCC among habitats in June and August (Fig. 1.4a and c), whereas in July there was a clear separation of habitats based on DGGE banding patters (Fig. 1.4b).

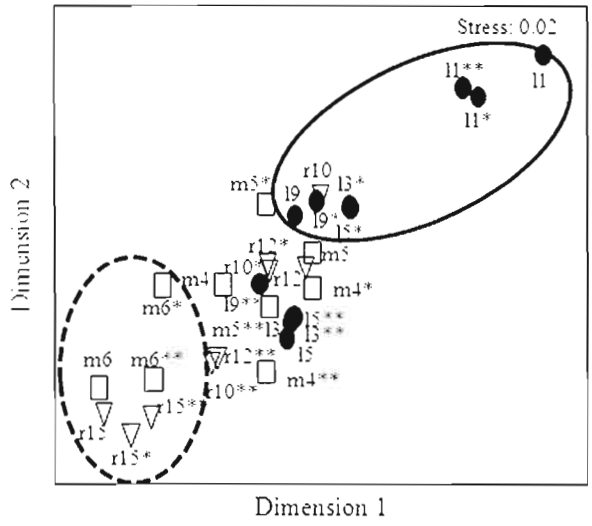


Figure 1.2 Distribution of sampling sites from MDS ordination of dissimilarity matrices (Euclidean distances) based on resources. Symbols denote the different sampling habitats: Black circles correspond to lakes, open squares to marshes, and open triangles to rivers. Sampling periods are represented as: no mark for June, * for July, and ** for august. Dashed circles indicate the grouping of headwater marsh and river and filled circles refers to lakes.

1.5.4 Links between resources and the different components of bacterial community structure

The links between resources, bacterial community metabolism, and the four different components of bacterial community structure were assessed through a Procrustes test that compares pairs of dissimilarity matrices (Table 1.3). The analysis was carried out for each sampling period separately to examine whether connections varied over the entire sampling period. Only BCM was significantly related to resource conditions for all three-study periods, suggesting that the ensemble of bacterial metabolism closely tracked resources. The other components of community structure did not show any consistent relationship with resources: None of the four components were correlated to the resource matrix in June and August, yet all but one of these bacterial community attributes (except physiological properties) showed significant correlations to the resource matrix in July, suggesting both large seasonal variations in the structure of bacterioplankton itself, and in the strength of its coupling to environmental forcing.

1.5.5 Links among the different components of bacterial community structure

Table 3 summarizes the comparison between the different aspects of the bacterial community in all possible combinations. Overall, our results show few significant relationships between the various aspects of community structure and no consistent patterns in these relationships. For example, there was a significant correlation in June between BCC and MC, suggesting a direct link between these two components of structure, but the relationship disappeared in July. Likewise, the physiological structure was significantly correlated to BCC only in one month (June).

1.5.6 Links between community metabolism and the different components of bacterial community structure

Overall, there were few significant relationships between the individual components of community structure and metabolism, and were weaker than those observed between BCM and the resources. We found that BCM was significantly related to MC in both June and July, and to the physiological structure in August, and no significant correlation was found between BCM and BCC (Table 1.3).

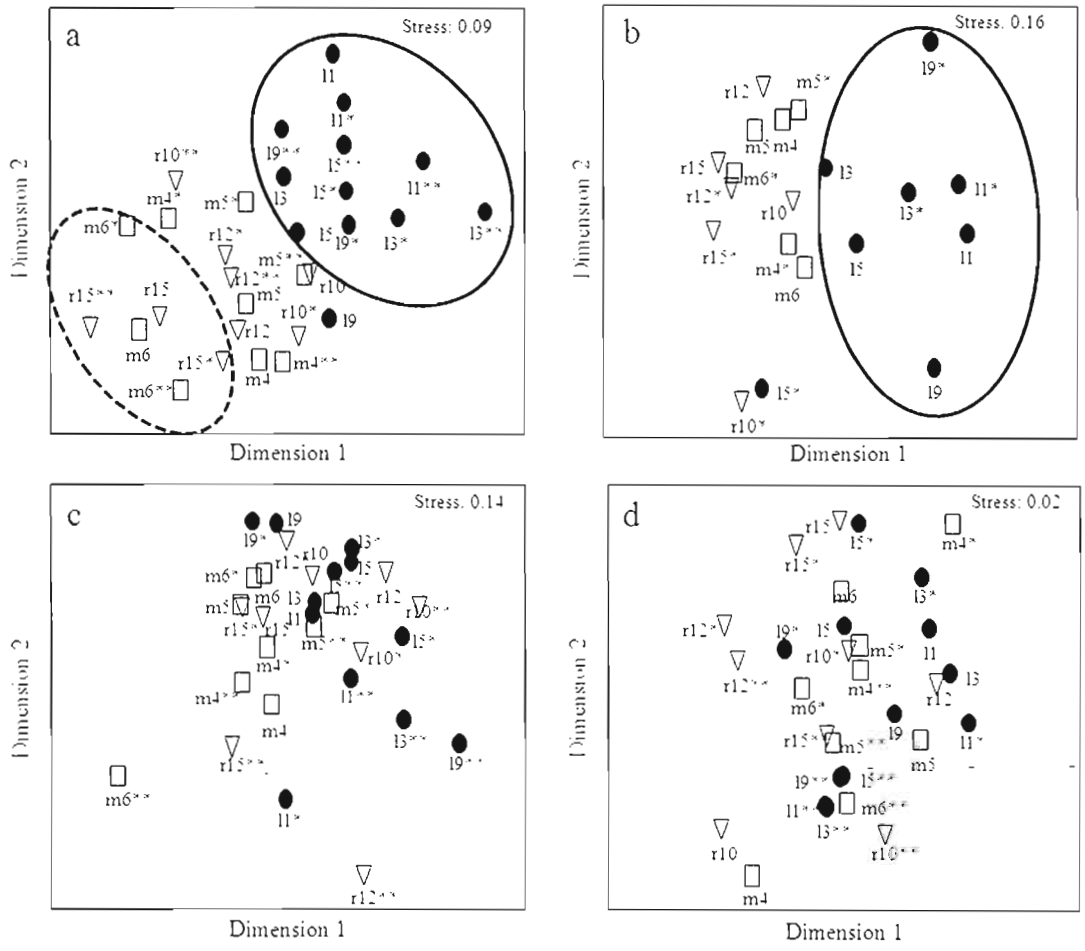


Figure 1.3 MDS ordination of dissimilarity (Euclidean distances) of the different sites in the watershed, in terms of bacterial community metabolism (a) metabolic capacities (b), physiology (c), and single-cell characteristics (d). Symbols denote the different sampling habitats: Black circles correspond to lakes, open squares to marshes, and open triangles to rivers. Sampling periods are represented as: no mark for June, * for July, and ** for august. Dashed circles indicate the grouping of headwater marsh and river and filled circles refers to lakes. Dashed circles indicate the grouping of headwater marsh, river and filled circles refers to lakes.

1.6 DISCUSSION

1.6.1 Resource tracking and ecosystem specificity of bacterioplankton metabolism and structure

Previous studies have reported strong coupling between the environment (resources in particular) and bacterial carbon metabolism (Alonso-Sàez et al., 2007; Bertilsson et al., 2007), yet most of these have focused on individual aspects of BCM (i.e. BP, growth rate) as they relate to specific resources variables (i.e. DOC or nutrients). Our own dataset reveals a large number of ecologically relevant relationships between the individual variables that we measured, but we treat these individual relationships elsewhere (Comte and del Giorgio, in prep).

Our multidimensional analysis shows that the different types of ecosystems sampled within our study watershed were clearly separated in terms of the ensemble of targeted resources, particularly lakes versus rivers and marshes. In parallel, we observed that the overall bacterial carbon metabolism also yielded clear habitat-type separation, suggesting a direct link between resources and the response of bacteria, not in terms of a single metabolic feature, but in the ensemble of C metabolism. This close coupling between overall bacterial community metabolism and the ensemble of resource conditions is further evidenced by the strong correlation between their respective dissimilarity matrices (Table 1.3). In this regard, bacterial community metabolism appears to closely track resource conditions, and to the extent that the ensemble of resources tends to differ between the major habitat-types, community metabolism reflects this distinction.

Bacterial metabolic capacities also yielded a clear separation of habitats, and were related to the metabolic matrix most of the study period, but were weakly related to resources, suggesting that this aspect of community structure presents some degree of ecosystem specificity, BCC, on the other hand, presented a higher level of ecosystem-specificity as it at times resulted in a clear separation of habitats, but was rarely correlated to resources and never to BCM. In contrast, other aspects of community structure, such as the

Table 1.3

Association metric (m^2) of Procrustes analyses between dissimilarity matrices of resources (Res, Euclidean distances), bacterial community metabolism (BCM, Euclidean distances), community composition (BCC, Bray-Curtis index), community metabolic capacities (MC, Euclidean distances), physiological structure (PS, Euclidean distances) and single-cell characteristics (SCC, Euclidean distances) for the different sampling periods. Significant correlations (in bold) are at $*P \leq 0.05$, $**P \leq 0.01$. nd (not determined).

June	*Res*	*BCM*	*BCC*	*MC*	*PS*	*SCC*
Res						
BCM	**0.49****					
BCC	0.70	0.72				
MC	0.71	**0.62***	**0.57***			
PS	0.92	0.91	**0.59***	0.74		
SCC	0.88	0.77	0.65	0.94	0.91	
July						
Res						
BCM	**0.53****					
BCC	**0.64***	0.77				
MC	**0.60***	**0.56***	0.75			
PS	0.79	0.85	0.73	0.77		
SCC	**0.62***	0.90	0.75	0.75	0.63	
August						
Res						
BCM	**0.55****					
BCC	0.92	0.9				
MC	nd	nd	nd			
PS	0.71	**0.63***	0.99	nd		
SCC	0.94	0.92	0.81	nd	0.72	

distribution of physiological states, were weakly correlated to the resource matrix (again only in July), and did not result in a clear separation of sites (Fig. 1.4, Table 1.3), suggesting little or no ecosystem-specificity.

These results suggest that bacterioplankton responses range from close tracking of resource conditions and a strong ecosystem specificity (metabolism, metabolic capacities), to weak tracking of resources but strong ecosystem-specificity (composition), to no clear relationship to either resources or to the ecosystem-type (physiological structure and single-cell characteristics).

1.6.2 Relationships between the different components of bacterial community structure and overall metabolism

There is now a large body of information concerning the direction and magnitude of change of various aspects of bacterial community metabolism in relation to the environment (e.g. Findlay et al., 1998; Smith and Prairie, 2004; Pace and Prairie, 2005). Yet we still do not understand the underlying regulation of these metabolic responses, and in particular, the role of the different components of bacterial community structure in mediating the observed overall metabolic response to shifts in the environment. There is evidence in the literature that shifts in metabolic performance of bacterioplankton communities can occur concomitantly with changes in community composition and metabolic capacities, for example, along gradients in dissolved organic matter (Eiler et al., 2003; Findlay et al., 2003; Kirchman et al., 2004; Judd et al., 2006), and there are examples of significant relationships between specific aspects of bacterial structure and of BCM (e.g. Lebaron et al., 2002; Nishimura et al., 2005; Bertilsson et al., 2007). Furthermore, some bacterial groups have been shown to have functional specialization (Cottrell and Kirchman, 2000b; Covert and Moran, 2001), so that a link between composition, function and overall metabolism would be expected.

Overall, our results show weak or no relationships between the various components of community structure, in spite of the fact that some of these components resulted in relatively similar ordination of sites. For example, while composition and metabolic capacities both allowed for clear differentiation between lakes and other systems, they were rarely significantly correlated to each other (Table 1.3). Such uncoupling between different

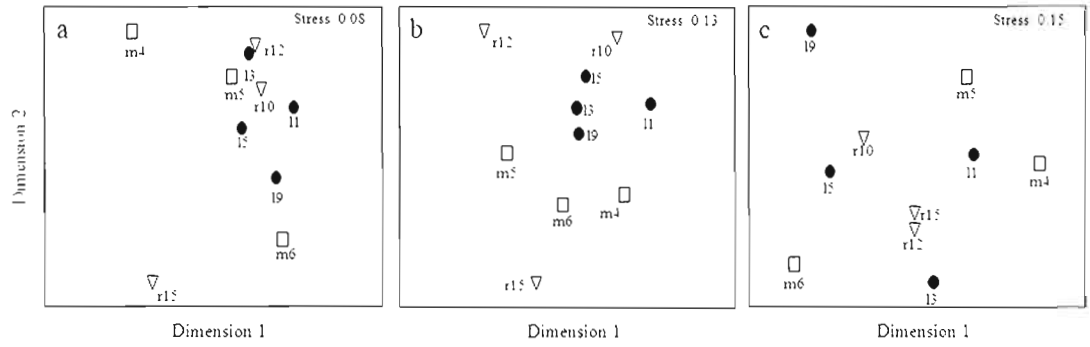


Figure 1.4 MDS ordination of dissimilarity (Bray-Curtis) calculated from DGGE banding patterns of the different habitat types in the watershed in June (a), July (b) and August (c) 2005. Symbols denote the different sampling habitats: Black circles correspond to lakes, open squares to marshes, and open triangles to rivers. Sampling periods are represented as: no mark for June, * for July, and ** for august.

bacterioplankton community characteristics has been reported by other studies before (Findlay and Sinsabaugh 2003, 2006; Langenheder, Lindström et Tranvik, 2005). These results suggest that these two components of community structure may be influenced by different factors. MC, on the one hand, appears to be more strongly related to resource conditions and thus appears to mediate the link between resources and BCM directly and consistently across systems. BCC, on the other hand, was never correlated to BCM and rarely to patterns in resource conditions, which suggests that this aspect of community structure may be influenced by other environmental factors that are more ecosystem-specific.

1.6.3 Temporal variations in the structure of bacterial communities

Our results showed that the interactions between components of bacterial community structure have an important temporal dimension. For example, we observed that the various aspects of community structure appeared to be related to each other and with resources only at certain times. Interestingly, during the month of July we observed a converging behavior from four out of five components of structure (excluding the physiological structure) in the sense that they were all significantly related to the resource matrix, and yet they were not correlated to each other. This result would suggest that resources may at times play a key role in shaping bacterioplankton structure at all levels (from single-cell characteristics to overall composition and metabolic capacities), but that this may not always be the case; conversely our results suggest that these different components of structure are generally related to each other not through strong deterministic links but rather by highly dynamic connections.

The differences in the strength of links between the various aspects of bacterial community structure and metabolism for the different sampling periods could be related to variations in the type and intensity of forces that regulate bacterial communities. It is now well known that both "top-down" and "bottom-up" forces impact on aquatic food web and more especially on multiple aspects of bacterial communities (Šimek *et al.*, 1997; Posch *et al.*, 1999; Findlay et al., 2003), yet the *in situ* dynamics of such interactions between biotic and abiotic forces and their combined effect on bacterial community metabolism and structure remained unclear. Several recent studies have examined whether bacterial communities present annual patterns in their different attributes and the factors that determine these patterns (Kent et al., 2004, 2007; Yannarell and Triplett, 2005; Fuhrman et al., 2006).

For example, Kent et al., (2004) reported recurrent patterns in the seasonal dynamics of bacterial diversity in a humic lake and observed that these patterns were mainly related to concomitant variations in both phytoplankton and zooplankton populations rather than in abiotic conditions. More recently, Kent et al., (2007) have further shown that patterns in bacterioplankton community composition were mainly driven by biological interactions (e.g. with phytoplankton). Corno and Jürgens (2008) reported that several aspects of bacterial community structure were impacted by predation and that the magnitude of this impact was mediated by the productivity of the system. In this regard, previous studies conducted in this same watershed reported short-term variations in the regulation mode of microbial communities (i.e. "top-down" to "bottom-up") (Gasol, et al., 1995), with a predominance of "top-down" control in late June during the clear water phase driven by dominance of *Daphnia*, clear "bottom-up" control in late summer and fall, and a combination of both during the remainder of the growing season.

In addition to "top-down" and "bottom-up" regulation modes, regional climatic factors may determine ecosystem stability by influencing stratification and water retention times, which in turn may affect the nature of the links that exist between resources and the various components of bacterioplankton structure. For example, it has been shown in high elevation lakes that the seasonal dynamics in bacterioplankton community composition tracked seasonal variations in lake environmental characteristics (e.g. thermal stratification stability and residence time), which were determined by climatic conditions (Nelson, *in press*). MacIntyre et al. (2006) have further shown that sporadic climatic events (wind storms, high precipitation) can induce profound modifications in both physical and chemical conditions of lakes even during thermal stratification. This increased mixing can in turn result in increased diffusion and movement of nutrients and organic matter, which might influence bacterial community structure and metabolism. Overall, climatic factors seem unlikely to directly affect the links between components of bacterial community structure but rather can indirectly induce changes in the different aspects of structure through shifts in physico-chemicals characteristics of aquatic systems. In this regard, Kent et al. (2007) have shown that regional meteorological factors explain very little of variations in BCC patterns in comparison to phytoplankton, but that biological interactions may bridge climatic forces and bacterial community responses in terms of composition.

Overall, the most consistent pattern that we observed was the recurrent strong relationship between bacterial community metabolism and the ensemble of resource variables. This pattern suggests that overall community C metabolism is indeed related to resources in a relatively predictable manner, despite a large variability in the different components of community structure that mediate this overall response of bacteria to resources. This in turn supports the idea that ecosystem processes (i.e. bacterial community metabolism) can be stabilized in response to variation in resources, through the existence of a range of species, and functional groups, that respond similarly to environmental change (Hooper et al., 2005). This idea was already proposed for bacterioplankton decades ago by Stevenson (1977), who argued that the apparent stability of the overall bacterial community output (i.e. carbon metabolism) in the environment did not necessary mean stability in the various features of the communities involved (i.e. identity, morphometry). There are examples of these phenomena in other types of microbial communities as well. For example, Fernàndez et al. (1999) followed an anaerobic bioreactor for over 2 years, and observed that the performance of the system, in terms of output, remained stable despite profound temporal changes in bacterial community structure, in terms of the dominant players and of their metabolic capacities.

1.7 CONCLUSION

Collectively, these results, suggest that bacterial communities from different freshwater ecosystems within the same watershed respond similarly in terms of bulk community C metabolism to the ensemble of resource variables. Yet these predictable end-point responses are mediated by complex shifts at different levels of community structure, some of which are ecosystem-specific, others showing no systematic patterns. In this regard, we found few significant relationships between the various components of bacterial community structure, and the links that did exist were weak and highly variable over time. One major corollary of this work is that similar environmental conditions trigger in different systems or at different times alternative pathways and multiple combinations of the various components of bacterioplankton community structure. Yet all these components nevertheless converge to yield comparable response in terms of overall community C metabolism (i.e. ecosystem functioning) to the main resources. While the final outcome can be relatively well constrained, the configuration of the components of structure, or the links that exist between them, are themselves difficult or impossible to predict on the basis of other attributes of community structure or of the resources conditions. These results point to the limits of our capacity to predict aspects of the structure and functioning of bacterioplankton communities, and we suggest that these connections should be more profitably addressed from a dynamic point of view rather than from a static perspective that assumes fixed, deterministic links.

CHAPITRE II

LINKING THE PATTERNS OF CHANGE IN COMPOSITION AND FUNCTIONAL CAPACITIES IN BACTERIOPLANKTON SUCCESSIONS ALONG ENVIRONMENTAL GRADIENTS.

Jérôme Comte and Paul A. del Giorgio

Published in *Ecology* (2010), 91(5): 1466-1476.

Groupe de Recherche Interuniversitaire en Limnologie (GRIL), Dépt. des sciences biologiques, Université du Québec à Montréal, CP 8888, Succ. Centre Ville, Montréal, Québec, Canada, H3C 3P8

AUTHOR CONTRIBUTIONS:

J. Comte designed and performed research, analyzed the data, created the figures, and wrote the paper. P.A. del Giorgio participated in developing the ideas, provided advice on experimental setup and analyses, and commented on the paper.

N.B : References cited in this chapter are presented at the end of the thesis.

2.1 RÉSUMÉ

Les liens qui existent entre la composition et les caractéristiques fonctionnelles des communautés bactériennes sont encore aujourd'hui un sujet de débats intenses, en dépit de plus d'une décennie de recherches. Dans cette étude, nous explorons trois facettes différentes des liens qui peuvent exister entre la composition et la fonction au sein de successions bactériennes qui s'établissent au fil de l'eau dans un bassin hydrographique complexe. Nous analysons la corrélation entre la composition et la fonction en termes des patrons absolus, et en termes de leur taux de changements par rapport au temps de transit de l'eau dans différentes transitions environnementales, et également par rapport aux variations des ressources dans ces mêmes transitions. Nos résultats montrent que les patrons absolus dans la composition (BCC, profils de DGGE) et des capacités fonctionnelles (FC, profils Biolog) des communautés n'étaient pas corrélés entre eux, mais que les taux de changements en BCC et FC le long des transitions étaient fortement corrélés l'un à l'autre. En outre, nous avons observé que la force et la forme de la relation entre les taux de changements de BCC et FC varient par rapport à la nature et à l'intensité du gradient considéré. Collectivement, ces résultats montrent que BCC et FC sont étroitement liés, mais d'une manière très dynamique, de sorte que leurs patrons absolus ne semblent pas être reliés. Ceci suggère un haut niveau de redondance fonctionnelle qui se produit tant au sein de la communauté existante, qu'au niveau de la métacommunauté à partir de qui les phylotypes sont sélectionnés pour occuper les nouvelles niches qui sont créées le long des transitions.

MOTS CLÉS: successions du bactérioplancton, fonction des bactéries, composition bactérienne, transitions environnementales, gradients de ressources, bassin versant.

2.2 ABSTRACT

The connections that exist between the composition of bacterial communities and their functional attributes are still a matter of intense debate, despite over a decade of intense studies. Here we explore three different facets of the links that may exist between bacterioplankton compositional and functional successions that occur along the water flow path in a complex watershed in southern Québec: We analyze the correlation between composition and function in terms of their absolute patterns, and in terms of their rates of change relative to transit time in environmental transitions, and relative to shifts in resources along the same transitions. Our results show that the absolute patterns in community composition (BCC, using DGGE profiles) and functional capacities (FC, using BIOLOG profiles) were not correlated, but that the rates of change in BCC and FC along the transitions were strongly correlated to each other. Further, we observed that the strength and shape of the relationship between the changes in BCC and FC varied relative to the type and intensity of gradient considered. Collectively, these results show that BCC and FC are strongly related but in a very dynamic manner, such that their absolute patterns do not appear to be connected. This in turn suggests a high level of functional redundancy that occurs both within the existing community, and in the meta-community from which phylotypes are selected to occupy the new niches that are created along the transitions.

KEY WORDS: bacterioplankton successions, bacterial function, bacterial composition, environmental transitions, resources gradients, watershed.

ACKNOWLEDGMENTS

The authors would like to thank L. Fauteux, M. Bergevin, and C. Beauchemin for their assistance in the field and in laboratory analyses. DOC data were provided by F. Guillemette. The authors are grateful to P. Peres-Neto for help with statistical analyses, to Y. Prairie and A. Parkes for stream data. We acknowledge J.M. Gasol and C. Pedrós-Alió and two anonymous reviewers for their comments and input in previous versions of the manuscript. This work was supported by grants from the National Science and Engineering Research Council of Canada.

2.3 INTRODUCTION

Aquatic bacteria are involved in all key biogeochemical processes, and play a major role in the trophic structure in all aquatic ecosystems. The metabolic and biogeochemical functions of these aquatic bacterial communities have been the focus of research for many decades now (Ducklow 2008). More recently, the widespread application of molecular techniques has revealed an unsuspected level of genetic diversity within aquatic prokaryotic communities (Pedrós-Alió 2006b). Yet the connections that exist between the various aspects of microbial diversity and community function in these aquatic bacterial assemblages have only recently been explored and are still a matter of intense discussion (Horner-Devine et al. 2006; Smith 2007; Allison and Martiny 2008).

The exploration of links between diversity and function in natural bacterial communities remains a major challenge, because of the extremely high diversity, functional and metabolic versatility, and complex structure and regulation of most natural communities. Laboratory microcosms and experimental systems have been extensively used to explore these links, and these involve the manipulation of both the structure of the bacterial community and the environmental conditions. Some of these studies have shown evidence of significant relationships between bacterial diversity and some aspects of community function (Horner-Devine et al. 2003; Bell et al. 2005), but these results are difficult, if not impossible, to extrapolate to natural systems. Studies carried out in natural communities, on the other hand, have yielded contrasting and inconclusive results, with evidence for both significant connections between composition, richness or diversity with some aspect of the function of the system (Findlay et al. 2003; Kirchman et al. 2004; Reinthaler et al., 2005; Alonso-Sáez et al. 2007; Bertilsson et al. 2007), and many others reporting weak or no links at all (Langenheder, Lindström et Tranvik 2005; Findlay and Sinsabaugh 2006).

Regardless of the approach, most studies have attempted to find direct correlations between composition and function, the underlying assumption being that any link, if it exists, must be causal and predicable. The apparently conflicting results that are found in the literature suggest in fact, that the connections between composition and functional capacities may only be expressed at certain temporal and spatial scales and not in others, and may emerge only under certain environmental conditions. Further, the nature of the connection itself may be different from the deterministic type of link that is generally assumed.

In previous work, we have shown that the various aspects of freshwater bacterial community structure, including composition and functional capacities, present different levels of ecosystem specificity, and that the connections between them are often weak and temporally variable (Comte and del Giorgio 2009). In this paper, we focus on the connections between composition and functional capacities in freshwater bacterioplankton communities using a broader approach that seeks, on the one hand, to explore this link over a wide range of freshwater environmental gradients and of temporal and spatial scales and to understand the extent to which the connection may be expressed differently under varying environmental conditions. On the other hand, we have assessed different facets of the relationship between composition and function, and not only a direct, deterministic link between them. The experimental design was based on examining a range of environmental transitions along the water flow path in a complex watershed in Southern Québec. These transitions varied in their type (i.e. river to lake, lake to wetland), in their intensity (i.e. the magnitude of change in resources), and in the temporal and spatial scales at which they occurred. We have chosen to measure community profiles of individual C substrate utilization, using BIOLOG Ecoplates (Garland et al. 2001), as a measure of functional capacity (FC), because the capacity to utilize organic substrates is at the base of the functioning of heterotrophic bacterioplankton communities, and all other biogeochemical and ecological functions necessarily depend on the acquisition of C and energy. Community composition (BCC) was determined using PCR-based DGGE profiles. We have assessed the correlation between the patterns in BCC and FC at the whole watershed scale, and have also assessed the rates of change of BCC and FC along these environmental transitions and how these rates of change relate to each other. We further examined how the type and intensity of environmental gradient experienced by bacteria influence the strength of coupling in the dynamics of BCC and FC.

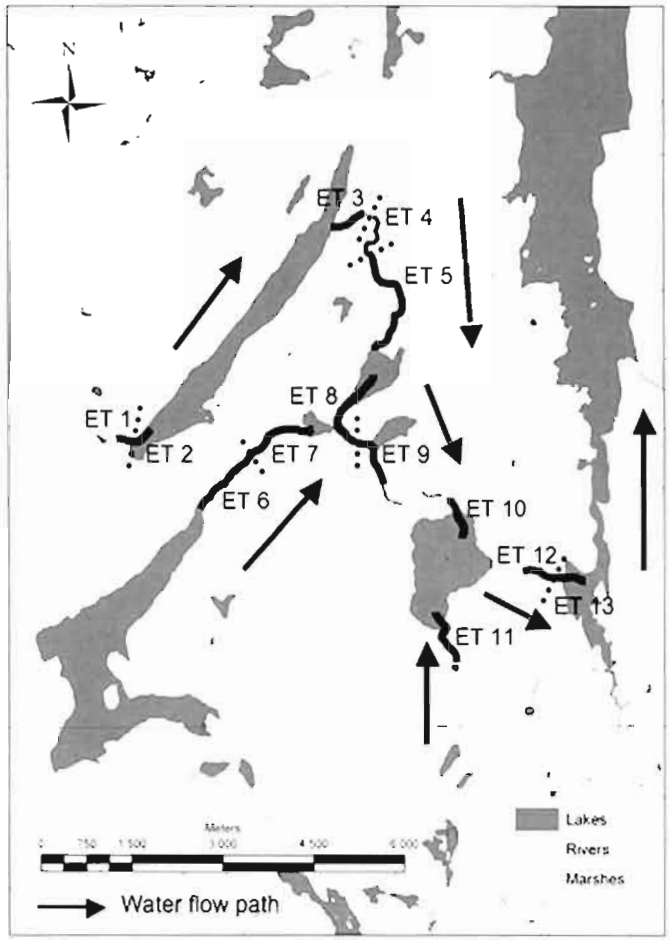


Figure 2.1 Aereal photograph of the study watershed, showing the different environmental transitions sampled (marked ET1 to ET13). Full line represents the length of the transition, and the dashed lines mark the limits of each transition. Each transition consisted of 3 to 4 sampling points along the flow path. The inset shows a detail of the water flow path within the watershed and across the different systems sampled.

2.4 MATERIALS AND METHODS

2.4.1 Sampling site and procedure

The study watershed is located 100 Km South-East of Montréal (Québec, Canada) (45° 30' 28.8"N, 73° 35' 16.8"W) and has a network interconnected lakes (ranging from oligo to meso-eutrophic), rivers and marshes (Fig. 2.1). We selected 13 distinct environmental transitions (ET), which cover the range of interface types between the major habitats that exist in the watershed (Table 2.1). These transition zones further varied in the nature of the resource gradient involved. Each ET was characterized by 3 or 4 sampling points along the water path, depending on the intensity of gradient. The systems sampled varied greatly in depth, from dozens of meters in lakes to less than one meter in some of the streams. Samples were always taken below the surface (at approximately 50 cm depth) using a diaphragm pump connected to clean plastic tubing, so as to be comparable among the different types of systems. The ensemble of 13 zones was sampled twice, in June and July 2005. The amount of time needed to sample a complete transition varied, but in all cases was below 1 hour. This sampling time was similar or shorter than the water transition time of most environmental transitions. Samples were processed within 2 hours of collection. Transition Times (TT), i.e. the average transit time of a water mass between two successive sampling points within a given transition, were calculated for rivers using the distance between the sites, and the mean measured discharge in the segment (data not shown). For lakes and marshes, we used the mean lateral current velocity following Kalff (2002) based on average wind speed (Environment Canada). The distance between sampling sites was determined from digital maps using ArcGIS software 9.0 (ESRI 2004).

2.4.2 Environmental variables

Concentrations of total phosphorus (TP) and nitrogen (TN) were measured by the persulfate digestion method (Cattaneo and Prairie 1995). Colorimetric analyses were carried out on spectrophotometer (Spectro Ultrospec 2100 pro spectrophotometer from Biochrom, Cambridge, UK) for phosphorus and on an Alpkem RFA300 Flow Solution IV autoanalyzer (OI analytical, College Station, TX, USA) for nitrogen. Concentrations of DOC were measured by high temperature persulfate oxidation on a OI 1010 TIC/TOC analyzer (OI

Table 2.1

Description of the characteristics of the 13 environmental transitions (ET) sampled during the summer of 2005. TT represents the water transit time (hours) of the transitions and their length (meters), is also provided. DOC, TP and TN refer to concentrations of dissolved organic carbon (mg L^{-1}), total phosphorus (μg L^{-1}) and nitrogen (mg L^{-1}) respectively. All values represent mean (min-max values) values, based on 3 replicates, except ET13, where there were missing DOC values.

ET	TT	Length	DOC		TP		TN	
			inlet	*output*	*inlet*	*output*	*inlet*	*output*
1	0.1	40.6	3.3 (2.6-3.8)	3.7 (3.2-4.3)	4.4 (4.1-4.6)	8.6 (5.7-10.7)	0.3 (0.3-0.4)	0.4 (0.3-0.5)
2	19.2	533.9	3.7 (3.2-4.3)	3.5 (2.2-2.8)	8.6 (5.7-10.7)	1 (0.4-1.3)	0.4 (0.3-0.5)	0.1 (0.1-0.2)
3	20	565.1	2.1 (2.1-2.2)	2.8 (2.6-3)	2 (0.9-2.9)	4.7 (2.6-6.3)	0.1 (0.1-0.2)	0.2 (0.16-0.17)
4	5.4	153.97	5.7 (4.4-7.9)	5.1 (4.9-5.7)	9.4 (7.1-12)	7.1 (6-8.8)	0.3 (0.2-0.4)	0.3 (0.2-0.3)
5	0.8	863.5	5.1 (4.9-5.7)	5.3 (3.9-7.3)	7.1 (6-8.8)	8.9 (7.1-11.5)	0.3 (0.2-0.3)	0.2 (0.2-0.3)
6	1	2366.8	4.6 (4.5-4.6)	5 (4.5-5.8)	7.3 (4.1-9)	13 (7.5-18.3)	0.2 (0.2-0.3)	0.4 (0.3-0.5)
7	11.9	335.3	5 (4.5-5.8)	6.2 (6.1-6.3)	13 (7.5-18.3)	10 (7.6-13.1)	0.4 (0.3-0.5)	0.3 (0.2-0.3)
8	56.4	1597.9	4.6 (4.4-4.7)	5.4 (5.1-5.6)	7.6 (4.2-9.7)	7.8 (3.8-13.7)	0.2 (0.15-0.2)	0.2 (0.17-0.24)
9	36.5	1722.5	5.4 (5.1-5.6)	6.4 (5.7-7.2)	7.8 (3.8-13.7)	8.4 (4.4-13.5)	0.2 (0.17-0.24)	0.3 (0.2-0.3)
10	21.6	627.4	6 (4.8-7.1)	6 (6-6.1)	9 (6.6-12.9)	5.3 (4.3-7.4)	0.3 (0.2-0.3)	0.2 (0.19-0.22)
11	36.5	938.4	10 (9-12)	6.1 (5.7-6.4)	21 (20-22)	6 (4.6-8.1)	0.4 (0.4-0.5)	0.2 (0.2-0.3)
12	0.05	94.6	5.9 (5.7-6.1)	6.1 (5.7-6.4)	16 (4.7-32.3)	11 (4.1-19.2)	0.3 (0.2-0.3)	0.2 (0.2-0.3)
13	6.27	175	6.1 (5.7-6.4)	6.7 †	11 (4.1-19.2)	11 (8.3-12.4)	0.24 (0.2-0.3)	0.3 (0.2-0.3)

analytical, College Station, TX, USA), in 0.2-mm filtered samples. In addition, water color was measured as absorbance at 440 and 280nm on a spectrophotometer.

2.4.3 Bacterial community composition

BCC was determined by denaturing gel electrophoresis (DGGE) of 16S rRNA gene. Details of the method are given in Comte and del Giorgio (2009). In brief, samples were extracted by adding successively CTAB buffer and 0.4% β-ME. The aqueous phase was extracted twice with equal volume of chloroform/isoamyl alcohol (24:1), and the resulting aqueous phase was combined with ½ volume of 5M NaCl and 1 volume of isopropanol. Precipitated DNA was washed with 70% ETOH, and resuspended in 25 µl of sterile water. Six ng of DNA extracts were amplified in 50-µl PCR reactions using the Taq PCR core Kit (Qiagen, Mississauga, ON, Canada) and GC clamp-358 F and 907 rM primers (HPLC purified, Sigma Genosys, Oakville, ON, Canada) using a touchdown cycle. PCR products (100 ng) were analyzed in 40-65% denaturant gradient gels for 16h at 100V and 60^0C using a Dcode (Biorad, Mississauga, ON, Canada) DGGE machine. Bands were stained (SYBR Gold, Molecular probes) and visualized under ultraviolet illumination. Gel pictures were analyzed using Quantity One software (Biorad) by estimating the relative contribution of each band to the total band signal in the lane. Bands located in the same position in the different lanes of the gel were matched and assumed to be similar populations.

2.4.4 Bacterial functional capacities

We determined carbon substrate utilization profiles measured using BIOLOG Ecoplates as a measure of bacterial functional capacities. Details of the methods are given in Comte and del Giorgio (2009). In brief, the 96-well Ecoplates were inoculated with unfiltered water samples (125µl). Immediately upon inoculation, the zero time-point absorbance of each plate was read at 595 nm. Color development (i.e. utilization of C substrate from bacteria) was followed using a microplate reader (Tecan Genios, Männedorf, Switzerland) for 3 to 7 days until maximum color development was reached. The overall color development of each plate was expressed as average well color development (AWCD), and this was computed each time the plates were read; we used the absorbance profiles corresponding to the time at which the AWCD was closest to the reference absorbance of 0.5 AWCD (± 0.2) (Garland et al. 2001).

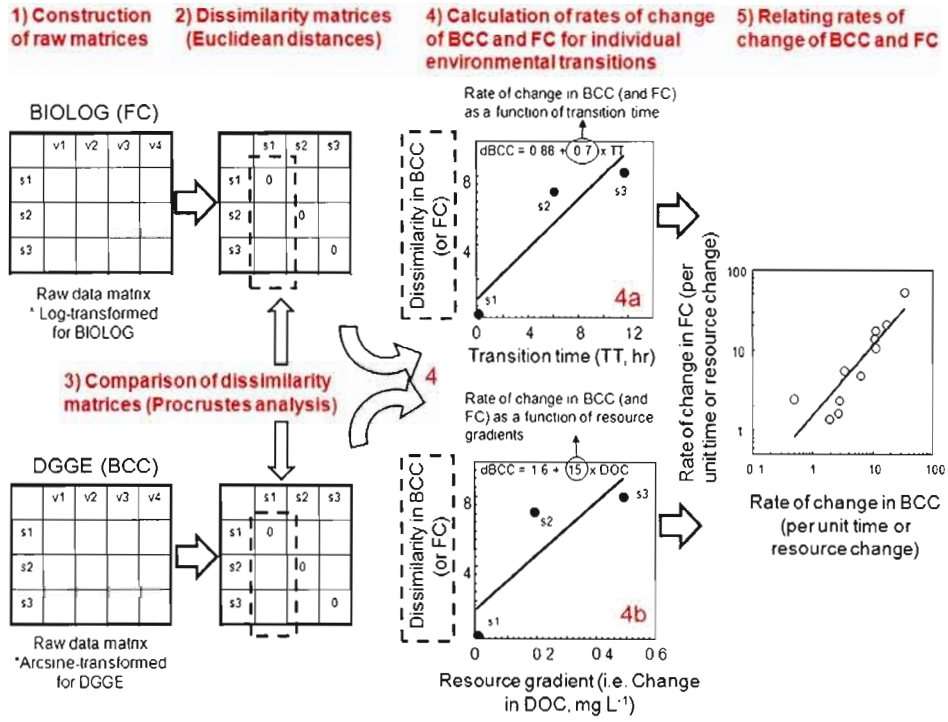


Figure 2.2 Schematic of the sequence of data processing and analysis. **Step 1**: The construction of raw data matrices, where rows correspond to individual sampling sites (s), and columns correspond to either the DGGE band fluorescence (BCC matrix), BIOLOG Ecoplates absorbance (FC matrix), or the chemical (Resource matrix) data (v). **Step 2:** Construction of site dissimilarity matrices (using Euclidean distances) based on DGGE, BIOLOG, and resource data. **Step 3:** Analysis of the correlation between the site patterns generated by BCC (DGGE) and by FC (BIOLOG), using Procrustes analysis. **Step 4:** An example of the calculation of rates of change of BCC for an individual transition (example shown of ET3, June 2005). The dissimilarity values of DGGE (dBCC) and FC (dFC) of successive sites relative to the initial sampling site for that particular transition were extracted from the respective dissimilarity matrices, and plotted as a function of transit time within the transition (**Step 4a**), to derive a rate of change in dissimilarity per unit transit time. The rate of change was also calculated as a function of the gradient in resources along the same transition (**Step 4b**, change in BCC relative to change in DOC concentration used in this example). **Step 5:** Analysis of the relationship between rates of change in BCC and FC, as functions of both transit time and of the various resource gradients, using least square regression.

2.4.5 Data processing

The different steps of data processing are summarized in Figure 2.2.

Construction of raw and dissimilarity matrices (Steps #1-2, Fig. 2.2) The raw data matrices (Step #1, Fig. 2.2) consisted of an equal number of rows, representing individual sampling sites, and a variable number of columns, representing either the absorbance value for a given substrate in the case of the FC matrix, and the relative fluorescence of a DGGE band in the case of the BCC matrix. In order to attain normality, the raw data were log10-transformed in the case of FC matrix, and arcsine-transformed in the case of the BCC matrix, the latter transformation being more appropriate for proportion data containing zero values. Additionally, data were standardized to the mean and standard deviation to minimize the influence of extreme values in the calculation of Euclidean distances. Site dissimilarity matrices based on BCC and FC were then built with Euclidean distances using Primer 5.2 software (Clarke and Gorley 2001) (Step 2). We did not combine the data from the two sampling periods, and we thus generated two separate BCC and two FC dissimilarity matrices. In addition, we constructed 2 resource matrices (one for each month) with the same sites and where the columns were TP, TN, DOC and absorbance at 280 and 440 nm.

Correlation between absolute patterns in BCC and FC (Step #3, Fig. 2.2) The site patterns generated by BCC and FC were analyzed using both multidimentional scaling and ANOSIM (Primer 5.2). The relationship between the absolute patterns in BCC and FC was analyzed using a Procrustes analysis (Matlab 7.5.0 software) (Kendall 1989), which is a modified Mantel test which finds the best superimposition that maximizes the fit between two dissimilarity matrices (Peres-Neto and Jackson 2001). The resulting metric of association (m^2) indicates the correlation between the dissimilarity matrices, with values between 0 (perfect correlation between the two) and 1 (no correlation between the two); the statistical significance of the m^2 was set at $P = 0.05$.

Calculation of rate of change of BCC and FC relative to transition time (TT) (Step 4, Fig. 2.2) For each environmental transition, we estimated the rates of change in BCC and FC as the increase in dissimilarity along the transition as a function of transition time (TT). The first sampling point of each transition was considered as the starting reference; we obtained from the matrices described in step #2 the values of dissimilarity of the successive sampling points

along the transition, relative to this initial point, and did this for both BCC and FC (Step #4a). We used the slope of the least square regression model of the relationship between dissimilarity and TT as an estimate of the rate of change of BCC (and FC) along each transition. We did this for each of the 13 transitions zones and for the two sampling periods, potentially generating a total of 26 estimates of rate of change for BCC, FC and resources, respectively. However, due to missing data in the raw matrices (resulting from sampling or analytical problems), the actual final number of rate estimates for each of these categories is lower, ranging from 12 to 17 points.

Calculation of rate of change of BCC and FC relative to changes in resources (Step #4b, Fig. 2.2) In addition to estimating the rate of change in BCC and FC relative to transition time, as described in step #4a, we estimated the rates of change of these two components relative to the change in resources within each transition zone. To do this, we used the same values of dissimilarity in BCC or FC along successive sampling stations, as described in step#2, but we analyzed them not as a function of transition time, but as a function of the differences in concentration in TP, TN and DOC in these same stations relative also to the reference point. The least square slope of this regression represents the rate of change in BCC and FC per unit change in any of these individual resources (Step #4b). In addition, we regressed the dissimilarities in BCC and FC along the transition as a function of the dissimilarities in the overall resource matrix, to derive a rate of change in BCC and FC relative to the change in the ensemble of resources.

Relationships between patterns of change in BCC and FC (Step 5, Fig. 2.2) The relationship between rates of change in BCC, and FC were analyzed by least square regression using JMP 7.0 software (SAS institute). The estimated rates of change were log-10 transformed to attain normality.

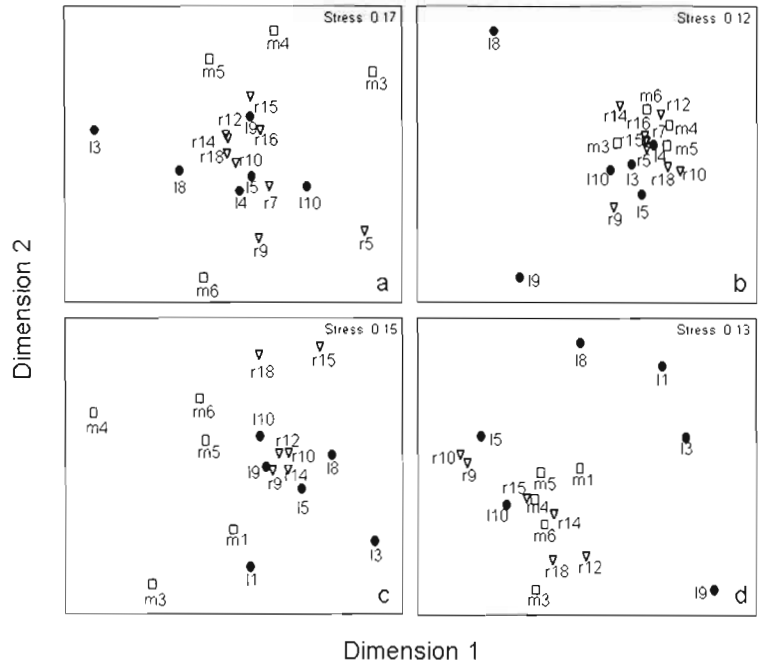


Figure 2.3 Multidimensional scale (MDS) ordination of all sampled sites in the watershed, based on the dissimilarity matrix of bacterial community composition (a-c), and functional capacities (b-d), for June and July 2005 respectively. Symbols denote the different sampled habitats: Black circles correspond to lakes, open squares to marshes, and open triangles to rivers.

2.5 RESULTS

2.5.1 Environmental heterogeneity

Bacteria experienced a range of environmental gradients along the water flow path in our study watershed. For example, in ET 2 and 6, total phosphorus changed more than in other zones, whereas in ET 8 the gradient was stronger for DOC (Table 2.1). In addition, ET 11 was the only environmental transition that presents the more drastic changes in all type of resources. In addition, for a given type of gradient, there was a large spatial and temporal variability in the intensity of change. For example, bacteria experienced changes in DOC concentrations in a relatively short period of time in ET 5 and 12, whereas ET 9 had a similar total change in DOC but over a much longer transition period (Table 2.1).

2.5.2 Comparison of absolute patterns of BCC and FC

Analysis of both BCC and FC matrices using multidimensional scaling revealed no discernable patterns between the different habitat types in June, whereas in July lakes and marshes appeared relatively well separated from each other, rivers lying between these two groupings (Fig. 2.3). The ordination based on BCC and FC dissimilarity was further tested using ANOSIM analyses, and showed significant differences in BCC ordination according to habitats in both June ($R = 0.252$, $P=0.007$) and July ($R = 0.272$, $P=0.001$), whereas no significant ordination in FC was observed. These results thus suggest some degree of habitat differentiation by BCC. In particular, marshes appeared significantly distinct from lakes ($R=0.266$, $P=0.043$) and rivers ($R=0.464$, $P=0.017$) in June and also in July ($R=0.44$, $P=0.002$ for lakes and $R=0.427$, $P=0.002$ for rivers). We compared the patterns of site dissimilarity generated by BCC and FC across the different environmental transitions in the watershed using a Procrustes analysis. No significant correlation between these two components was found in June (June: $m^2 = 0.91$, $P = 0.35$) and only a weak connection was observed in July ($m^2 = 0.8$, $P = 0.07$).

2.5.3 Comparison of rates of change of BCC and FC

We used the rates of change in BCC and FC (calculated relative to transit time as described in Methods section) to assess whether BCC and FC were correlated in terms of the magnitude of change along transitions, rather than in terms of their absolute patterns.

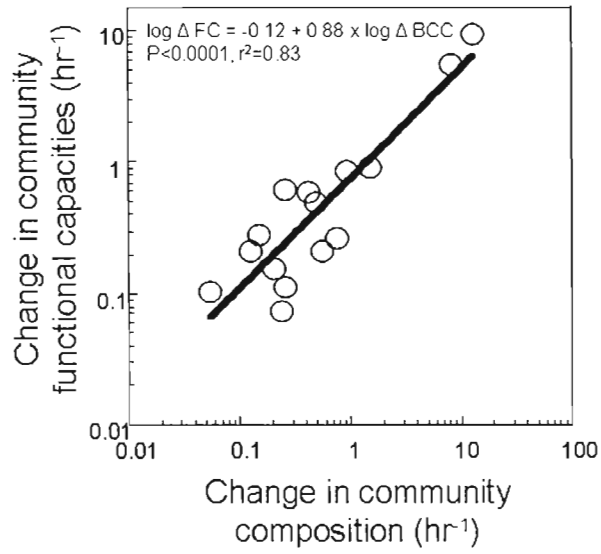


Figure 2.4 The relationship between rates of change in bacterial functional capacities (Δ FC) and community composition (Δ BCC), both calculated relative to the transit time. Each point represents the rate of change (per hour) determined for an individual transition and a single date in June or July 2005. The data are log10-transformed, and the line represents the least square regression fit.

There was a highly significant positive relationship between the rates of change in both components (Fig. 2.4). The log-log slope of this relationship was significantly lower than 1, suggesting that BCC varies proportionately more than FC along the transitions.

2.5.4 Influence of the type and intensity of environmental gradient on the relationship between BCC and FC

We used the rates of change of both BCC and FC as a function of the change in resources (as described in Methods above) to assess how the type of gradient influences the coupling between changes in BCC and FC. Our results show that both BCC and FC respond to shifts in resources in the transition zones, but differ in their response to these gradients (Fig. 2.5). There was a strong positive relationship between the rates of change of BCC and FC relative to the change in the overall resource matrix (Fig. 2.5a). This relationship would suggest that the link between changes in BCC and FC shown in Figure 2.4 is not limited to a temporal dimension in terms of transition time, but that it is also linked to the response of both components to changes in resources along the transitions. The rates of change in BCC and FC relative to changes in individual resources, rather than to changes in the overall resource matrix, were also related but the strength of this connection varied greatly with the type of gradient considered. The strongest relationship between BCC and FC was observed with rates of change calculated relative to changes in DOC concentration (Fig. 2.5b). The rates of change in BCC and FC calculated relative to changes in TP were also correlated, but the relationship was weaker than that based on DOC (Fig. 2.5c). Finally, the rates of change in BCC and FC calculated on the basis of TN were not significantly correlated to each other (Fig. 2.5d).

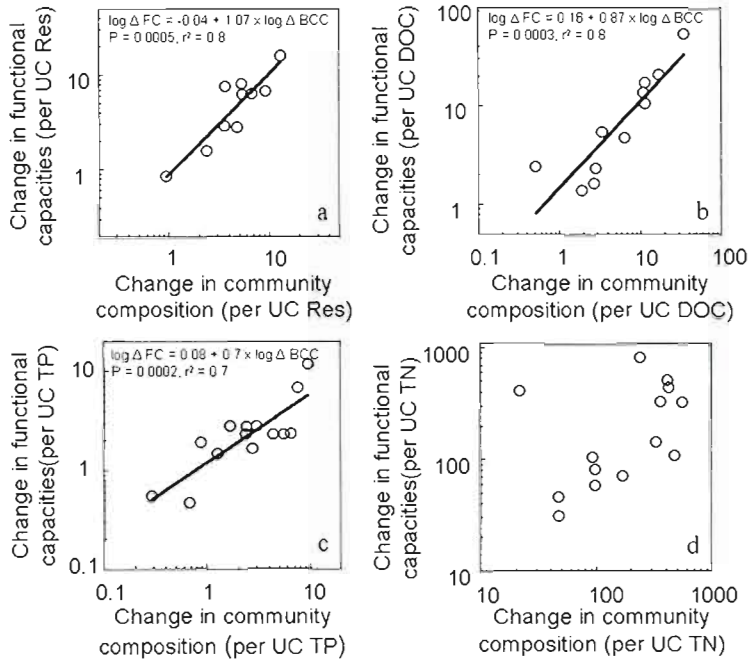


Figure 2.5 The relationship between rates of change in bacterial community functional capacities (Δ FC) and in community composition (Δ BCC), both rates calculated relative to changes (Unit Change = UC) in (a) the overall resource matrix (UC Res), (b) in DOC (UC DOC), (c) in TP (UC TP), and (d) in-TN (UC TN). Each circle represents the rates of change determined for an individual transition and for a single date in June or July 2005. The data are log10-transformed, and the line represents the least square regression fit.

2.6 DISCUSSION

There is little question that the metabolic performance and functional capacities of bacterial communities are related to their composition, in the sense that not all possible combinations of phylotypes available from a regional pool could conceivably result in similar outcomes under a given set of environmental conditions. The question then is not if these functional capacities are related to the composition of the community, but rather how these are connected. This question has been intensively explored in recent years using a variety of approaches and with very contrasting results (Gray and Head 2001; Allison and Martiny 2008). Part of the problem lies in the different conceptual and technical approaches taken. For example, studies have explored the link between community function and species richness, diversity, and the actual taxonomic composition, but these are very different aspects of community structure (Horner-Devine et al. 2006). This has lead to efforts to develop alternative ways to assess the patterns in diversity and its connections to aspects of community or ecosystem function (Bohannan and Hughes 2003).

Our approach is a variation of distance-decay relationships, which have been extensively explored in plant and animal ecology (Horner-Devine et al. 2006), and more recently been applied to microbial communities (Franklin and Mills 2003), but rather than focusing on the decline in similarity with distance, we examine how this dissimilarity varies as a function of both transit time and of the environmental gradients within the transitions that occur along the water flow path. Our results show overall weak connections between the absolute patterns in BCC and FC, although there was evidence that there may be a seasonal component in the coupling between functional capacities and community composition. In contrast, our results show a strong relationship between the rates of change in BCC and FC relative to transit time in the environmental gradients that we studied. Further, we show that the changes in both BCC and FC are directly associated to environmental gradients, suggesting that the functional response to resource and other gradients is at least in part mediated by changes in composition.

Several studies have discussed the limitations of fingerprinting methods, and in particular of DGGE, as descriptors of taxa richness and diversity in natural microbial communities (Hewson and Fuhrman 2004; Woodcock et al. 2006; Bent et al. 2007; Blackwood et al. 2007). These molecular techniques inevitably have a threshold of absolute

abundance below which taxa are not detected (Blackwood et al. 2007; Casamayor et al. 2000); this threshold seems higher for DGGE than for other fingerprinting methods (Casamayor et al. 2002). All the evidence suggests that DGGE detects mostly the numerically dominant taxa in the community (Casamayor et al. 2000; Lindström 2000). Other fingerprinting techniques, such as ARISA and TRFLP appear to have lower thresholds of detection and typically yield a higher taxa resolution (Casamayor et al. 2002; Hewson and Fuhrman 2004; Donavaro et al. 2006), but still have the same basic limitations. DGGE, as well as the other fingerprinting techniques, are thus clearly inappropriate to assess the overall taxa richness in microbial samples, or to determine overall microbial diversity. However, the DGGE approach, while admittedly limited in terms of resolution, does allow for large-scale comparisons of samples, and targets the taxa that are most likely to be responsible for a large fraction of the observed ambient activity (Bernard et al. 2000). In the case of our study, which focused on linking the changes in bacterial composition and bulk community function along gradients, the inclusion of rarer taxa through the use of more sensitive approaches would have probably not yielded increased insight into these connections. The composition matrix we used was based not on presence/absence of bands (taxa) alone, but rather on their relative contribution to total community DNA in each sample. The inclusion of rare and low abundant taxa, which may vary inconsistently between samples, would have had either little impact on the actual patterns, or in fact, could have even weakened the patterns and links that we found, by adding noise to the community response signal involving the dominant players.

Likewise, the use of techniques based on substrate utilization profiles, such as BIOLOG Ecoplates, has greatly increased in the last decade (reviewed by Preston-Mafhan et al. 2002). Several studies have discussed the problems related to enrichment and selection that occur during the incubations, and have questioned the extent to which the resulting profiles can be used to characterize natural communities (Konopka et al. 1998; Preston-Mafhan et al. 2002). It has been shown that the composition of the community that develops during these incubations may be very different to that of the ambient community, and may also differ between plates (Garland 2000). In addition, the substrates provided in those plates do not necessary represent those available or most common in situ (Garland 2000). Interestingly, in our own experiments we have consistently noted that replicate plates of the same sample yield very similar profiles, and that these profiles remain relatively stable at

least over the short- to medium-term within a given system (data not shown), suggesting that even though there may be selection and enrichment of certain taxa during incubation, bacterial communities from different habitats, or from the same habitat in time, have very distinct and repeatable BIOLOG profiles. In this study, we are not assuming that the substrate utilization profile that we measured using BIOLOG represents exactly the profile that the in situ community expressed. Rather, we exploit the fact that different communities consistently develop distinct BIOLOG profiles, with the underlying assumption that these distinct BIOLOG profiles must reflect some property of the ambient community, in terms of number and numerical distribution of taxa and metabolic capabilities in the natural assemblage, which are then selected for during the BIOLOG incubation.

Previous studies of the connection between microbial diversity and function have focused on patterns in species richness, rather than on community composition, as we have done here. Our analysis shows no relationship between the number of bands and any of the patterns we have reported here (data not shown). This would suggest that, contrary to previous reports (Horner-Devine et al. 2003; Bell et al. 2005), the adjustments in function that occur along transitions are more strongly linked to changes in the identity and relative distribution of the main players rather than to changes in the total number of dominant phylotypes.

Our results further suggest a relatively high level of functional redundancy in these bacterial communities, which is expressed at two different levels: On the one hand, we have shown in Fig. 4 that there is a 3-order of magnitude range in rates of change in FC compared to a 4-order of magnitude range in BCC, indicating that changes in function are associated with proportionally higher changes in BCC, which suggests a relatively high degree of functional redundancy within these communities wherein variations in FC are not proportional to changes in BCC, especially at low levels of environmental change. On the other hand, these results would suggest a high level of redundancy within the regional meta-community. Our study system represents a network of connected aquatic habitats and their respective interfaces within a single watershed, therefore characterized by a common regional bacterial meta-community (Yannarell and Triplett 2005). In previous studies in this area we have shown that each habitat type within this network (lakes, rivers, marshes) presents some level of ecosystem specificity in terms of community composition (Comte and del Giorgio

2009). Our present work shows a higher degree of uncoupling between changes in BCC and FC at low rates of change (Fig. 5b), but that in transitions with more drastic environmental gradients, changes in composition and function appear to be more strongly coupled (Fig. 5a). This would suggest that in steeper gradients functional adjustments cannot be carried out within the existing range of capacities (i.e. existing functional redundancy) and require changes in community composition. The lack of significant correlation between the absolute patterns in BCC and FC would indicate that there are multiple combinations of phylotypes available from the regional pool that can fulfill these adjustments and carry out similar functions, in addition to that which exists within the community at any given point in time and space.

It is unlikely that dispersal played an important role is shaping the patterns in composition that we observed, because the study systems are highly interconnected within a relatively small area. In addition, previous work in this region has shown no evidence that dispersal influences lake bacterial community composition (Beisner et al. 2006). Rather, our results point to strong local environmental forcing and that the gradients within our watershed further select from within the regional pool of phylotypes those that can potentially occupy the new niches that are created along the transitions. The importance of local factors in determining species sorting has recently been emphasized (Beisner et al. 2006; Van der Gucht et al. 2007).

In our study, the functional capacities appeared strongly related to DOC, and whereas the gradient in DOC appeared to elicit adjustments in function that were clearly mediated by changes in composition, the actual configuration of the community was probably determined not by the DOC gradient itself, but by the combined influence of other resources and possibly other physical and Biological processes. In this regard, the slopes of the log-log relationships between BCC and FC suggest differences in their response to each of the gradients studied: The slopes based on changes in the ensemble of resources (Fig. 5a) as well as that based on DOC concentrations (Fig. 5b) were not significantly different from unity, suggesting that the changes in BCC and FC relative to both the overall resource and DOC gradients are of similar magnitude. However, the slope of the relationship based on changes in TP is significantly lower than unity (Fig. 5c), suggesting that BCC is more responsive to changes in TP. There is thus indication that the magnitude of change in BCC relative to changes in TP,

for example, was greater than that of FC, which suggests that phosphorous availability may play an important role in shaping community composition beyond any influence of DOC. A corollary of this would be that while DOC gradients may drive the adjustments in function (expressed as changes in substrate uptake capacities), in the context of widespread functional redundancy in terms of C metabolism, other gradients may act on the species sorting to determine the final configuration of the community that attains this adjustment. It has been well established that bacterial communities respond to changes in quality and quantity of organic matter (Crump et al. 2003; Eiler et al. 2003) and nutrients (Lindström 2000; Schäfer et al. 2001), but how these interact to shape the resulting configuration of the community remains unclear.

Our results suggest that DOC plays a key role in shaping these freshwater bacterial successions, and of modulating the connections between composition and function. There is evidence that not only the DOC concentration changes along these transitions, but its quality as well (F. Guillemette per. com.) as the water moves across these interfaces, and thus bacteria must react to both quantitative and qualitative changes in organic resources. It is thus not surprising that DOC appears to be a major regulating factor of the functional capacities that we chose to examine here, which are based on substrate consumption profiles. There are many other functional categories that we could have potentially examined, related to other types of functions such as nutrient cycling, the utilization of various energy pathways and others. It is likely that DOC will play a lesser role in shaping the change in other functional categories, but the main conclusions of our work probably will still apply.

A scenario of significant functional redundancy both at the community and meta-community levels in terms of C utilization, coupled to some degree of specialization on other environmental requirements, would lead to the observed co-variation in terms of rates of change between composition and functional capacities, but to the lack of correlation in their actual absolute patterns. It is clear from our results then that widespread functional redundancy does not imply lack of connection between composition and function, but rather that redundancy influences how the connection between composition and function is expressed.

2.7 CONCLUSION

Collectively our results show that while it may be possible to predict the magnitude of change in bacterioplantkon function and composition from each other or from the environment, the actual outcome is much more difficult to constrain. These results in turn point to the limits of our capacity to predict aspects of the structure and functioning of bacterial communities in aquatic ecosystems, and probably apply to other communities as well.

CHAPITRE III

COMPOSITION INFLUENCES THE PATHWAY OF THE METABOLIC RESPONSE OF FRESHWATER BACTERIAL COMMUNITIES TO RESOURCE GRADIENTS

Jérôme Comte and Paul A. del Giorgio

Submitted to *The ISME Journal*.

Groupe de Recherche Interuniversitaire en Limnologie (GRIL), Dépt. des sciences biologiques, Université du Québec à Montréal, CP 8888, Succ. Centre Ville, Montréal, Québec, Canada, H3C 3P8.

N.B : References cited in this chapter are presented at the end of the thesis.

3.1 RÉSUMÉ

Le métabolisme des communautés de Bactérioplancton est à la base du fonctionnement des écosystèmes aquatiques, et fortement réactif aux changements de l'environnement. Cependant, les processus sous-jacents de cette réponse restent mal compris. Dans cette étude, nous explorons le rôle que joue la composition des communautés dans l'élaboration de la réponse métabolique des bactéries à des gradients de ressources le long d'écotones dans un bassin hydrographique complexe au Québec. Nos résultats montrent que la réponse est médiée par des changements complexes dans la structure des communautés, mais l'analyse par équations structurelles a révélé deux voies principales, l'une impliquant des ajustements au niveau de l'activité des phylotypes existants, et l'autre, le remplacement des phylotypes dominants. Ces voies distinctes de réponse n'ont pas été déterminées par le type ou l'intensité des gradients de cause, comme nous l'avions supposé, mais il semblerait plutôt que certaines configurations de composition peuvent être intrinsèquement plus plastique que d'autres. Nos résultats suggèrent que la composition des communautés détermine le niveau global de la plasticité à l'échelle de la communauté, mais que la composition elle-même peut être influencée par des facteurs indépendants des gradients environnementaux eux-mêmes, de sorte que la réponse des communautés bactériennes à un type de gradient peut alterner entre l'ajustement et le remplacement des phylotypes. Nous concluons que la composition de la communauté influe sur le type de la réponse à ces communautés bactériennes, et non sur la performance métabolique elle-même, qui est déterminée par l'environnement et qui peut être assurée par de multiples configurations alternatives.

MOTS CLÉS: Successions du bactérioplancton, métabolisme du carbone, composition des communautés, analyses d'équations structurelles, gradients de ressources.

3.2 ABSTRACT

Bacterioplankton community metabolism is central to the functioning of aquatic ecosystems, and strongly reactive to changes in the environment, yet the processes underlying this response remain unclear. Here we explore the role that community composition plays in shaping the bacterial metabolic response to resource gradients that occur along aquatic ecotones in a complex watershed in Québec. Our results show that the response is mediated by complex shifts in community structure, but structural equation analysis revealed two main pathways, one involving adjustments in the level of activity of existing phylotypes, and the other the replacement of the dominant phylotypes. These contrasting response pathways were not determined by the type or the intensity of the gradients involved, as we had hypothesized, but rather it would appear that some compositional configurations may be intrinsically more plastic than others. Our results suggest that community composition determines this overall level of community plasticity, but that composition itself may be driven by factors independent of the environmental gradients themselves, such that the response of bacterial communities to a given type of gradient may alternate between the adjustment and replacement pathways. We conclude that community composition influences the pathways of response in these bacterial communities, but not the metabolic outcome itself, which is driven by the environment and which can be attained through multiple alternative configurations.

KEY WORDS: Bacterioplankton successions, carbon metabolism, community composition, path analysis, resource gradients.

ACKNOWLEDGMENTS

We thank L. Fauteux, P. Tremblay-Dauphinais, M. Bergevin and. C. Beauchemin for assistance in the field and lab. F. Guillemette did bacterial respiration measurements on mass spectrometry, bacterial growth efficiency and carbon demand calculations and DOC analyses. We also thank A. Parkes and Y. Prairie for providing us physical data on streams, B. Shipley for his inputs in SEM analyses, and E. Lindström and S. Langenheder for their comments on a previous version of the manuscript. This work was supported by grants from the National Science and Engineering Research Council of Canada to PdG.

3.3 INTRODUCTION

After decades of research on microbial processes in aquatic systems there is now evidence that bacterioplankton communities are extremely sensitive and reactive to changes in environmental conditions (Ducklow, 2008). For example, even slight changes in resources and other conditions (e.g. salinity, temperature) often elicit large responses in terms of community metabolism in both marine (Apple *et al.*, 2008) and freshwater (Vrede, 2005) bacterial communities. The direction and magnitude of change in this overall metabolic response have been intensively studied and is in general relatively well understood (Lennon & Cottingham, 2008). Less well understood are the mechanisms involved in this response. One interesting feature of bacterioplankton communities is that total cell abundance (and biomass) tends to vary much less, both spatially and temporally, than either bacterioplankton metabolism, or the environmental factors that influence bacteria. For example, bacterial abundance in temperate lakes generally ranges from 1 to 6 x 10^6 cells ml^{-1}, and yet community growth rates and bacterial production may vary by several orders of magnitude (Cotner & Biddanda, 2002). The same pattern has been observed in marine systems (del Giorgio & Gasol, 2008). If the change in community metabolism is not primarily driven by shifts in abundance or biomass, it follows that there must necessarily be profound changes in other aspects of community structure (Fisher *et al.*, 2000; Alonso- Sácz *et al.*, 2007; del Giorgio & Gasol, 2008).

The structure of bacterioplankton communities is complex and includes a wide range of properties, such as the morphological and metabolic characteristics of individual cells, and the relative distribution of these within the community. Changes in overall community metabolism may result from shifts in the total number or size of cells, in the intrinsic level of activity of the cells, in the proportion of cells with different levels of activity, or most likely, by a combination of the above (del Giorgio & Gasol, 2008). In turn, these shifts in cell abundance, morphometry and physiological state may or not be associated to changes in the composition of the community. Determining the role that community composition plays in shaping the response of bacterioplankton to environmental change is indeed one of the central questions in contemporary microbial ecology (Reed & Martiny, 2007; Allison & Martiny, 2008), but this role has typically been elusive to discern, precisely due to the difficulty

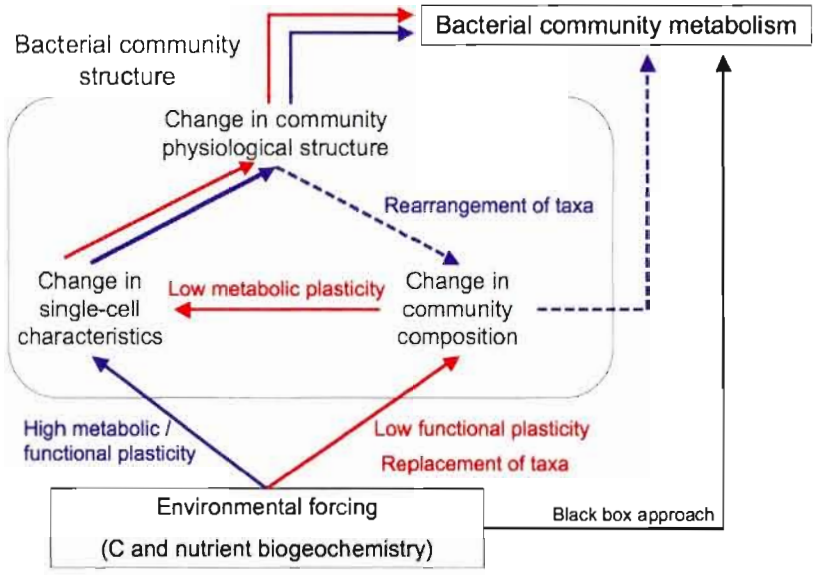


Figure 3.1 Conceptual scheme of the components of community structure that mediate the response of freshwater bacterial communities, in terms of overall metabolism, to changes in resources. This scheme assumes two major pathways in this response: In the Adjustment pathway (lines in blue), changes in resources predominantly trigger shifts in the level of activity and other cellular properties of the existing dominant phylotypes; these changes in single-cell characteristics then trigger shifts in the physiological structure of the community (i.e. the proportion of highly active versus less active bacteria), which then result in changes in community metabolism. In the Replacement scenario (red lines) resource gradients trigger predominantly shifts in the composition of the community, in terms of the dominant phylotypes, which have intrinsically different single-cell characteristics. In both scenarios, the final metabolic response is mediated by shifts in the physiological structure, that is, the distribution of activity and physiological states within the community. In either scenario, the response may or not include changes in biomass or cell abundance. The Adjustment scenario is associated to either modest resource changes, or to communities that are intrinsically plastic. The Replacement scenario is associated to either steep resource gradients, or to communities that are dominated by phylotypes that are collectively less plastic. We quantitatively test variations of these two basic scenarios using path analysis (Appendix A).

in untangling the concomitant shifts that occur at other levels of community structure. This is the objective of the work presented here.

Conceptual approach – The response of freshwater bacterial communities to changes in the environment can be conceptualized into two simplified scenarios: the response can be initiated by changes (1) at the single-cell level, in terms of metabolic adjustments of the existing phylotypes (referred to as Adjustment scenario), or (2) at the compositional level of the community (referred to as Replacement scenario) (Figure 3.1). The adjustment scenario corresponds to situations wherein the dominant phylotypes are generalists, and have a high degree of functional and metabolic plasticity, and / or the degree of environmental change is small relative to the existing metabolic breadth in the community. The resulting adjustments at the level of single-cell activity then propagate to determine shifts in the physiological structure of the community, in terms of the distribution of cells with different levels of activity, which in turn determine the overall metabolic performance of the community (Figure 3.1).

In the Replacement scenario, the dominant taxa cannot accommodate the changes in the environment due to either a lower overall level of functional and metabolic plasticity, or to stronger environmental gradients. This response in community composition occurs as changes in the actual identity of these dominant phylotypes, conceivably through selective activation and inactivation of phylotypes already present in the metacommunity pool. In this replacement scenario, changes in the identity of taxa generate shifts in the single-cell characteristics, because the new taxa have different intrinsic metabolic properties, which would then induce shifts in the physiological structure and eventually in community metabolism.

The conceptual model shown in Figure 3.1 no doubt represents an oversimplification, since both scenarios probably coexist, but it does provide a framework to explore the relative importance of compositional changes versus metabolic versatility in shaping the response of bacterial communities to environmental forcing, and how this may change both across types and intensities of gradients, and temporally within a given system. In order to quantitatively assess the pathways shown in Figure 3.1, we must be able to establish connections between the various components of community structure, and between these and both the environment and community metabolism. In a previous (Comte & del Giorgio 2009) we showed that the

Table 3.1
Description of the variables included in each of the dissimilarity matrices considered in this study.

Dissimilarity matrices	Variables
Resource conditions (Res)	Concentrations of Total nitrogen, phosphorus and dissolved organic carbon; water absorbance at 440 and 280 nm
Bacterial community metabolism (BCM)	Rates of bacterial biomass production (BP, ^{3}H-Leucine), growth (BGR, ^{3}H-Thymidine), ratio BP:BGR, respiration, growth efficiency, carbon demand, ATP content
Bacterial community composition (BCC)	Relative contribution of each band to the total intensity of the lane
Bacterial physiological structure (PS)	Specific rates of BP, BGR, respiration, ATP content, proportion of high DNA content (HNA), respiring, injured, intact and dead cells
Bacterial single-cell characteristics (SCC)	Fluorescence and side scatter values of HNA, respiring cells, fluorescence values of injured, intact and dead cells

connections between the absolute patterns in the various components of structure, including community composition and functional capacities, were often weak or non-existent. These observations, however, do not mean that these components are uncoupled, and in later studies we have shown that community composition and functional capacities are in fact highly correlated to each other, but only in terms of their rates of change along environmental gradients, not in their absolute patterns (Comte & del Giorgio, 2010).

In this paper, we examine how the rates of change in four key components of structure relate to each other, to shifts in the main resources and to changes in community metabolism, and in turn we use these relationships to quantitatively explore the two main scenarios proposed in Figure 3.1. We further assess whether these pathways change with the intensity of environmental gradients and with time. We selected a temperate watershed that contains a diversity of freshwater habitats (lakes, rivers and marshes) that are interconnected, and focused within this watershed on a series of environmental transitions that differ in the nature and intensity of the resource gradients involved, thus generating a range of potential outcomes in terms of bacterial successions originating from the same regional metacommunity (Comte & del Giorgio, 2010).

We targeted four major aspects of bacterioplankton structure: Bacterial abundance (BA), community composition (BCC), the distribution of physiological states (PS), and single-cell characteristics (SCC) of bacteria (Table 3.1). The end-point response was an ensemble of ecologically-relevant aspects of community metabolism (BCM, Table 3.1). In turn, the environmental gradients were described by a set of the main bacterial resources variables (RES, Table 3.1). We thus generated three matrices (BCC, PS and SSC) plus an individual variable (BA) that describe community structure; one matrix that describes the overall metabolic response (BCM), and a resource matrix (RES) that characterizes the actual environmental transitions. We determined the rates of change in the six categories along each of these transitions, and then used the resulting relations between these rates of change to reconstruct the sequence of events using structural equation modeling.

3.4 MATERIALS AND METHODS

3.4.1 Sampling sites and procedure

Thirteen interfaces within a watershed containing interconnected lakes, rivers and marshes were sampled during the growing season in 2005. Details of sampling locations are provided in Comte & del Giorgio (2009). For each of the 13 environmental transitions (ET), the distance between sampling sites as well as the average transit time (TT) of water masses between two successive sampling points was estimated, as described in Comte & del Giorgio (2010).

3.4.2 Chemical analyses

DOC concentrations were measured with a TIC TOC 1010 Analyzer (OI analytical), DOC absorbance was determined at 280 and 440 nm using a Biochrom spectrophotometer; total phosphorus and nitrogen were measured spectrophotometrically following persulfate digestion.

3.4.3 Bacterial community metabolism

Bacterial production (BP) was assessed as the rate of incorporation of both ^{3}H-leucine, and ^{3}H-thymidine into DNA; Bacterial respiration (BR) was determined using membrane-inlet mass spectrometry following procedures provided in Comte & del Giorgio (2009). Estimates of bacterial growth efficiency and bacterial carbon demand (BCD) were derived from measurements of BP and BR. Intracellular ATP concentration was assessed by bioluminescence.

3.4.4 Bacterial community composition

Bacterial DNA was extracted using CTAB buffer and chloroform/isoamyl alcohol and amplified in PCRs reactions (Comte & del Giorgio, 2009). The resulting products were separated into bands by DGGE and further comparison of banding profiles for different samples identified matching bands. Bacterial community diversity was examined by the Shannon index of diversity H' (Shannon, 1948), which was calculated using the relative fluorescence of bands on the DGGE banding patterns. We further assessed the changes in

presencc–absence of bands at the different locations of each environmental transition in comparison to the banding pattern of the corresponding head location of these transitions.

3.4.5 Flow cytometry analyses

Details of cytometric approaches are provided in Comte and del Giorgio (2009). Bacterial abundance was assessed using SYTO 13, and High- and Low-DNA cells were discriminated; cells with depolarized and damaged membranes were enumcrated using DiBAC4(3) and BacLight Live/Dead viability kit respectively; respiring and polarized cells were enumerated using CTC and DiOC6(3), respectively.

3.4.6 Construction of dissimilarity matrices

For 3 of the 4 components of community structure (BCC, PS and SCC), in addition to bacterial community metabolism and resources, we constructed a raw data matrix where rows represent sites at each sampling date, and columns correspond to the variables measured for that particular category. For each raw matrix, data were succcssively normalizcd and standardized, and then a site dissimilarity matrix was generated based on Euclidcan distances, for each of the categories listed in Table 1. No dissimilarity was constructed for BA as we directly used cells numbers from flow cytometry estimates.

3.4.7 Calculation of rates of change

This approach has been developed and described in detail in Comte and del Giorgio (2010). Briefly, for each environmental transition (ET), we plotted the dissimilarity between the first sampling point and the successive sampling sites within that transition as a function of the transit time between each sampling point, and used the slope of the resulting least square regression model as an estimate of rates of change (per hour) in the 5 categories (BCM, BCC, SCC and PS). In the case of BA, we estimated the rates of change by plotting the actual difference in abundance along the transition as a function of transition time. We further estimate rates of change in bacterial diversity, on the basis of the difference in H' values, and rates of change in DGGE bands replacement, by calculating the difference in shared bands between two samples. These two latter categories have not been included in path analyses.

3.4.8 Structural equation modeling

We conducted structural equation modeling (SEM) (Shipley, 2002) to identify the sequence of causal relationships that may exist between rates of change in resources, in the components of community structure, and in community metabolism, and in particular, determine the position that composition has in this sequence.

In all the causal structures (referred to as Directed Acyclic Graphs, DAG) that we tested, changes in resources represent the independent variable, whereas BCM represents the final, dependent response variable, such that resources and BCM do not share a direct link, and the community structure variables were placed between these endpoints; the models tested varied in terms of the position of these community structure variables relative to the endpoints and to each other. We tested two broad categories of models that target the two main scenarios described in Figure 3.1, and which differ mainly in the position of BCC: The replacement and adjustment scenario. Within each of these two broad scenarios there are multiple alternative structures, and we tested a total of 9 potential models (Appendix A). The principle and output of SEM analyses are explained in Appendix B.

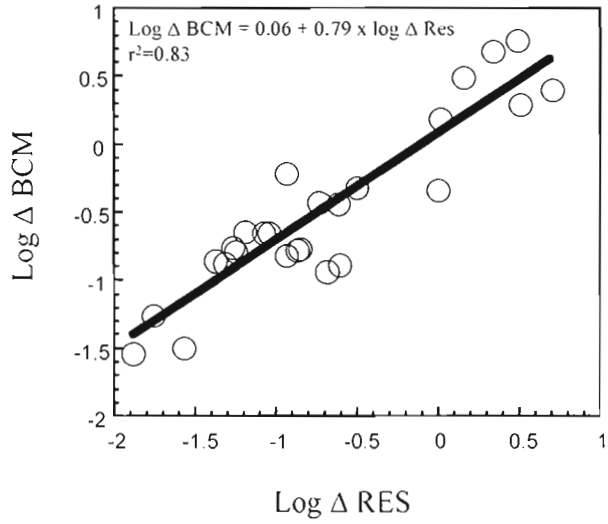


Figure 3.2 Rates of change in bacterial community metabolism (Δ BCM) as a function of changes in the overall resource matrix (Δ RES). These rates of changes were calculated relative to the transit time of each environmental transition. Each circle represents the rates of change determined for an individual transition and for a single date from June to August 2005. The data are log10-transformed, and the line represents the least square regression fit.

3.5 RESULTS

3.5.1 Metabolic response of bacterial communities to environmental gradients

The 13 transitions studied differed greatly in the type and intensity of the environmental gradients involved, and also in the temporal and spatial scales at which they occur (Appendix C). Along the different transitions, the rate of change in the ensemble of metabolic variables was strongly positively correlated to the rate of change in the ensemble of resources (Fig. 3.2).

3.5.2 Response of community structure to changes in resources

The four components of community structure also appeared to respond to changes in resources along these transitions, and the rates of change for BA, PS, SSC and BCC were positively correlated to the rate of change in resources; the strongest correlations were observed with SSC and BCC (Fig. 3.3a and b). The magnitude of change of these various aspects of community structure varied seasonally as well, and differed from each other, and whereas the rate of change in SCC and BA varied by around 3 orders of magnitude, the rates of change in BCC varied only by 2 orders of magnitude along the same gradients.

3.5.3 Relationships between components of bacterial community structure

The rates of change of the four components of community structure were significantly correlated to each other, albeit with very different degrees of strength. For example, changes in PS were strongly correlated to changes in SSC (Fig. 3.3c), but much less so to changes in BA (Fig. 3.3d). Likewise, the rates of change in BCC were positively correlated to both SSC and PS, but the relationship was much stronger with the former (data not shown). The log-slopes of these relationships varied greatly, suggesting that these components of structure do not necessarily vary proportionally to each other. For example, while the slope of the SSC vs BCC relationship was exactly 1, the slopes of the relationships between PS or SSC and BA were significantly lower than 1, suggesting a larger degree of uncoupling between these variables.

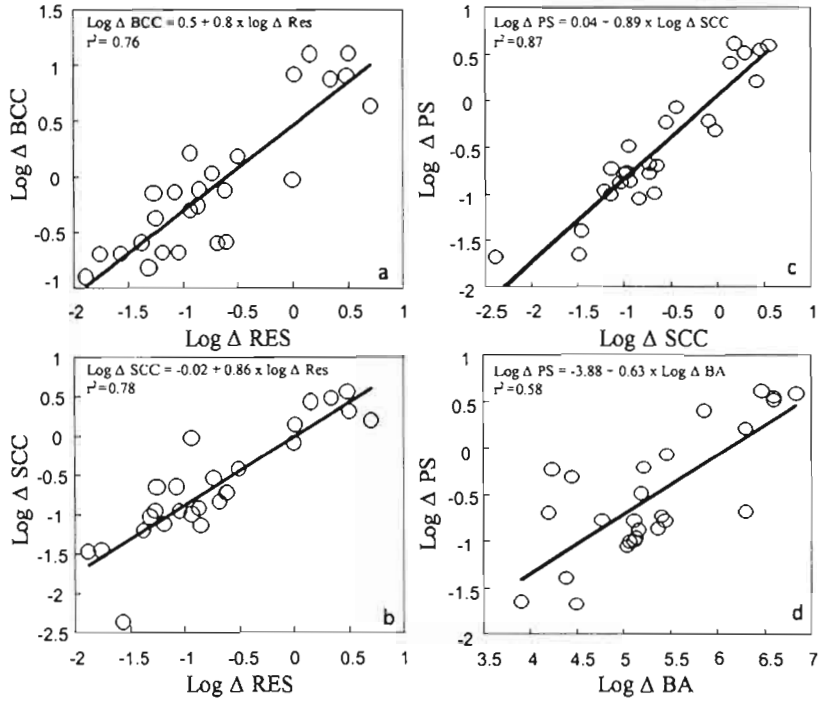


Figure 3.3 Rates of change in bacterial community composition (Δ BCC, Figure 3.3a) and single-cell characteristics (Δ SCC, Figure 3.3b) as functions of rates of change in the overall resource matrix (Δ RES); rates of change in bacterial community physiological structure (Δ PS) as a function of changes in bacterial single-cell characteristics (Δ SCC, Fig. 3.3c) and abundance (Δ BA, Fig. 3.3d). Each point represents the rates of change determined for an individual transition and for a single date from June to August 2005. The data are log10-transformed, and the line represents the least square regression fit.

3.5.4 Relationships between changes in community structure and the overall metabolic performance of the community

The rates of change in BCM were more strongly correlated to the rates of change in PS (Fig. 3.4a) and to BCC (Fig. 3.4b), whereas the relationship with the rates of change in BA was much weaker (Fig. 3.4c).

3.5.5 SEM analysis of pathways of community response to environmental change

Our SEM analyses based on the entire data set resulted in the rejection of all nine alternative models that we tested. We explored the possibility that the pathways may differ temporally by testing the nine alternative models for each of the three sampling periods separately, and this analysis resulted in very different patterns for the three months: All nine models were rejected for the month of June, although the individual variables were highly correlated to each other in this period. In July, all the models related to Scenario A were rejected, but one of the models from Scenario B (B-DAG4) was significant (χ^2=17.9, p=0.1, df=8), (Fig. 3.5a). The variability in the different factors was well explained by the structure of B-DAG4 (78 to 97% explained), except for BA (51%). The path coefficient between BA and BCM was not significant suggesting that changes in BA did not have a significant direct effect on changes in BCM (Fig 3.5a). Interestingly, in this pathway BCC is not within the main sequence leading from resources to BCM, but changes in BCC were nevertheless significantly related to both changes in PS and BCM (Fig. 3.5a). Finally, for August all the models related to Scenario B were rejected, but one of the models for Scenario A (A-DAG4) was significant (χ^2=15.7, p=0.14, df=8, Fig. 3.5b). Changes in BA explained only a very small fraction of the variations in BCM, and as was observed in July, and the proportion of the variance in BA explained by the model was low (45%).

We further tested whether the type of predominating resource gradient might influence the pathways of response, by considering the changes in the individual resources rather than in the overall resource matrix. Interestingly, the results now show that in June, one of the structures associated to Scenario B (B-DAG4) was highly significant (χ^2=13, p=0.26, df=8) based on changes in DOC alone (Fig. 3.5c), and all variables were well described by the model (from 81 to 98% of variations explained). In July, no DAG based on the changes in individual resources fit the data, suggesting that no single resource variable

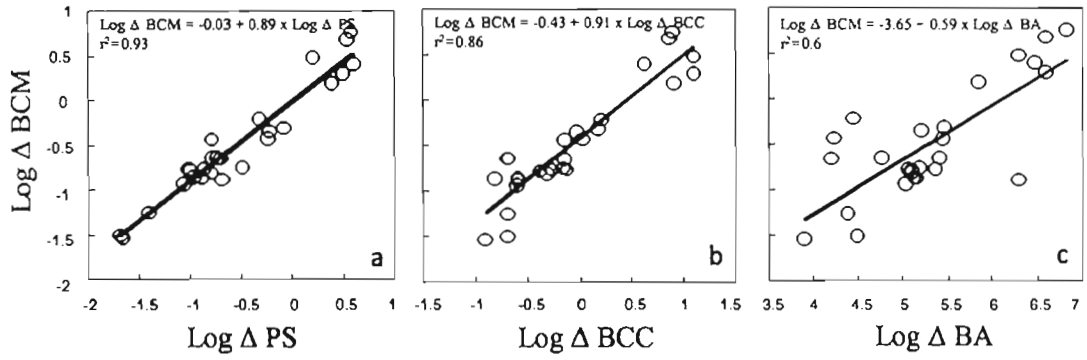


Figure 3.4 Rates of change in bacterial community metabolism (Δ BCM) as a function of the changes in bacterial community composition (Δ BCC, Fig. 4a), physiological structure (Δ PS, Fig. 4b), and bacterial abundance (Δ BA, Fig. 4c). Each circle represents the rates of change determined for an individual transition and for a single date from June to August 2005. The data are log10-transformed, and the line represents the least square regression fit.

drove the bacterial response. In August, the same structure that was significant based on the ensemble of resource variables (A-DAG4), was also significant based on changes in DOC alone (χ^2=17.7, p=0.1, df=8) (Fig. 3.5d).

Finally, we explored whether the intensity of the resource gradients may influence the pathways of response, by grouping our environmental transitions into two equal-sized categories based on their magnitude of change in resources (high and low), and testing the nine alternative models on each of these groupings separately. All 9 models were rejected for the data in the "Low" environmental change category, but three models, all belonging to Scenario B (B-DAG2, 3 and 4), were significant for the "High" environmental change category, with model B-DAG4 providing the best fit to the data (χ^2=12.4, p=0.22, df=8).

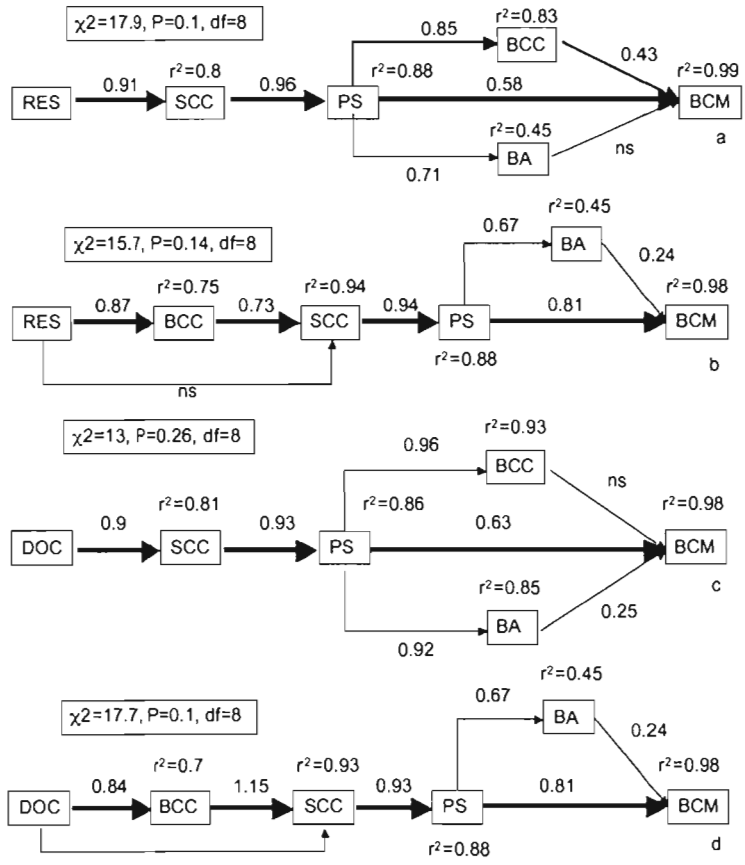


Figure 3.5 Results of structural equation modeling to determine potential pathways leading from changes in resources to the final bacterial metabolic response. Figures 3.5 a and b show the pathways that were significant for the various months of study, based on the changes in the overall resource matrix: The model that was significant for July (B-DAG4, Fig. 3.5a) followed the Adjustment scenario (response mediated by shifts in single-cell activity and characteristics), whereas the model that was significant for August (A-DAG4, Fig. 3.5b) followed the Replacement scenario (response mediated by shifts in community composition). Figures 3.5 c and d show the pathways that were significant based on changes in dissolved organic carbon (DOC) alone, rather than on the ensemble of resources: In June there was a significant model (B-DAG4) that followed the Adjustment scenario (Fig. 3.5c), whereas in August there was a significant model (A-DAG4) that followed the Replacement scenario (Fig. 3.5d). Arrows represent causal relationships between variables with the value of the standardized path coefficient (the standard deviation change of a variable given a standard deviation change the corresponding causal variable). Arrows in bold represent the most probable sequence between the different variables. Coefficients refer to the significance of the F statistic (i.e. different from 0) showing that the causal relationship cannot be rejected. NS represents non-significant causal relationships. The proportion of variation explained for each variable (r^2) takes into account the total effect (direct and indirect) of the ensemble of the preceding variables.

3.6 DISCUSSION

One of the main issues in contemporary ecology has been the extent to which the diversity and composition of communities play a role in shaping their overall performance and their responses to environmental forcing (Loreau, 2000; Giller *et al.*, 2004; Cardinale *et al.*, 2007). Microbial ecology studies to date have addressed this issue using either experimental manipulations of communities or resources (Bell *et al.*, 2005; Judd *et al.*, 2006; Langenheder *et al.*, 2006; Reed & Martiny, 2007; Salles *et al.*, 2009), or along natural gradients (del Giorgio & Bouvier, 2002; Kirchman *et al.*, 2004; Alonso-Sáez *et al.*, 2007; Comte & del Giorgio, 2010), and have generally focused on very specific links, for example between community composition and environmental parameters (i.e. Fuhrman *et al.*, 2006), growth rate (i.e. Bertilsson *et al.*, 2007), or single-cell characteristics (i.e. Longnecker *et al.*, 2005).

There are two fundamental assumptions underlying most of these studies: 1) That the link between community composition or diversity and community function or metabolism, if it exists, should be deterministic, such that a particular configuration of the community should correspond to a particular level of metabolic activity or function; and 2) that this potential influence of community composition is exerted directly, such that it can be detected by simple statistical comparisons of composition and metabolism or function. There are strong reasons to think that these two key assumptions are unjustified: On the one hand, natural aquatic bacteria may be extremely plastic (Meyer *et al.*, 2004; Buchan *et al.*, 2005; Mou *et al.*, 2008), aquatic bacterial communities are extremely diverse (Zwart *et al.*, 2002; Venter *et al.*, 2004; Lozupone & Knight, 2007), and there is evidence for widespread functional redundancy in these communities (Fernàndez *et al.*, 1999; Mills *et al.*, 2003; Wohl *et al.*, 2004; Langenheder *et al.*, 2005); it would be thus extremely unlikely that a particular level of metabolic activity or functional capacity would correspond to one, and only one, community configuration in terms of composition. On the other hand, if community composition were to play a role in the overall metabolic response of the community, this must necessarily be mediated by shifts at the level of the individual cell activity and in the

distribution of activity within the community, such that one cannot be understood without considering the other (Schimel *et al.*, 2009).

In this study, we attempted to circumvent these two key assumptions: We do not assume an a priori deterministic relationship between BCC and metabolism, and we do not assume that the potential link between BCC and metabolism is direct, but rather that it is mediated by changes at the single-cell level and at the level of the physiological structure of the community. We confirmed that bacterial community metabolism closely tracks environmental change in these freshwater transitions, which is in agreement with previous reports by us (Comte & del Giorgio, 2009) and others (Ducklow, 2008; Lennon & Cottingham, 2008). We established that the overall metabolic response of freshwater bacterial communities was not explained by shifts in bacterial abundance, but rather mediated by changes in the community structure. We further established that the four major aspects of bacterioplankton community structure (bacterial abundance, single-cell characteristics, physiological structure and community composition) tended to covary with each other, but only in terms of their rates of change along environmental transitions, not in their absolute patterns. This is important, because had we focused only on the absolute patterns in the various components, rather than on their rates of change, we would probably have not detected links between them, and we would thus been unable to explore the potential pathways of response.

The two basic scenarios (Adjustment and Replacement) that we tested admittedly represent extremes in the potential response pathways, and there is little question that both likely coexist, and thus the SEM results need to be interpreted with caution: The fact that no structure is significant could suggest that the models selected simply do not reflect the structure of the data, that several pathways coexist at a given time, or that the pathways change in time and space. Our results would suggest the latter. Our path analyses showed that no model fit the entire data set, suggesting that there is no single type of pathway of bacterial response that is prevalent in these aquatic systems. We found evidence, however, that both the Adjustment and Replacement scenarios actually operate, and in fact, may alternate temporally within a given set of systems. In June there was no model that fit the data, in July an Adjustment model fit the data and the sequence of responses to changes in resources was clearly mediated by shifts at the single-cell level of existing dominant phylotypes. In August

of bacteria led to changes in single-cell characteristics, which then propagated to influence the physiological structure and finally BCM. In support of this conclusion, we observed that the rates of change in the presence and absence of DGGE bands were indeed highest in August, relative to July and June (Appendice D).

The alternating scenarios that we observed may be the result of changes in the dominant resource to which bacteria are responding. The fact that for June the only significant model was one based on changes in DOC rather than in the ensemble of resources would suggest that at certain times there may be a single, key resource that drives the response of bacterial communities, whereas during other periods bacteria respond to multiple factors. Previous studies have also suggested a major role of DOC in controlling bacterial community performance (e.g. Findlay *et al.*, 2003; Kirchman *et al.*, 2004; Judd *et al.*, 2006; Kritzberg *et al.*, 2006; Bertilsson *et al.*, 2007).

In June, the response of the community to shifts in DOC was driven by adjustments at the single-cell level. The analyses of the rates of change in H' and of the DGGE band replacement further suggests that during this period, a rearrangement of the different phylotypes rather than their replacement predominated (Appendice D). Interestingly, DOC also appeared to play a key role in driving the changes in bacterial metabolism in August, as it did in June, yet the pathway of response was different and followed the Replacement scenario. This would suggest that a similar type of environmental forcing may elicit very different pathways of response at different times, some involving community composition, and some not.

This result may be linked not to the type of gradient per se but rather to its intensity, and we had originally hypothesized that the Replacement scenario should occur under steeper resource gradients, whereas the Adjustment scenario should prevail along less pronounced gradients, but our data do not support this hypothesis. When we separately analyzed transitions with stronger and weaker gradients, we found exactly the opposite result: The data from steeper gradients clearly fit pathways associated to the Adjustment scenario, whereas the weaker gradients did not fit any particular pathway of response. The fact that these bacterial communities appear to be able to respond through metabolic adjustments to even the strongest gradients would suggest that there is a potentially very large degree of metabolic versatility in these communities, most likely as the result of dominance by functional

generalists, as has been hypothesized for bacterioplankton communities in general (Button *et al.*, 2004; Mou *et al.*, 2008). The question is then, why does the response at times involve changes in BCC, even in circumstance of relatively mild resource change?

We postulate here that the key determinant for the type of response may be linked to intrinsic properties of the communities, specifically the level of metabolic and functional plasticity of the dominant phylotypes. Phenotypic plasticity, defined as the capacity for single genotypes to change their chemistry, physiology, development, morphology, or behavior in response to environmental cues (Futuyma & Moreno, 1988; Agrawal, 2001), is clearly a property of individual taxa or phylogenetic entities. It has been hypothesized, however, that the degree of plasticity of interacting members of the same community or of interacting players in a food web might not be independent from each other, and that there may be common and reciprocal regulation of phenotypic plasticity within communities and food webs (Agrawal, 2001).

There are many factors, in addition to resources, that can influence the seasonal phylogenetic succession of bacterioplankton, including temperature or physical conditions (e.g. Nelson, 2009), and biological interactions, such as grazing (Šimek *et al.*, 2001; Corno & Jürgens, 2008) or viral infection (Weinbauer & Rassoulzadegan, 2004; Bouvier & del Giorgio, 2007); it is possible that these factors indirectly influence the response of bacterial communities to changes in resources by somehow favoring, at different points of the succession, phylotypes that have different niche breadths and degrees of metabolic plasticity towards the same set of environmental factors (e.g. DOC type and concentration). In this regard, our results could suggest that niche breadth and phenotypic plasticity, as they relate to resource utilization, may be co-selected in the dominant phylotypes, such that some community assemblages may be characterized by an overall higher degree of plasticity than others. In this context, plasticity at the community level is akin to the notion of "Resistance" discussed by Allison and Martiny (2008). Importantly, our results would further suggest that the resource gradients themselves may not be responsible for determining either community composition or its associated level of plasticity, such that it is difficult to predict the pathway of response on the basis of the type or intensity of the gradient.

In this conceptual framework, community composition always plays a role in the response, because it is what determines the overall level of community plasticity, which in turn

In this conceptual framework, community composition always plays a role in the response, because it is what determines the overall level of community plasticity, which in turn determines whether the response to an environmental gradient will be through the Adjustment or Replacement scenario. A complicating factor in this scheme is that there also appears to be significant functional redundancy in these microbial communities, since we observed a large degree of compositional change that was not linked to either shifts in resources, or to changes in either single-cell characteristics or in physiological structure. This would suggest that there are many possible community configurations that might share a similar level of overall metabolic plasticity.

The framework that we propose has conceptual implications on how we view the links between composition, diversity and the functioning of these microbial communities, since it suggests that there may be emergent properties of these communities, such as the collective metabolic plasticity, that are linked to community composition but that are regulated by factors that are different that those that influence the overall metabolic performance of the community. It has also practical implications on how we design experiments and field studies to explore potential links between diversity and function in these communities, and especially how we interpret experimental results and empirical patterns observed in natural aquatic systems, because the lack of apparent coupling between composition and function that has often been observed in microbial communities in no way implies that composition does not play a role, but rather that this role probably does not conform to the preconceived notions and assumptions of most of these studies.

CHAPITRE IV

CONTRIBUTION OF METABOLIC PLASTICITY AND FUNCTIONAL REDUNDANCY IN SHAPING FRESHWATER BACTERIOPLANKTON COMMUNITIES RESPONSE TO CHANGING HABITAT CHARACTERISTICS

Jérôme Comte, Lisa Fauteux and Paul A. del Giorgio

In preparation for publication in *Ecology Reports*.

Groupe de Recherche Interuniversitaire en Limnologie (GRIL), Dépt. des sciences biologiques, Université du Québec à Montréal, CP 8888, Succ. Centre Ville, Montréal, Québec, Canada, H3C 3P.

N.B: References cited in this chapter are presented at the end of the thesis.

4.1 RÉSUMÉ

Il est maintenant reconnu que les communautés bactériennes aquatiques sont très sensibles et réactives aux changements dans les ressources. Cependant, il est encore incertain dans quelle mesure cette réponse est médiée par des changements dans la composition de la communauté, principalement en raison de passation de marchés et l'expansion des niveaux de la plasticité au niveau de la communauté, et aux différents degrés de redondance fonctionnelle. Dans le cadre d'une métacommunauté, nous avons évalué le degré de plasticité et de redondance functlonelle dans les communautés bacteriennes de différents habitats au sein d'un bassin versant, et relié ces estimations aux patrons de la réponse métabolique observés. Nous avons effectué des expériences de transplantations où chaque habitat a servi à la fois de source pour le milieu et l'inoculum. Les résultats montrent une grande convergence au cours du temps en termes de métabolisme entre les communautés bactériennes autochtones et transplantées, ce qui suggère une forte influence des caractéristiques du milieu. Les différentes composantes de la structure des communautés réagissent différemment à ces croisements, et en particulier il n'y avait qu'une faiblc convergence en termes de la composition de la communauté. Cette absence de relation entre la similitude dans le temps de la performance du métabolisme et la composition des communautés est en partie expliquée par un niveau élevé de redondance fonctionnelle au sein des communautés. En outre, nos résultats montrent que ces communautés présentent différents niveaux de plasticité. En particulier, les bactéries de lac semblent moins performantes (taux de production de biomasse bactérienne plus faible) quand elles sont transplantées dans d'autres milieux aquatiques que dans leur propre milieu, suggérant que ces communautés sont intrinsèquement moins plastiques. En revanche, les bactéries de la rivière et du marais semblent plus plastiques (c-a-d taux plus élevés de production bactérienne lorsqu'ils sont placés dans l'eau du lac que ceux de l'environnement duquel ils sont originaires). Ceci suggère que la différence dans la composition du carbone organique entre les lacs et les autres habitats peuvent influencer la structure des communautés locales bactériennes dans les habitats avec une forte influence terrestre, en sélectionnant les taxons qui sont soit plus spécialisés d'un point de vue fonctionnel ou qui présentent une grande niche écologique.

MOTS CLÉS: bacterioplancton, métabolisme du carbone, structure de la communauté, plasticité métabolique , redondance fonctionnelle, métacommunauté

4.2 ABSTRACT

It is now recognized that aquatic bacterial communities are very sensitive and responsive to changes in resources. However, it is still uncertain to what extent this response is mediated by changes in the composition of the community, mainly due to contracting and expanding levels of plasticity at the community level, and to varying degrees of functional redundancy. In the context of a metacommunity, we assess the degree of apparent plasticity and of functlonal redundancy in the communities from different habitats in a watershed, and link these to the resulting patterns in metabolic response. .We conducted transplant experiments where each habitat served as a source of both a medium and inocula. The results show a significant convergence over time in terms of metabolism between transplanted and autochtonous bacterial communities, suggesting a strong influence of the ambient water characteristics. The various components of community structure respond differently to these crosses, and in particular there was only a weak converge in terms of community composition. This lack of relationship between the similarity in time in metabolic performance and community composition is partly explained by a high level of functional redundancy within the communities. In addition our results further show that these communities present different levels of plasticity. In particular, lake bacteria appeared to perform worst (lower rates of bacterial biomass production) when transplanted in other aquatic environment than in their own medium suggesting that these communities are intrinsically less plastic. In contrast, bacteria from the river and marsh seemed more plastic (i.e. higher rates of bacterial production when placed in lake water than those in the environment they originate). This suggests that the difference in the organic C composition between lakes and the other habitats may influence the structure of local bacterial communities in habitat with a high terrestrial influence, by selecting taxa that are either more functionally specialized or that present a wide niche breadth.

KEY WORDS: bacterioplankton, carbon metabolism, community structure, metabolic plasticity, functional redundancy, metacommunity

ACKNOWLEDGMENTS

We thank P. Tremblay-Dauphinais, A. Guindon, M. Bergevin A. Parkes and. C. Beauchemin for assistance in the field and lab. Wc also thank R. Vogt for his comments on earlier version of the manuscript. This work was supported by grants from the National Science and Engineering Research Council of Canada to PdG.

4.3 INTRODUCTION

Understanding the forces that shape bacterioplankton communities is a major challenge in microbial ecology, since heterotrophic bacteria are key players in many aspects of aquatic ecosystems functioning (Azam 1998, Pomeroy et al. 2007, Ducklow 2008). Previous studies have shown that the overall metabolic performance of bacterial communities closely tracks resource distribution and variability in a deterministic and predictable manner (Ducklow 2008, Lennon and Cottingham 2008, Comte and del Giorgio 2009). Less clear were the processes that sustain such a relationship (e.g. Comte and del Giorgio, 2009). Results from both field studies (Crump et al. 2003, Yannarell and Triplett 2004, Jones et al. 2009, Nelson 2009) and laboratory experiments (Eiler et al. 2003, Judd et al. 2006, Kritzberg et al. 2006) have shown that shifts in organic matter source (e.g. DOC) can induce changes both in community composition and aspects of community metabolism, suggesting that the overall community metabolic response may be mediated by changes in community structure. In this regard, it has been recently shown that the metabolic response of bacterioplankton communities to resource gradients was mediated by changes in various components of community structure including community composition (chapter III).

A major issue in contemporaneous microbial ecology is the extent to which composition plays a role in determining community metabolism and function (Bell et al. 2005, Salles et al. 2009, Comte and del Giorgio 2010). Recently, the response of bacterial metabolic successions established along resource transitions within a watershed has been shown to result at certain times from adjustments in both the cellular characteristics and the physiological structure and in others from a replacement of the dominant taxa (chapter III). The fact that BCC matters in certain times and not in others suggest there are other environmental forces than resources that influence the response of bacterial communities by selecting taxa that present: (i) a high level of functional redundancy in bacterial communities, i.e. different bacterial phylotypes performing similar functions (Naeem 1998, Wohl et al. 2004), (ii) variable levels of metabolic plasticity, i.e. the ability of a single bacterium to perform several different functions (e.g. Agrawal 2001). Among these forces, the contribution of local (e.g. trophic interactions) versus external factors (e.g. dispersal) in determining the configuration of bacterial communities have received increasing attention

since the metacommunity concept (i.e. a set of different local communities that are linked by dispersal) has been introduced in aquatic microbial ecology (e.g. Leibold and Norberg 2004).

From a metacommunity perspective, a watershed comprises a variety of habitats and interfaces (i.e. rivers, lakes, marshes), each of these habitats representing different local physico-chemical (conductivity, DOC, nutrients, pH, residence time, temperature, mixing regime, UV exposure) and biological (phytoplankton, protozoan grazers, virus) conditions. Each habitat patch is comprised of unique local communities that are connected to other such communities through dispersal along the water flow path. Two paradigms have received particular attention in the last decade: (i) the species-sorting, which assumes that environmental heterogeneity rather than dispersal mostly explains differences in community structure between local habitat patches and (ii) the mass effect, which assumes that dispersal is high enough to shape bacterial community structure, and thus that species-sorting effect is less important (Logue and Lindström 2008). Many studies have found evidence for species-sorting (Beisner et al. 2006, Van der Gucht et al. 2007, Logue and Lindström 2010), mass-effect (Lindström et al. 2006, Crump et al. 2007, Nelson 2009) and a combined effect of both (Langenheder and Ragnarsson 2007) in bacterial communities. Significant effects of dispersal on the structure of bacterioplankton communities have been observed mostly in lakes with short retention time (e.g. Lindström et al. 2006), suggesting that in systems with long retention time species-sorting may be more influential than dispersal.

When exposed to identical environmental conditions (e.g. resources), the species-sorting model predicts that different local communities from the same metacommunity, would tend to become more similar in terms of community composition and may also generate changes in the overall community metabolic performance. On the other hand, community metabolism might be unrelated to community composition since bacterial communities present high level of functional redundancy or metabolic plasticity. Yet, the extent of functional redundancy and metabolic plasticity within bacterioplankton communities is largely unknown. In order to integrate these two intrinsic properties of bacterial communities in a metacommunity framework, we conducted transplant experiments where local bacterial communities, within a watershed, were inoculated in water originating from other habitat types and which differ in terms of their main resources (Table 4.1). Previous studies conducted in the same watershed have shown that along the environmental

transitions that connect these different systems, a portion of the community (based on DGGE banding patterns) was common between the different patch habitats whereas there were more specific taxa (bands) to each of them suggesting that bacteria actually disperse from one habitat to another (Comte and del Giorgio 2010). Yet, another study conducted in the same region has shown that local conditions override the effects of dispersal (Beisner et al. 2006). Based on these results, we hypothesize that habitat characteristics may be responsible for shaping the structure of bacterioplankton communities and thus determining their overall performance (BCM). Specifically, we tested whether transplanted bacterial communities converged in terms of community metabolism, physiological structure, single-cell characteristics and composition in comparison to the autochthonous bacterial communities. The experimental design further allowed testing the significance of community composition, functional redundancy and metabolic plasticity in determining the overall metabolic response of bacterial communities to changes in resources. To test the significance of metabolic plasticity, we calculated the rates of changes in BCC and BCM of transplanted communities over time during the regrowth experiments and expressed the rate of change in BCM per unit of change in BCC. We assumed that a high ratio between the rates of change between BCM and BCC would indicate a high level of metabolic plasticity, i.e. great variability in BCM in comparison to little variation in BCC, suggesting that the existing phylotypes can handle large environmental changes. In contrast, a high level of functional redundancy would have been observed in cases where identical treatments in dialysis bages would have lead to random compositional configuration of bacterial communities.

Table 4.1

Characteristics of the studied aquatic ecosystems. Values represent *in situ* mean concentrations of dissolved organic carbon (DOC), total phosphorus (TP) and total nitrogen (TN), average water temperature (Temp) and conductivity (Cond), mean rates of bacterial production in terms of ^{3}H-leucine uptake (BP) and bacterial abundance (BA).

Site	DOC ($mg\ L^{-1}$)	TP ($\mu g\ L^{-1}$)	TN ($mg\ L^{-1}$)	Temp (°C)	Cond ($mS\ cm^{-1}$)	BP ($\mu gC\ L^{-1}\ h^{-1}$)	BA ($10^{6}\ mL^{-1}$)
Lake Bowker	2.1	2	0.14	21.5	0.056	0.21	1.5
Marsh	5.1	7.1	0.26	21.8	0.057	1.15	4.1
River	10.2	21.6	0.45	16.8	0.046	1.83	3.7
Lake Fraser	6.	6	0.22	19.3	0.057	1.57	4.2

4.4 METHODS

4.4.1 Sampling and experimental set-up

We replicated 2 types of transplant experiments in June and July 2006, where a headwater aquatic system served as either a medium or an inoculum source for its recipient. All of these aquatic systems originate from the same watershed located 100 Km South-East of Montréal (Québec, Canada) (45° 30' 28.8"N, 73° 35' 16.8"W), and differ in their nutrient and dissolved organic carbon concentrations (Table 4.1). In the first series of experiments, the headwater system was an oligotrophic lake (lake Bowker) and the recipient system was a marsh located few kilometers downstream from the outlet of the lake. In the second series of experiments, the headwater system was a river with a high terrestrial influence (i.e. high input in allochtonous organic matter from the catchment, F. Guillemette pers. communication) and the recipient system was its receiving lake (mesotrophic lake Fraser). Water samples were taken at 0.5 m depth and transported to the laboratory in acid washed bottles within 3 hours for analysis.

Experiments were carried out in the laboratory using dialysis bags (Spectrum labs, MWCO: 12-14 kDa). The bags allow diffusion of nutrients and smaller organic C compounds, thus circumventing the effects of nutrient exhaustion in longer-term incubations. Bags were cut to a length of 37 cm (final volume of 600 mL), thoroughly washed in hot tap water, rinsed overnight, and then soaked for at least 3h in Nanopure water before use.

Water from each location was divided into two fractions: a medium and an inoculum. Water for the preparation of the medium was sequentially filtered through a 142 mm precombusted glasfiber filter with a pore size of 3 µm (A/D glass fiber filter, Pall Corporation), and finally through a 0.2 µm pore-size filter Capsule membrane (Acropak 1000 supor capsule membrane, Pall Corporation) to ensure that organisms are removed from the water.

Each medium (594 ml) was inoculated with 6 ml of an unfiltered inoculum in dialysis bags on day 0 of experiments. Each treatment was conducted in duplicate and incubated at 20°C in the dark in a tank filled with 40L of the corresponding unfiltrated medium water. Tank water was renewed at day 3 to avoid accumulation of the ambient nutrients. Duplicate bags were removed at 4 time points: 0, 2, 3, and 5 days.

Figure 4.1 summarizes the different cross combinations established among habitats.

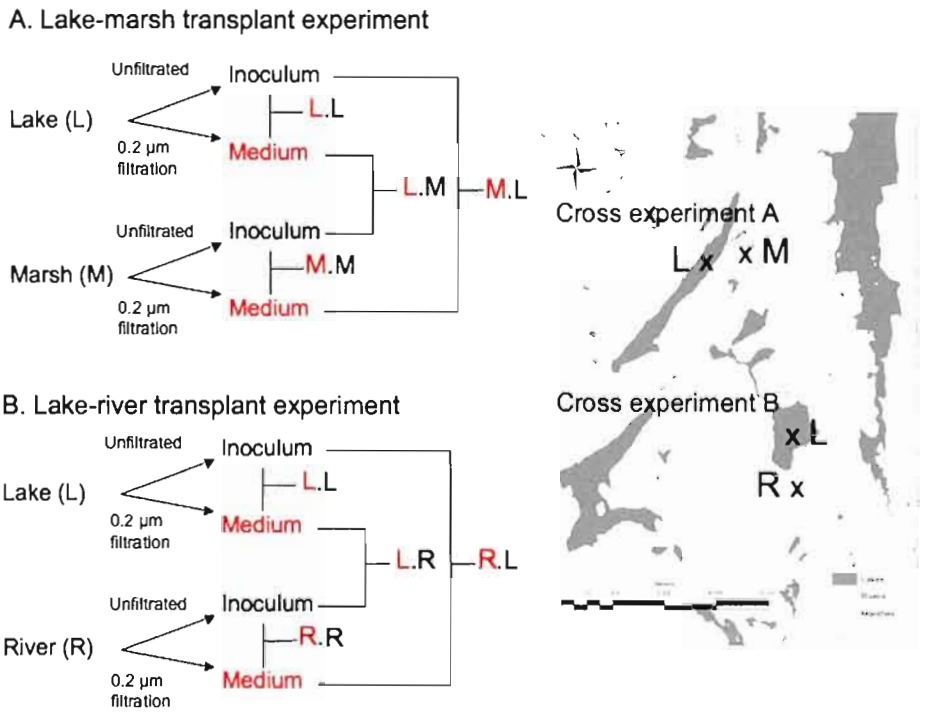


Figure 4.1 Experimental design of the study - Two-way factorial design with two series of experiments (Lake-Marsh and Lake-River), each replicated two times (June and July 2006). Experimental duration was 5 days with a replacement of ambient water in the tanks occurring after day 3. Samples were taken at days 0, 2, 3 and 5.

The first type of cross experiment, named experiment A (C1 and C3 in June and July respectively) consisted of (i) inoculating the upstream oligotrophic lake water (lake Bowker) with bacteria originating from the downstream marsh (L.M indicating that lake water receive marsh bacteria), (ii) inoculating marsh water with lake bacteria (i.e. M.L), (iii) inoculating each medium with its own microbial assemblages (L.L and M.M) and (iv) including unfiltrated water from both sites (lake and marsh). The second type of cross experiment, named experiment B (cross C2 and C4) consisted of (i) inoculating the upstream river water with bacteria from the receiving lake (lake Fraser) (R.L), (ii) inoculating lake water with river bacteria (L.R), (iii) inoculating each medium with its own microbial assemblages (L.L and R.R) and (iv) including unfiltrated water from both sites (lake and river). Controls with only sterile medium but no inocula were also prepared for each experiment.

4.4.2 Environmental variables

Water temperature and conductivity were measured using a LS-600 probe (YSI) in the field and temperature was further determined at day 3 of the experiment. DOC concentrations were measured with a TIC TOC 1010 Analyzer (OI analytical). After persulfate digestion, concentrations of total phosphorus and nitrogen were measured by colorimetry on a spectrophotometer and Alpkem RFA300 Flow Solution IV autoanalyzer (OI analytical) respectively.

4.4.3 Bacterial community metabolism

Bacterial community production (BP) was assessed as the rate of incorporation of ^{3}H-leucine, assuming a leucine to C conversion factor of 3.1 (Kirchman 1993). The rate of incorporation of ^{3}H-thymidine into DNA was measured using the procedure described by Fuhrman and Azam (1982).

4.4.4 Bacterial functional capacities

Bacterial community carbon substrate utilization profiles were characterized at the beginning and end of the experiments using BIOLOG Ecoplates. Details of the methods are given in Comte and del Giorgio (2009). In brief, the 96-well Ecoplates were inoculated with 125µl water samples. Color development (i.e. utilization of C substrate from bacteria) was followed by measuring the plate absorbance at 595 nm using a microplate reader (Tecan Genios) for 3 to 7 days until maximum color development was reached. The overall color development of each plate was expressed as average well color development (AWCD), and

this was computed each time the plates were read; we used the absorbance profiles corresponding to the time at which the AWCD was closest to the reference absorbance of 0.5 AWCD (± 0.2) (Garland et al. 2001).

4.4.5 Bacterial community composition

BCC was determined by denaturing gel electrophoresis (DGGE) of PCR amplified 16S rDNA. DNA was extracted by cell lysis in CTAB buffer and subsequent extraction with chloroform/isoamyl alcohol (Comte & del Giorgio 2009). PCR reactions were performed using GC clamp-358 F and 907 rM primers (HPLC purified, Sigma Genosys). PCR products were separated into bands by electrophoresis for 16 h at 100V and 60°C on acrylamide gels with gradients of 40 to 65% denaturants. Gels were analyzed using Quantity one software (Biorad) and comparison of banding profiles for different samples identified matching bands.

4.4.6 Bacterial single-cell activity and characteristics

A series of measures of bacterial single-cell activity and integrity were performed using a FACScalibur flow cytometer (Becton Dickinson), using 1 μm beads solution as internal standard. Total bacterial abundance was determined using SYTO 13 staining. High and Low-DNA fractions were further discriminated on the basis of their green fluorescence (FL1) and side scatter signals (SSC). Respiring cells were enumerated using CTC on the basis of the orange fluorescence of CTC (FL2) and the SSC. Cells with compromised membranes were enumerated using DiBAC4 (3), from a cytogram on the basis of their FL1 and SSC signals. Cells with intact membrane potential were monitored using DiOC6 (3) and discriminated on the basis of their green fluorescence of (FL1) and light SSC emission. The percentages of HNA, LNA, CTC+, compromised and intact cells were calculated relative to the total bacterial counts obtained by SYTO-13 staining.

4.4.7 Construction of dissimilarity matrices

We constructed raw data matrices for each of the 4 components considered in the study (BCM, PS, SCC and BCC), where rows represent the different treatments at the different time points during the experiment, and columns correspond to the variables measured for each component. For example, columns in the physiological matrix refer to the percentage of cells in terms of the different aspects listed in Table 4.1. In the case of BCC matrix, each column corresponds to the relative contribution of each band to the overall fluorescence of the sample. For each raw matrix, data were successively $\log_{10}$-transformed,

except for the BCC and PS data, which were arcsine transformed, normalized, and standardized after which a dissimilarity matrix between the different treatments was generated based on Euclidean distances (Primer 5.2 software).

4.4.8 Calculation of rates of changes

For each treatment of each experiment, we estimated the change in dissimilarity of the community in terms of BCM, MC, BCC, PS and SCC using the Euclidean distance between the time-zero of the experiment and the successive time points. We then plotted distance values as a function of time, and used the slope of the resulting least square regression model (Jmp 7.0 software) as an estimate of rates of changes (per hour) in the 4 categories. In order to test the extent of bacterial metabolic plasticity, rates of change in BCM, MC, PS and SCC were expressed per unit of change in BCC by dividing the ratio between the rates of change in the various components of community structure and in BCC. In this regard, a high ratio would indicate a high level of plasticity because large variations in metabolic and functional properties are associated with low level of variability in community composition.

4.4.9 Statistical analyses

Cluster analyses based on Euclidean distances between treatments were used to describe trajectories of samples in terms of BCM, BCC, SCC and PS over time, and also to compare the trajectories of transplanted bacteria with bacteria in their own medium.

The convergence over time (in terms of the 4 categories described above) of samples inoculated with different inocula was quantified by plotting the Euclidean distance between samples at each time as a function of time. For example, in the transplant experiment C1 (and C3) the distance between L.L and L.M (and between M.M and M.L) was measured at T0, 2, 3 and 5 days in terms of BCM and then plot the corresponding distances against the corresponding time points using least-square regression models (Jmp 7.0). The resulting slope was considered as an estimate the dissimilarity of the community. We did the same for experiments C2 and C4 with distances between R.R and R.L, and between L.L and L.R. Differences in dissimilarity estimates as well as differences in the rates of change in terms of BCM, MC, PS, and SCC during experiments were analyzed using successively a one-way ANOVA and HSD Tukey test (Jmp 7.0). We conducted the same analyses to test significant differences in the rates of changes in BCM between the treatments.

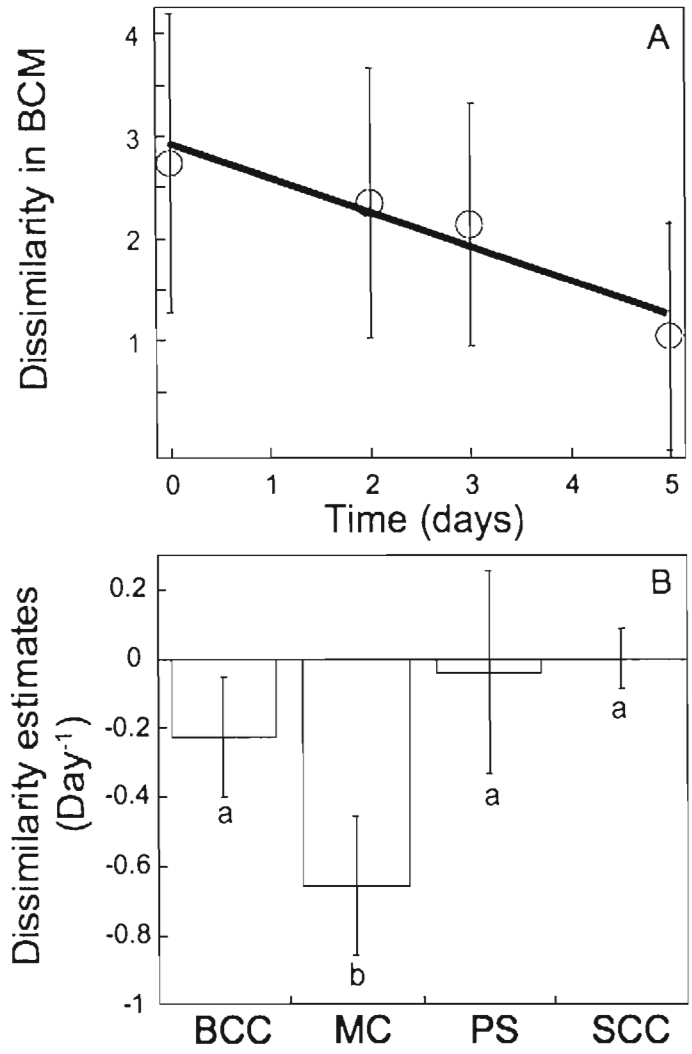


Figure 4.2 (A) Relationship between the dissimilarity in bacterial community metabolism (BCM) between native and transplanted communities, as a function of time. Each point represents the average Euclidean distance between transplanted and autochtonous bacteria in terms of metabolism for all experiments. (B) Comparison of dissimilarity estimates between bacterial community composition (BCC), metabolic capacities (MC), physiological structure (PS) and single-cell characteristics (SCC). Dissimilarity estimates are the slope of the relationship between dissimilarity for the different aspects of community structure and time. Significant differences between components of structure are given (Tukey's HSD test following one-way ANOVA, $p<0.05$) (B).

4.5 RESULTS

4.5.1 Heterogeneity in ecosystem characteristics

The characteristics of the different aquatic habitats sampled are summarized in Table 4.1. Systems used in transplant experiment A (Lake Bowker-marsh) differed greatly in terms of the main resources. For example, [DOC] in the marsh was twice as high as in lake Bowker. Similar differences were observed between systems used for the second transplant experiment (lake Fraser-river). For instance, [TP] was three times higher in the river than in the pelagic zone of lake Fraser (Table 4.1). These systems also presented clear differences in the total abundance and biomass production rates of bacteria as assessed by the uptake of 3H-leucine. These metabolic differences parallel previously observed differences in community composition between these habitats (Comte and del Giorgio 2009). Lake Bowker and the marsh presented a similar range of temperature and conductivity, whereas the river was colder and had a lower conductivity than lake Fraser.

4.5.2 Role of resources in shaping BCM and aspects of community structure

The response of bacterial communities to environmental change was driven by environmental factors (Fig 4.2A). The negative relationship between dissimilarity in BCM and time ($r2=0.9$; $p<0.04$), suggests that transplanted and indigenous bacteria converged in overall metabolic performance. For example, in the first transplant experiment A (June 2006), bacterial production rate measured in the treatment L.L was, at time 0, twice as low as treatment L.M (0.08 and 0.16 $\mu gC\ L^{-1}\ h^{-1}$ respectively), whereas at time 5 of the experiment, production rates were more similar (4.8 and 4.6 $\mu gC\ L^{-1}\ h^{-1}$ respectively). In addition, the results show that lake bacteria tend to perform worst when transplanted in marsh or river water, whereas bacteria that originate from the latter two habitats appeared to perform better in lake water. For example, in June, marsh bacteria inoculated in lake water had 3H-leucine uptake rates of 4.8 $\mu gC\ L^{-1}\ h^{-1}$ after 5 days of incubation, whereas production rates of marsh bacteria in their own environment were 3.7$\mu gC\ L^{-1}\ h^{-1}$. In contrast, production rates of lake bacteria in lake water were in the order of 4.6 $\mu gC\ L^{-1}\ h^{-1}$ whereas in marsh water these rates were about 3.6 $\mu gC\ L^{-1}\ h^{-1}$.

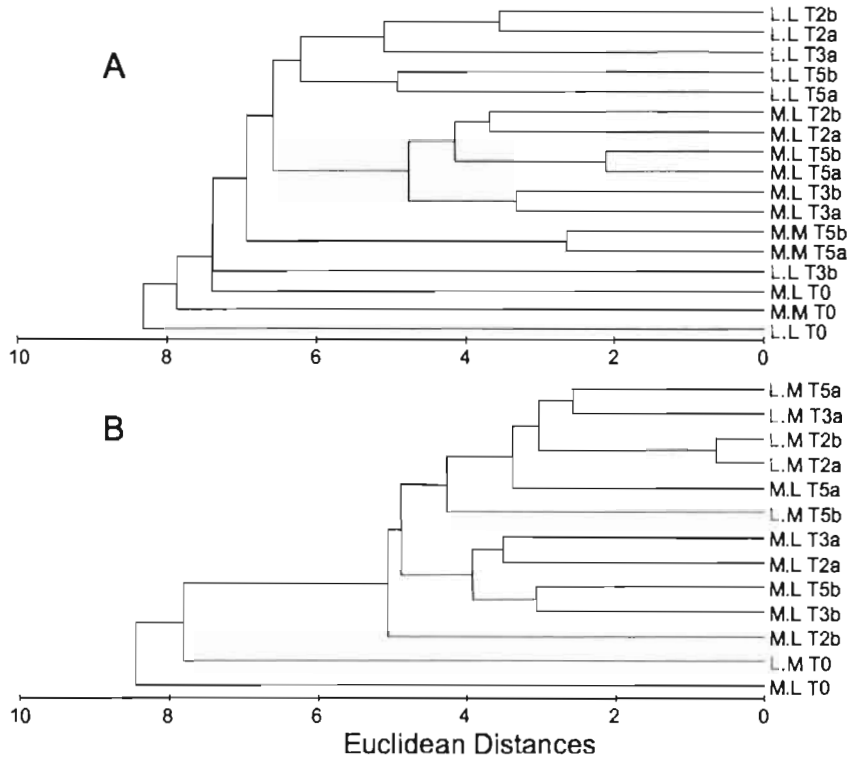


Figure 4.3 Patterns in bacterial community composition (A), as assessed by DGGE and based on Euclidean distances, at the different stages of the transplant experiments. (B) Cluster analyses of community composition during M.L, M.M and L.L treatment in June 2006 and during M.L and L.M treatment in July 2006. Duplicates were included for each treatment. The first letter refers to the source of the medium (lake medium is presented in red). The second letter corresponds to the source of the inoculum. L and M mean lake and marsh respectively.

Figure 4.2B presents the slope of the relationship between the dissimilarity in BCC, MC, PS and SCC with time, and shows that components of community structure presented different responses to environmental change. The two communities showed a clear convergence in their metabolic capacities over time, whereas this response was weaker for community composition and almost null for PS and SCC. ANOVA shows that only MC was significantly different from the response of the other components ($F=8.74$, $N=16$, $p=0.0024$).

4.5.3 Role of composition in the response of bacterial communities to changing environment

Cluster analyses based on DGGE banding patterns shows that replicate bags had very similar community fingerprints. Figure 4.3A shows an example of transplant experiment A conducted in June. This result indicates that the environment exerts a consistently strong sorting effect on taxa. For example, after five days of incubation, lake bacteria inoculated in marsh water tended to be phylogenetically closer to marsh bacteria in their environment than lake bacteria in lake water (Fig. 4.3A). Similar patterns were observed for the other treatments and, for a given treatment, were consistent between both experimental series (i.e. June and July). Importantly, Figure 4.3B shows for instances that BCC patterns in M.L and L.M treatments are separated into two distinct clusters.

4.5.4 Role of bacterial functional redundancy and metabolic plasticity

At the end of the experiments, the divergence observed between autochtonous and transplanted communities in terms of BCC was related to divergence in their metabolic performance (Figure 4.4). The shape of the relationships further indicate that there is first a lag phase where increases in the compositional distinction between the two communities were associated with small differences in their metabolism. This suggests a high level of functional redundancy within these communities, and also the existence of an environmental threshold, above which community composition and metabolism are more tightly linked. Interestingly, the standard deviation bars show a high variability in BCM at low levels of divergence whereas at high levels of divergence, a higher variability is observed in BCC.

The degree of metabolic plasticity is significantly different between the various components of community structure and metabolism (ANOVA $F=19.37$, $N=56$, $p<0.0001$,

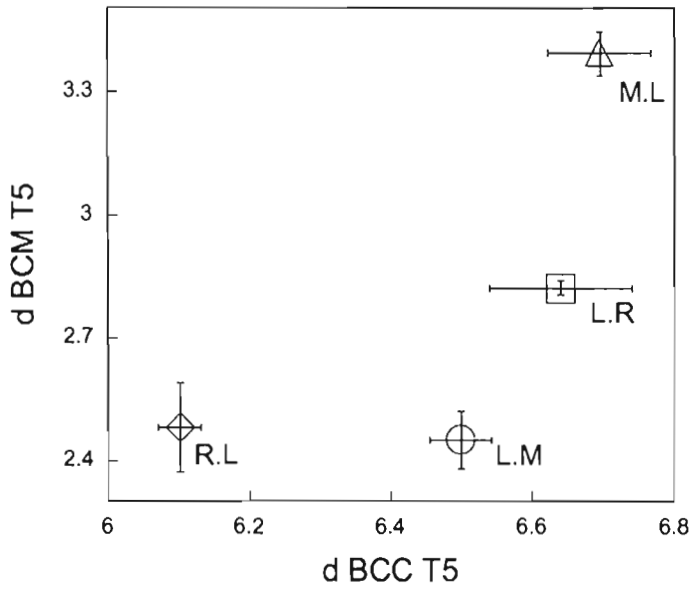


Figure 4.4 Relationship between the dissimilarity in bacterial community metabolism (d BCM) between native and transplanted communities, as a function of their corresponding dissimilarity in community composition (d BCC) at the final stage of transplant experiments. Each point represents the mean of Euclidean distances between transplanted and indigenous bacteria in terms of both community metabolism and composition, for each type of transplant experiment. The first letter refers to the medium and the second to the inoculum. R, L and M mean river, lake and marsh respectively. Bars represent the standard deviation measured. Values of Standart deviation have been reduced ten times to simplify the reading of the figure.

Fig. 4.5A). In particular, BCM is shown to present the lowest degree of plasticity (i.e. lowest BCM/BCC ratio) whereas MC present the highest (i.e. highest MC/BCC ratio). PS and SCC show intermediate levels of plasticity (HSD Tukey test, Fig. 4.5A). Figure 4.5B shows that the ratio between rates of change in BCM per unit change in BCC were significantly lower for lake bacteria in comparison to bacteria originating from the marsh and the river (ANOVA $F=17.67$, $N=16$, $p<0.0001$).

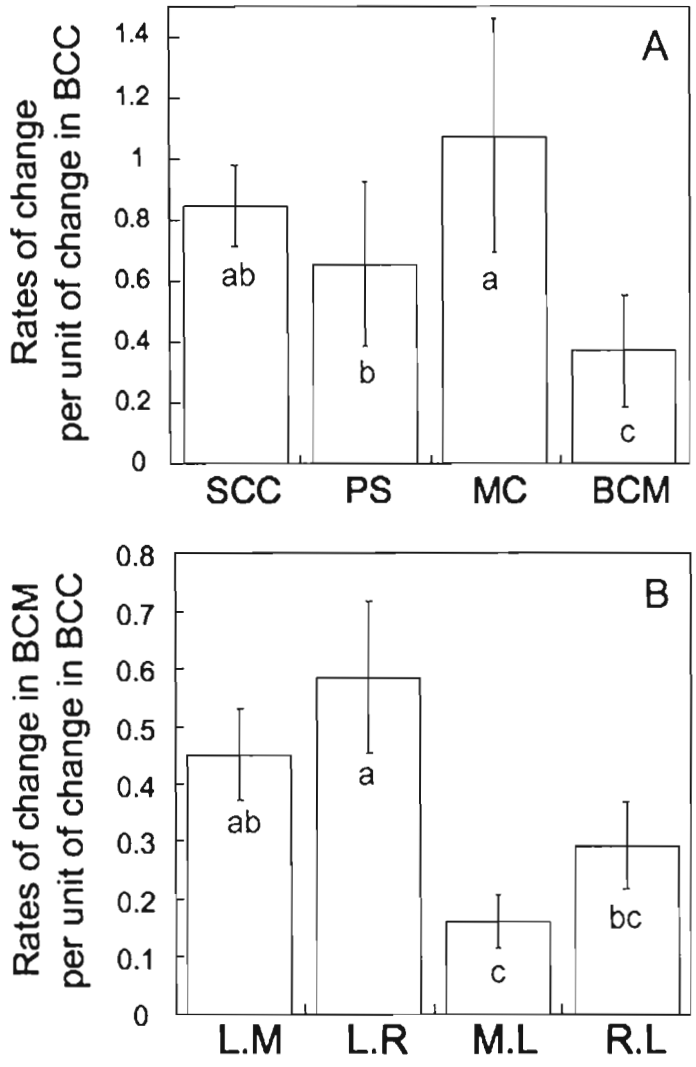


Figure 4.5 Comparison between the rates of change in single-cell characteristics, physiological structure, metabolic capacities and community metabolism per unit of change in community composition during the regrowth experiments (A). Bars represent the mean (± standard deviation) value of rates of change, all transplant experiments included, for each particular component. (B) Rates of change in bacterial community metabolism per unit of change in community composition in the different types of transplant experiments. Mean values from duplicate cultures (± standard deviation) are shown. Letters on bars represent significant differences between either components of structure (A) or treatments (B) (Tukey's HSD test following one-way ANOVA, $P<0.05$).

4.6 DISCUSSION

The main objective of this study was to determine the extent to which patch habitat characteristics determine the overall performance of local aquatic bacterial communities. To achieve this goal, we performed transplant experiments between habitats that are known to present clear differences in the main resources. For example, DOC concentration in lake Bowker was half that of the marsh, and total phosphorus in the river was more than 3 times higher than in lake Fraser (Table 4.1). These habitat patches also differ in the composition of bacterial communities (Comte and del Giorgio 2009) although the communities share common taxa due to a high degree of connectivity and dispersal. These experimental approaches, variations of which have been attempted in microbial communities before (e.g. Reed and Martiny 2007), allows to disentangle the influence of the environment and community composition on bacterial community metabolism, and to test whether the metacommunity concept applies to bacterial communities, i.e. the importance of species-sorting or mass effect in determining the configuration and performance of these communities. However, most studies to date that have used similar approaches have conducted the experiments on unconnected systems, often from different regions (e.g. Langenheder, Lindström et Tranvik 2005, 2006), and few have used an approach at the scale of the same regional watershed (e.g. Kirchman et al. 2004). The main advantage of the latter is that we account for cross-regional differences in BCC that have previously been reported (Yannarell and Tripplet 2005) and allow working in a metacommunity framework.

One crucial issue for our experiments was to verify that the incubation conditions did not impose a certain community composition, i.e. dominance of opportunistic taxa that develop during the experiments. Indeed, if the same bacterial taxa within the overall genetic pool were favored by the conditions in the cultures, then local and transplanted communities would systematically present high levels of similarity to each other. These similarities would be probably higher than those between the ambient communities at the original sampling sites, where environmental conditions selected for distinct community composition. Our results show that this was not the case, since the different treatments were separated into two distinct clusters all along the experiment incubation, and there was no evidence that experimental conditions selected a particular group of bacteria (Fig. 4.3B).

In this study, we hypothesized that our set of 4 different local bacterial communities were drawn from a regional metacommunity. Previous studies in the same geographical area have shown that the different local bacterial communities present some compositional similarities to each other suggesting that dispersal acts in these systems by transferring taxa from one patch of habitat to another (Comte and del Giorgio 2010). Yet other studies have shown, in this region, that local environmental gradients were strong enough to have an impact on the structure of the local communities, and thus dilute potential dispersal processes (Beisner et al. 2006). According to the species-sorting framework, taxa disperse throughout at the watershed scale but are selectively expressed by the environment at the local scale, such that under converging environmental conditions, the communities should present some degree of convergence in BCC. In this regard, several studies have reported examples of species-sorting in microbial communities (e.g. Beisner et al. 2006, Vand der Gucht et al. 2007, Logue and Lindström 2010).

Our results clearly show that after 5 days of incubation, transplanted bacteria perform with similar rates of production as autochtonous bacteria suggesting that the environment exerts a strong influence on the community performance (Fig. 4.2A). Such results are in accordance with Lennon and Cottingham (2008) who found that resource availability and quality have strong effects on bacterial metabolism and production in particular. The convergence in BCM that we report here is also in accordance with previous studies conducted in the same systems that demonstrated that BCM closely tracks resources (Comte and del Giorgio 2009, chapter III). Interestingly, we observed that the metabolic performance of lake bacteria was diminished when transplanted in either marsh or river water relative to the native communities, whereas the inverse was not true. The fact that lake bacteria perform worst (i.e. lower rates on bacterial production) when transplanted in other aquatic environment than they do in their own environment could suggest on the one hand that lake bacteria are less plastic or that the marsh (or river) resources and conditions might be preferentially selecting for the bacterial taxa that are already there. In contrast, the fact that marsh and river bacteria perform better (i.e. higher rates of bacterial production) in lake water could suggest that these bacteria are more plastic or that there are resources and/or conditions in the lake environment that are favourable to marsh and river communities (e.g. organic carbon composition). These points will be discussed below.

We hypothesized that this metabolic convergence should result from a common convergence in the different aspects of community structure and in particular BCC, which would indicate a strong effect of local conditions rather than the source of the inoculum. But our results do not support this hypothesis. Instead, they show that the different components of community structure respond differently to the changing environmental conditions (Fig. 4.2B). For example, we show a significant convergence in terms of the functional capacities in terms of C utilization profiles, between transplanted and indigenous communities, whereas there was a very weak convergence in terms of community composition and no convergence at all in terms of the physiological structure and single-cell characteristics (Fig. 4.2B). These results suggest that the source of the inoculum, and not the medium of the inoculum, is of major importance in shaping the community structure. If the medium was determinant, we would have seen that communities convergence in terms of BCC with time as well as in the physiological structure and single-cell characteristics, something that was not observed. These lacks of convergence in BCC, PS and SCC further suggest: (i) few replacement of the dominant members and/or (ii) replacement of the domainant members by other phylotypes that present similar intrinsic characteristics (Fig. 4.2). In both cases, these changes combine in a overall convergence in terms of the community performance and function.

In the context of a metacommunity, the weak convergence in BCC could result from the large size of the genetic regional pool, where the chances of obtaining the same configuration in parallel regrowth experiments is very low. The same environmental conditions would then result in multiple combinations of the dominant taxa. In contrast, if the size of the genetic pool is small, then the same environmental conditions should yield roughly repeatable bacteria community composition. Our results support the latter scenario since all our bag replicates clustered together (Fig. 4.3A).

A large degree of metabolic versatility or plasticity in these communities can have also influenced the degree of convergence of BCC that we measured, resulting for example in the dominance of generalist taxa, as has been previously argued (Button *et al.* 2004, Mou *et al.* 2008). Plasticity is defined as the capacity for a given taxa to modify its physiological, chemical and morphological properties in response to the ambient environmental conditions (Agrawal 2001). There is now evidence that at least some aquatic bacteria are capable of broad physiological and morphological adjustments to changes in environmental conditions

(Hahn et al. 2003, Jaspers and Overmann 2004, Meyer et al. 2004, Buchan, Gonzalez and Moran, 2005, Schimel, Balser and Willenstein 2009, chapter III). In the context of our experiments, a high level of plasticity would lead to either small changes in composition under very different environmental conditions, i.e. small changes in BCC of transplanted communities, or to communities that are similar in terms of BCC but which perform very differently. To explore these possibilities, we tested the plasticity of bacterial communities by calculating the rates of change in single-cell characteristics, in the physiological structure and in community metabolism over time per unit change in BCC. A high ratio would then indicate a high metabolic plasticity (or resistance *sensu* Allison and Martiny 2008) since it is associated with large variations in metabolic and functional properties of bacteria but only small variations in BCC. Our results show a high overall level of bacterial plasticity, but that the level of plasticity relative to the various components of community structure appeared to be very different (Fig. 4.5A). For example, Figure 4.5A shows that bacteria presented an overall significantly lower degree of plasticity in terms of BCM than all components of community structure. Within the community structure, bacteria present a higher level of plasticity in terms of MC and SCC than in PS.

In addition, these different levels of plasticity occur simultaneously with a high level of functional redundancy in these communities at the regional scale. Our results show the existence of environmental thresholds that determine the connection between BCC and BCM (Fig. 4.4). Indeed, Figure 4 shows that below certain environmental thresholds, there is a lag phase where relatively large divergence in bacterial community composition between the transplanted and indigenous communities was not accompanied with changes in the metabolic performance of bacteria. Beyond a certain threshold of dissimilarity in terms of BCC, there was increased metabolic distinction between the two communities. Importantly, the standard deviation around the mean estimates of Euclidean distances presented in Figure 4 suggests that these environmental thresholds determine the configuration of local communities based on the levels of plasticity and redundancy of the new selected taxa. Below this environmental threshold the new selected taxa appeared extremely plastic (i.e. more variation in BCM than in BCC) whereas above the same environmental thresholds, the new taxa appeared less plastic but more redundant, suggesting that there are many possible community conformations that might share a similar level of overall metabolic plasticity.

taxa appeared less plastic but more redundant, suggesting that there are many possible community conformations that might share a similar level of overall metabolic plasticity.

We have further explored whether redundancy and plasticity were ecosystem-specific, i.e. testing whether there are systematic differences in terms of these two features between habitat types (e.g. lakes versus marsh and river). Our results show that lake bacteria present a significantly lower degree of plasticity than bacteria originating from the two other aquatic habitats, i.e. the marsh and the river (Fig. 4.5B). These results are in accordance with other results described above that show that lake bacteria rates of biomass production were lower when they were inoculated in marsh (or river) water than when they grow in lake water. In contrast, marsh and river bacteria perform better (i.e. higher rates of biomass production) in lake water than they do in the ambient water they originate. Possible explanations of these patterns, can be on the one hand, the strong influence of terrestrial inputs in organic matter that are rich in humic compounds into the marsh and the river in our drainage basin system (F. Guillemette pers. communication). This allochtonous DOC is usually thought to present a structure that is more complex than the algal derived-organic matter that is more prevalent in the lake DOC pool (Søndergaard and Middelboe 1995; Guillemette and del Giorgio submitted). The differences in the nature of the organic C pools may influence the structure of local bacterial communities, by selecting taxa that are either more functionally specialized or present a wider niche breadth. In this regard, Jones et al. (2009) have recently observed that the relative contributions of terrestrial vs aquatic carbon to the bulk DOC pool represented an important selective force on bacterial community composition. It is thus likely that bacterial communities from marsh and river are predominantly composed of generalist bacteria that present a wide niche breadth. For example, the *Cytophaga-Flavobacterium* cluster that is known to be able to consume high molecular weight molecules are predominantly present in rivers compared to lakes (Kirchman 2002). In contrast, lake bacterial communities appear to be composed of taxa that are less plastic. On the other, internal processes of lakes such as the water residence time (WRT), can also account for the significant difference in lake bacterial plasticity. Previous studies have shown that bacterial community structure, and particularly BCC, was significantly related to lake WRT (Lindström et al. 2006, Nelson 2009). In particular, Lindström et al. (2006) have showed that lakes with WRT above 200 days tend to be

"protected" from the influence of external forces, i.e. dispersal. Our two lakes have WRT of 3270 and 131 days (lake Bowker and Fraser respectively) and therefore would be in the low and intermediate external control categories of lakes respectively, as defined by Lindström and coworkers (2006). This would suggest that the influence of dispersal is moderate and thus that the local gradients are strong enough to have an impact to structure the communities. Yet dispersal can still be important as it can let communities track changes if the local environmental conditions change. In this regard, our results show that the plasticity of bacteria originating from lake Fraser was higher than those from lake Bowker, suggesting that dispersal may contribute to the response of lake Fraser bacteria with a higher input of river bacteria that remain active in lake water and that probably present a wider niche breadth.

Collectively the results of this study show that species-sorting processes seem to be important in structuring local bacterioplankton communities from the same regional metacommunity. We have shown that the characteristics of the community such as plasticity and redundancy play a central role in determining the type of response of bacteria to changes in environmental conditions. Further studies are needed to address these questions with a higher level of resolution in describing the composition and diversity of bacterial communities, in particular, in tracking the diversity of the dispersal pool at the regional metacommunity level. There is some evidence that rare opportunist taxa can contribute to the overall community performance (Szabó et al. 2007), yet there is few evidence that rare or less active taxa in local communities are the same that become dominant after changes in environmental conditions (Yannarell, Steppe and Paerl 2007). Understanding the functional significance of the low abundant and rare taxa constitute a major challenge in aquatic microbial ecology and will greatly contribute to our understanding of the mechanism involved in bacterial response to changing environmental conditions and to a larger extent, to a prediction of these responses and their implications in terms of aquatic ecosystems functioning.

CONCLUSION

L'objectif principal de cette thèse est de décrire les processus qui déterminent la réponse métabolique des communautés de bactérioplancton dulçaquicoles face à des gradients dans l'environnement. L'hypothèse sous-jacente de cette thèse est que la réponse finale des communautés bactériennes aux gradients de l'environnement que nous mesurons sur le terrain (mesures des taux de production et respiration bactérienne) résulte de changements à l'intérieur de la structure même de la communauté (ex : structure physiologique, capacités fonctionnelles, composition). Différents aspects relatifs à cette hypothèse ont été adressés spécifiquement dans chacun des chapitres de cette thèse et comprenaient en autres : Des mesures du métabolisme des communautés bactériennes ainsi que de divers aspects de la structure des communautés le long de gradients de ressources, l'utilisation d'approches alternatives pour décrire les connections qui existent entre ces différents composants et en particulier entre la composition et les capacités fonctionnelles des communautés bactériennes, une description de la séquence des relations de causes et effets qui déterminent cette réponse et une exploration du rôle de la composition de la communauté dans cette réponse et des caractéristiques de l'habitat dans la détermination de la configuration des communautés.

Collectivement, les résultats de cette thèse indiquent que la médiation de la performance des communautés bactériennes par les ressources s'effectue par une série de changements au sein des composantes de la structure de ces communautés. Les résultats issus de l'approche basée sur les patrons absolus révèlent que ces changements peuvent être soit directionnels, spécifiques aux écosystèmes, ou aléatoire, ce qui se traduit par un manque de connexions significatives entre les patrons absolus de ces différentes composantes (chapitre I). Ces résultats complètent ceux du chapitre II qui montrent une absence de relation entre les patrons absolus de la composition et des capacités fonctionnelles des communautés (chapitre II). Ces résultats suggèrent que la nature des relations entre les ressources, la structure et le métabolisme des communautés n'est pas déterministe, à savoir que l'on ne peut associer par exemple, une certaine configuration compositionnelle à un certain niveau de performance métabolique. Une des plus grandes contributions de cette thèse à l'écologie microbienne aquatique a été de montrer que la nature de la relation entre la composition et la fonction des communautés (chapitre II) mais également entre les ressources, les composantes de la

structure et la performance métabolique des communautés bactériennes (chapitre III) est en fait mesurable sur une base dynamique, basée sur la magnitude des changements de ces différents composants le long des transitions environnementales qui composent le bassin versant et qui connectent les différents types d'habitats.

Des études antérieures menées dans le même bassin versant ont montré que le long des transitions environnementales une partie de la communauté (basé sur les profils de bandes DGGE) est commune entre les différents habitats alors que certains taxons apparaisent plus spécifiques à chacun d'eux, ce qui suggère que les bactéries effectivement disperser d'un habitat à un autre et donc que chaque communautés locales fait partie d'une métacommunauté régionale (Comte et del Giorgio, 2010). Cependant, les résultats présentés dans le chapitre IV montrent que les caractéristiques de l'habitat locales ont une plus grande influence que la dispersion sur la structure des communautés bactériennes même si cette dernière n'a pas été explicitement mesurée. .À cet égard, le type et l'intensité du gradient peuvent influencer la forme et la puissance de la relation entre la composition et la fonction des communautés (chapitre II). En particulier, la variabilité temporelle et spatiale dans la disponibilité du carbone organique dissous semble jouer un rôle clé dans le contrôle des changements dans le métabolisme des communautés bactériennes le long des écotones (chapitre III). La réponse des communautés apparaît ainsi être médiée par deux principaux types de réponse : D'une part des ajustements du niveau d'activité des phylotypes dominants et d'autre part, par le remplacement même des phylotypes dominants (chapitre III).

Un des résultats les plus inattendus est que le type de réponse n'apparaît pas être déterminé par le type, ni l'intensité des gradients, mais plutôt par la plasticité métabolique de la communauté, qui à son tour semble être déterminée par des facteurs indépendants des gradients eux-mêmes (chapitre III). Par ailleurs, un haut niveau de redondance fonctionnelle a été observé tant au sein de la communauté existante, qu'au sein de la métacommunauté, à partir de laquelle les phylotypes sont sélectionnés pour occuper les nouvelles niches qui sont créées le long des transitions environnementales (chapitre II). De plus, les résultats des expériences de transplantation (chapitre IV) indiquent l'existence d'un seuil dans les conditions de l'environnement qui détermine le niveau global de redondance fonctionnelle, et une spécificité écosystémique dans la plasticité métabolique.

Collectivement, les résultats de cette thèse montrent que dans un contexte de métacommunauté, les conditions environnementales locales ont une grande influence sur la détermination de la performance métabolique globale de la communauté, et que la composition des communautés bactériennes joue toujours un rôle dans cette réponse en déterminant le niveau de plasticité de la communauté, mais que ce rôle n'est qu'apparent lorsque la réponse implique un remplacement des phylotypes dans les communautés qui sont intrinsèquement moins plastiques.

En conclusion, cette thèse a permis de mieux comprendre les facteurs déterminant la réponse des communautés du bactérioplancton aux gradients de l'environnement en estimant notamment l'influence des processus locaux à la définition de la configuration des communautés bactériennes, et en décrivant de quelle façon la composition des communautés peut contribuer à la performance globale de la communauté. Les travaux de cette thèse ont des implications tout d'abord conceptuelles sur la façon dont nous voyons les liens entre la composition, la diversité et le fonctionnement de ces communautés microbiennes. Ils suggèrent qu'il puisse y avoir des propriétés émergentes de ces communautés, comme le niveau de plasticité métabolique de la communauté, qui sont liées à la composition des communautés, mais qui sont contrôlées de façon indépendante par d'autres facteurs que ceux qui influencent la performance des taxons présents. D'autre part, cette thèse a également des implications quant à l'intérêt d'utiliser les systèmes microbiens pour tester des théories écologiques. Les résultats issus de cette thèse montrent en effet que les communautés microbiennes ne sont pas uniquement des modèles biologiques « simples » qui permettent d'aborder des questions fondamentales complexes (ex : Bohannan et Lenski, 2000), mais plutôt des communautés particulièrement pertinentes pour tester des théories écologiques telles que la distribution biogéographique, la relation taxon-surface et diversité-stabilité des écosystèmes. Les communautés bactériennes apparaissent sous l'influence de processus évolutifs et écologiques incluant la différenciation des niches écologiques et la dispersion, qui sont communément observés chez les plantes ou les animaux (ex : Martiny *et al.*, 2006). Les résultats présentés dans cette étude ont aussi des implications pratiques sur la façon dont nous concevons des expériences et des études de terrain dans le but d'explorer les liens possibles entre la diversité et la fonction de ces communautés. Les résultats ont également des implications sur notre façon d'interpréter les résultats d'expériences et de modèles

empiriques observés dans les systèmes aquatiques naturels. Par exemple, l'absence de relation entre la composition et la fonction des communautés qui a souvent été observée dans les communautés microbiennes n'implique en aucune façon que la composition ne joue pas un rôle, mais plutôt que ce rôle n'est probablement pas conforme aux idées préconçues et les hypothèses de la plupart de ces études (ex : relation causale directe et déterministe entre la composition et la fonction des communautés).

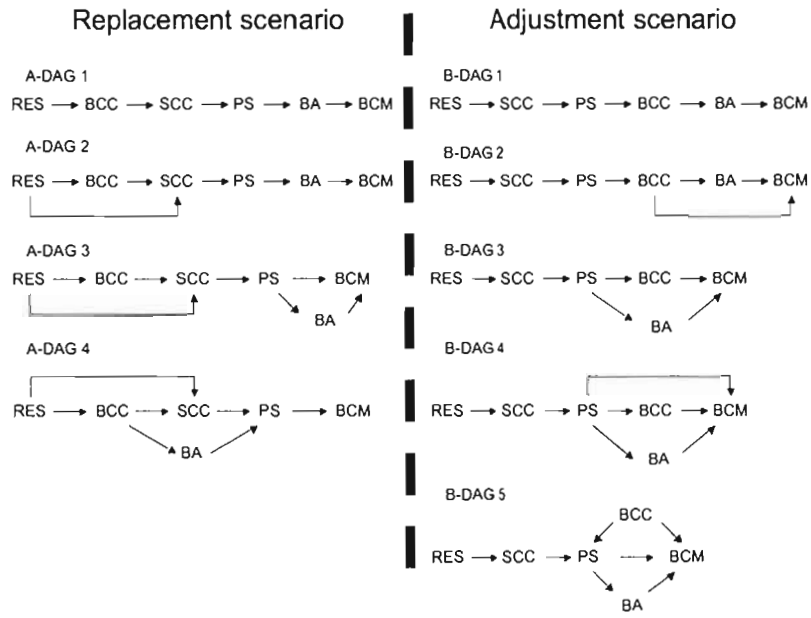
Cette thèse a, à son tour, soulevé deux grandes questions fondamentales. La première concerne l'étendue de la diversité totale à l'échelle régionale. La deuxième adresse plus particulièrement le potentiel fonctionnel de cette diversité régionale. L'application des techniques de biologie moléculaire a révélé un niveau insoupçonné de diversité génétique au sein des communautés microbiennes aquatiques à travers une vaste gamme d'écosystèmes aquatiques (Venter *et al.*, 2004 ; Lozupone et Knight, 2007). Pourtant, la répartition spatiale du bactérioplancton est encore controversée, avec d'une part, la théorie de l'ubiquité des microbes (ex : Fenchel et Finlay, 2004) et d'autre part, des exemples de patrons biogéographiques (Martiny *et al.*, 2006) et d'endémisme (Brown et Donachie, 2007) chez les microbes. À l'échelle d'un bassin versant tel qu'étudié, il serait important de pouvoir étudier la diversité à plusieurs échelles (diversité alpha, bêta et gamma), c'est-à-dire inclure des aspects de la diversité régionale et locale. Dans cette thèse seule la diversité bêta (entre écosystèmes ou le long de gradients environnementaux) a été considérée. Inclure la diversité à l'échelle locale d'un habitat ou global à l'échelle du bassin versant permettrait de mieux comprendre dans quelle mesure les caractéristiques des habitats sont importantes par rapport aux processus régionaux pour déterminer la diversité à l'échelle locale. De plus, décrire la diversité à différent niveau de résolution permettrait de connaître l'étendue de la diversité qui est exprimée *versus* la diversité « cachée » que les techniques d'empreintes tel que la DGGE (utilisées dans cette thèse) ne permettent pas de décrire car détectent uniquement les phylotypes dominants (Pedrós-Alió, 2006b ; Höfle *et al.*, 2008). Ainsi le but serait de savoir si chaque habitat d'une même région hydrographique possède le même pool génétique et donc le même réservoir de diversité potentielle, ou si chaque habitat présente une diversité totale différente dépendant par exemple de l'importance de la dispersion.

L'autre question soulevée par cette thèse est étroitement liée à la première et concerne le potentiel fonctionnel de cette diversité « cachée ». En plus de la distribution de la

diversité, la distribution de l'activité métabolique au sein des communautés bactériennes connaît également des controverses. Comme il a été abordé en introduction, l'activité semble être distribuée selon un continuum d'états physiologiques allant de très actif, à peu ou pas actif voire mort cellulaire. Il est cependant difficile de pouvoir explorer la diversité et le rôle de ces organismes peu abondants ou peu actifs notamment en raison des limites de détections de l'ADN ou l'ARN des méthodes utilisées. Cependant, certaines études ont démontré expérimentalement que l'augmentation de la température et de la concentration des éléments nutritifs pouvaient entraîner une forte proportion de bactéries qui paraissaient inactives à le devenir rapidement (Choi, Sherr et Sherr, 1999). De plus, des études complémentaires ont montré que des taxons rares ou peu abondants pouvaient contribuer significativement à la performance globale de la communauté après une perturbation dans l'environnement (Szabó *et al.*, 2007 ; Yannarell, Steppe et Paerl, 2007) ce qui renvoit à la notion de résilience d'un écosystème (Allison et Martiny, 2008). On peut facilement étendre ces résultats à l'étude de notre bassin versant ou le long des transitions où des processus d'activation ou d'inactivation de groupes bactériens ont dû opérer. Décrire cette partie non exprimée de la diversité et étudier son potentiel fonctionnel et métabolique permettrait d'évaluer son rôle de réservoir phylogénétique en réponse aux gradients de l'environnement.

APPENDICE A

Structure of the different models tested to describe the pathways sequence involved in the response of bacteria to changes in resources (chapitre III).



RES, BCC, SCC, PS, BA and BCM represent the rates of changes in resources, community composition, single-cell characteristics, physiological structure, total abundance and community metabolism respectively. Arrows represent causal relationships between variables. The same models have been tested with changes in DOC as independent variable. Two scenarios are presented: A- Replacement, i.e. BCC responds first to changes in resources and induces shifts in single-cell activity and characteristics (A-DAG1). Other interactions were considered: (i) a direct link between RES and SCC, that is not mediated by BCC (A-DAG2); (ii) same as previous, but the influence of PS on BCM is not mediated by BA (A-DAG3); (iii) shifts in BCC may cause changes in both BA and SCC, both of which exert an influence over PS and subsequently overall BCM (A-DAG4). B- Adjustment, i.e. changes in resources induce metabolic and physiological acclimation of the existing phylotypes (B-DAG1). Other interactions were considered: (i) a direct link between BCC and BCM that is not mediated by BA (B-DAG2), (ii) an indirect path connecting PS and BA which in turn influences BCM independent of BCC (B-DAG3), (iii) a direct link between PS and BCM, which is not mediated by either BA or BCC (B-DAG4); (iv) a path between BCC and both PS and BCM. In this case, BCC is independent from other parameters of community structure and thus exclusively under the influence of external forces (B-DAG5).

APPENDICE B

Description of path analyses output used in chapter III.

The output of the SEM analysis is presented in the form of a path (i.e. Figure 5), showing the components of the model in their proposed sequence; the r^2 shown above each component represents the proportion of its variability that is explained by the ensemble of preceding components located along the path; the strength of causal links between two connected components (i.e. path coefficient) is represented by the numbers above the arrows. Here we have used the standardized estimates, (values are standard deviations from the mean) so that these can be compared between each other. Path coefficients refer then to the standard deviation change in one component given a standard deviation change in the preceding connected component. Significance of a proposed structure is assessed using a χ^2 test that compares the fit between the observed and predicted components of the covariance matrix (Shipley 2002). The P value indicates the probability of having observed the minimum residual differences between the observed and expected covariance by randomness or sampling variation. SEM tests the null hypothesis that the observed and predicted covariances are identical except for random sampling variation. Thus, when P is smaller than an established significance threshold (0.05), the model should be rejected as an explanation of the observed data (Shipley 2002). SEM analyses are asymptotic, such that a low number of observations results in a high probability of rejecting a significant structure. To account for this when we separated the data set into monthly groups (from 8 to 9 observations each), we used a Monte-Carlo test that corrects the χ^2 likelihood distribution. In addition, to correct for the influence of the non-normality of variables on the statistics of the models, we used a Sattora-Bentler correction that generates "robust" χ^2 estimates.

We tested all the alternative models presented in Appendice A for the entire data set using multigroup analyses, which fix the model structure but allow the free parameters to vary among different groups (i.e. the three sampling periods), thus allowing us to assess whether the same structure fit the data for the different periods. In cases when multigroup analyses yielded no significant structures, we further tested the alternative models for each sampling period separately. All SEM analyses were conducted using EQS 6.1 software (mvsoft).

APPENDICE C

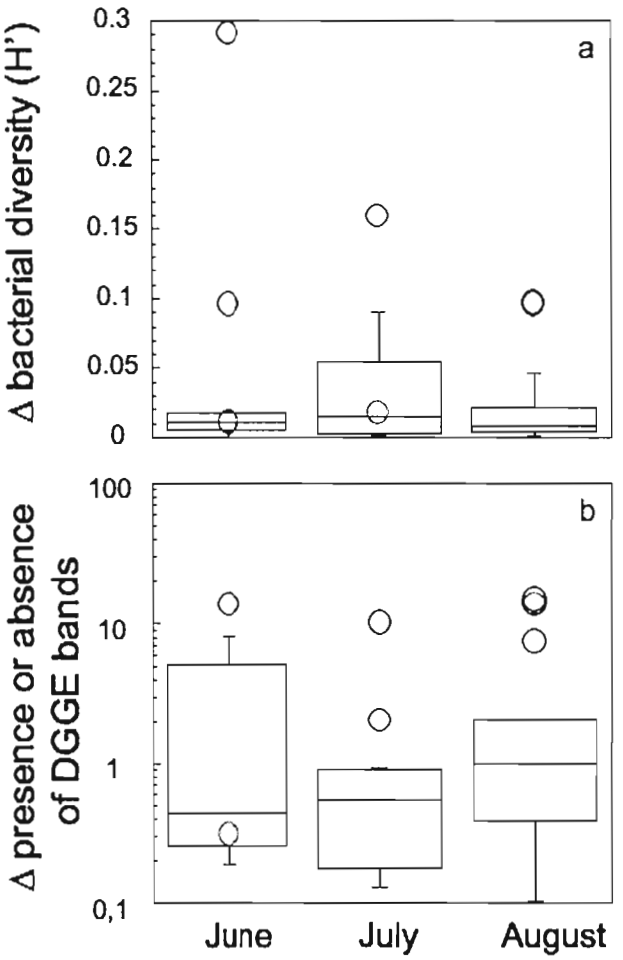
Some Biotic and abiotic characteritics of environmental transitions studied in chapter III.

ET	TT	L	DOC		TP		BP	
			inlet	*output*	*Inlet*	*output*	*inlet*	*output*
1	0.1	40.6	3.3 (2.6-3.8)	3.7 (3.2-4.3)	4.4 (4.1-4.6)	8.6 (5.7-10.7)	0.29 (0.003-0.7)	0.44 (0.35-0.52)
2	19	533.9	3.7 (3.2-4.3)	3.5 (2.2-2.8)	8.6 (5.7-10.7)	1 (0.4-1.3)	0.44 (0.35-0.52)	0.06 (0.03-0.08)
3	20	565.1	2.1 (2.1-2.2)	2.8 (2.6-3)	2 (0.9-2.9)	4.7 (2.6-6.3)	0.04 (0.03-0.05)	0.92 (0.7-1.06)
4	5.4	153.9	5.7 (4.4-7.9)	5.1 (4.9-5.7)	9.4 (7.1-12)	7.1 (6-8.8)	2.4 (1.76-3.04)	1.07 (0.57-2)
5	0.8	863.5	5.1 (4.9-5.7)	5.3 (3.9-7.3)	7.1 (6-8.8)	8.9 (7.1-11.5)	1.07 (0.57-2)	0.77 (0.05-1.52)
6	1	2366.8	4.6 (4.5-4.6)	5 (4.5-5.8)	7.3 (4.1-9)	13.1 (7.5-18.3)	0.24 (0.1-0.43)	2.43 (0.36-4.17)
7	12	335.3	5 (4.5-5.8)	6.2 (6.1-6.3)	13.1 (7.5-18.3)	10 (7.6-13.1)	2.43 (0.36-4.17)	0.65 (0.15-0.93)
8	56	1597.9	4.6 (4.4-4.7)	5.4 (5.1-5.6)	7.6 (4.2-9.7)	7.8 (3.8-13.7)	0.25 (0.15-0.4)	0.69 (0.14-1.49)
9	36	1722.5	5.4 (5.1-5.6)	6.4 (5.7-7.2)	7.8 (3.8-13.7)	8.4 (4.4-13.5)	0.69 (0.14-1.49)	1.2 (0.49-1.89)
10	22	627.4	6 (4.8-7.1)	6 (6-6.1)	9 (6.6-12.9)	5.3 (4.3-7.4)	2.06 (1.37-2.53)	0.34 (0.23-0.55)
11	36	938.4	10 (9-12)	6.1 (5.7-6.4)	21 (20-22)	6 (4.6-8.1)	5.03 (3.12-7.89)	0.25 (0.15-0.38)
12	0.1	94.6	5.9 (5.7-6.1)	6.1 (5.7-6.4)	16 (4.7-32.3)	10.6 (4.1-19.2)	1.57 (0.28-3.97)	1.17 (0.38-2.65)
13	6.3	175	6.1 (5.7-6.4)	6.7 †	10.6 (4.1-19.2)	10.7 (8.3-12.4)	1.17 (0.38-2.65)	1.62 (0.14-2.98)

ET represents the number of the environmental transition considered; TT and L refer to the water transit time (hours) and length (meters) of the transitions respectively. DOC and TP refer to concentrations of dissolved organic carbon (mg L^{-1}) and total phosphorus (µg L^{-1}) respectively. BP corresponds to bacterial production and assessed as uptake of tritiated leucine (µgC L^{-1} hr^{-1}). All values represent mean (minimal-maximal) estimations calculated from 3 replicates excepting ET 13† due to missing values in DOC concentrations.

APPENDICE D

Temporal variability of the observed rates of change (a) in the bacterial diversity measured by the Shannon index and (b) in the presence or absence of bands in the DGGE banding pattern (chapter III).



BIBLIOGRAPHIE

Agrawal, A. A. 2001. Phenotypic plasticity in the interactions and evolution of species. *Science* 12: 221-236.

Allison, S. D., et Martiny, J. B. H. 2008. Resistance, resilience, and redundancy in microbial communities. *Proceedings National Academy Science U. S. A.* 105: 11512-11519.

Alonso-Sáez, L. et Gasol, J. M. 2007. Seasonal Variations in the Contributions of Different Bacterial Groups to the Uptake of Low-Molecular-Weight Compounds in Northwestern Mediterranean Coastal Waters. *Applied and Environmental Microbiology* 73: 3528-3535.

Alonso-Sáez, L., Arístegui, J., Pinhassi, J., Gómez-Consarnau, L., González, J. M., Vaqué, D., et al. 2007 Bacterial assemblage structure and carbon metabolism along a productivity gradient in the NE Atlantic Ocean. *Aquatic Microbial Ecology* 46:43-53.

Apple, J. K., Smith, E. K. et Boyd, T. J. 2008. Temperature, salinity, nutrients, and the covariation of bacterial production and chlorophyll-a in estuarine ecosystems. *Journal of Coastal Research* 55: 59-75.

Azam, F., Fenchel, T., Field, J.G., Gray J.S., Meyer, L.A., et Thingstad, F. 1983. The ecological role of water column microbes in the sea. *Marine Ecology Progress Series* 10: 257-263.

Azam, F. 1998. Microbial control of oceanic carbon flux: The plot thickens. *Science* 280: 694-696.

Azam, F., et Worden, A. Z. 2004. Microbes, molecules and marine ecosystems. *Science* 303: 1622-1624.

Barberan, A. et Casamayor, E. O. 2010. Global phylogenetic community structure and β-diversity patterns in surface bacterioplankton metacommunities. *Aquatic Microbial Ecology* 59: 1-10.

Beisner, B. E., Peres-Neto, P. R., Lindström, E. S., Barnett, A., et Longhi, M. L. 2006. The role of environmental and spatial processes in structuring lake communities from bacteria to fish. *Ecology* 87:2985-2991.

Bell, T., Newman, J. A., Silverman, B. W., Turner, S. L., et Lilley, A. K. 2005. The contribution of species richness and composition to bacterial services. *Nature* 436: 1157-1160.

Bengtsson, J. 1998. Which species? What kind of diversity? Which ecosystem function? Some problems in studies of relations between biodiversity and ecosystem function. *Applied Soil Ecology* 10: 191-199.

Bent, S. J., J.D. Pierson, and L. Forney J. 2007. Measuring species richness based on microbial community fingerprints: the Emperor has no clothes. *Applied and Environmental Microbiology* 73:2399-2401.

Bernard, L., H. Schäfer, F. Joux, C. Courties, G.Muyzer, P. Lebaron. 2000. Genetic diversity of total, active and culturable marine bacteria in coastal seawater. *Aquatic Microbial Ecology* 23:1-11.

Bernhard, A. E., Colbert, D., McManus, J., et Field K. G. 2005. Microbial community dynamics based on 16S rRNA gene profiles in a Pacific Northwest estuary and its tributaries. *FEMS Microbiology Ecology* 52: 115-128.

Bertilsson, S., Eiler, A., Nordqvist, A., et Jørgensen. N. O. G. 2007. Links between bacterial production, amino-acid utilization and community composition in productive lakes. *The ISME journal* 1: 532-544.

Blackwood, C. B., D. Hudleston, D. R. Zak, et J. S. Buyer. 2007. Interpreting Ecological Diversity Indices Applied to Terminal Restriction Fragment Length Polymorphism Data: Insights from Simulated Microbial Communities. *Applied and Environmental Microbiology* 73:5276-5283.

Bohannan, B. J. M., et Lenski, R. E. 2000. The relative importance of competition and predation varies with productivity in a model community. *The American Naturalist* 156: 329-340.

Bohannan, B. J. M., et Hugues, J. 2003. New approaches to analyzing microbial biodiversity data. *Current Opinion in Microbiology* 6:282–287.

Bouvier, T.C., et del Giorgio, P.A. 2002 Compositional changes in free-living bacterial communities along a salinity gradient in two temperature estuaries. *Limnology and Oceanography* 47: 453-470.

Bouvier, T.C et del Giorgio, P.A. 2007. Key role of selective viral-induced mortality in determining marine bacterial community composition. *Environmental Microbiology* 9:287-297.

Bouvier, T.C., del Giorgio, P.A., et Gasol, J.M. 2007. A comparative study of the cytometric characteristics of High and Low nucleic-acid bacterioplankton cells from different aquatic ecosystems. *Environmental Microbiology* 9: 2050-2066.

Brown. M. V et S. P Donachie. 2007. Evidence for tropical endemicity in the Deltaproteobacteria Marine Group B/SAR324 bacterioplankton clade. *Aquatic Microbial Ecology* 46: 107–115.

Buchan, A., Gonzalez, J.M. et Moran, M.A. 2005. Overview of the marine roseobacter lineage. *Applied and Environmental Microbiology* 71: 5665-5677.

Button, D.K., Robertson, B., Gustafson, E. et Zhao, X. 2004. Experimental and theoretical bases of specific affinity, a cytoarchitecture-based formulation of nutrient collection proposed to supercede the Michaelis–Menten paradigm of microbial kinetics. *Applied and Environmental Microbiology* 70: 5511–5521.

Cardinale, B. J. Srivastava, D. S., Duffy, J. E., Wright, J. P., Downing, A., Sankaran, M. et Jouseau, C. 2006. Effects of biodiversity on the functioning of trophic groups and ecosystems. *Nature Letters* 443: 989-992.

Cardinale, B.J., Wright, J.P., Cadotte, M.W., Carroll, I.T., Hector, A., Srivastava, D.S., et al. 2007. Impacts of plant diversity on biomass production increase through time because of species complementarity. *Proceedings National Academy Science U. S. A* 104:18123-18128.

Cardinale, B.J., Bennett, D.M., Nelson, C.E. & Gross, K. 2009. Does productivity drive diversity or vice versa? A test of the multivariate productivity–diversity hypothesis in streams. *Ecology* 90: 1227-1241

Carrero-Colón, M., Nakatsu, C. H., et Konopka, A. 2006. Effect of nutrient periodicity on microbial community dynamics. *Applied and environmental microbiology* 72: 3175-3183.

Casamayor, E. O., H. Schäfer,L. Bañeras, C. Pedrós-Alió, and G. Muyzer. 2000. Identification and spatio-temporal differences between microbial assemblages from two neighboring sulfurous lakes: comparison by microscopy and denaturing gradient gel electrophoresis. *Applied and Environmental Microbiology* 66:499–508.

Casamayor, E. O., Massana, R., Benlloch, S., Øvreås, L., Díez, B., Goddard V. J., *et al.* 2002. Changes in archaeal, bacterial and eukaryal assemblages along a salinity gradient by comparison of genetic fingerprinting methods in a multipond solar saltern. *Environmental Microbiology* 4:338-348.

Castle, D. et Kirchman, D. L. 2004. Composition of estuarine bacterial communities assessed by denaturing gradient gel electrophoresis and fluorescence in situ hybridization. *Limnology and Oceanography: Methods* 2: 303-314.

Cattaneo, A., and Y. T. Prairie. 1995. Temporal variability in the chemical characteristics along the Rivière de l'Achigan: How many samples are necessary to describe stream chemistry? *Canadian Journal of Fisheries and Aquatic Sciences* 52: 828–835.

Choi, J. W., Sherr, B. F. et Sherr, E. B. 1999. Dead or alive? A large fraction of ETS-inactive marine bacterioplankton cells, as assessed by reduction of CTC, can become ETS-active with incubation and substrate addition. *Aquatic Microbial Ecology* 18 : 105-115.

Choi, K. H., et Dobbs, F. C. 1999 Comparison of two kinds of Biolog microplates (GN and ECO) in their ability to distinguish among aquatic microbial communities. *Journal of Microbial Methods* 36: 203-213.

Cohan, F. M. 2002. What are bacterial species? *Annual Review of Microbiology* 56:457-487.

Comte, J., et del Giorgio, P.A. 2009. Links between resources, C metabolism, and the major components of bacterioplankton community structure across a range of freshwater ecosystems. *Environmental Microbiology* 11:1704-1716.

Comte, J. et del Giorgio, P.A. 2010. Linking the patterns of change in composition and functional capacities in bacterioplankton successions along environmental gradients. *Ecology* 95:1466-1476.

Corno, G. et Jürgens, K. 2008. Structural and functional patterns of bacterial communities in response to protist predation along an experimental productivity gradient. *Environmental Microbiology* 10: 2857-2871.

Cotner, J. B. et Biddanda, B. A. 2002. Small players, large role: Microbial influence on biogeochemical processes in pelagic aquatic ecosystems. *Ecosystems* 5:105-121.

Cottingham, K. L., Brown, B. L., et Lennon, J. T. 2001. Biodiversity may regulate the temporal variability of ecological systems. *Ecology Letters* 4: 72-85.

Cottrell, M.T., et Kirchman, D.L. 2000a. Community Composition of marine bacterioplankton determined by 16S rRNA gene clone libraries and fluorescence *in situ* hybridization. *Applied and environmental microbiology* 66: 5116-5122.

Cottrell, M.T., et Kirchman, D.L. 2000b. Natural assemblages of marine Proteobacteria and members of the *Cytophaga- Flavobacter* cluster consuming low- and high-molecular weight dissolved organic matter. *Applied and Environmental Microbiology* 66: 1692–1697.

Covert, J. S. et Moran, M. A. 2001. Molecular characterization of estuarine bacterial communities that use high- and low-molecular weight fractions of dissolved organic carbon. *Aquatic Microbial Ecology* 25: 127-139.

Crump, B. C., Kling, G. W., Bahr, M., et Hobbie, J. E. 2003. Bacterioplankton community shifts in an Arctic lake correlate with seasonal changes in organic matter source. *Applied and Environmental Microbiology* 69: 2253-2268.

Crump, B. C., Hopkinson, C. S., Sogin, M. L., et Hobbie J. E. 2004. Microbial Biogeography along an Estuarine Salinity Gradient: Combined Influences of Bacterial Growth and Residence Time. *Applied and Environmental Microbiology* 70: 1494-1505.

Crump, B. C. et Hobbie, J. E. 2005. Synchrony and seasonality in bacterioplankton communities of two temperate rivers. *Limnology and Oceanography* 50: 1718-1729.

Crump, B. C., Adams, H. E., Hobbie, J. E., et Kling, G. W. 2007. Biogeography of bacterioplankton in lakes and streams of an arctic tundra catchment. *Ecology* 88: 1365-1378.

Curtis, T. P et Sloan, W. T. 2004. Prokaryotic diversity and its limits: microbial community structure in nature and implications for microbial ecology. *Current Opinion in Microbiology*, 7: 221–226

Dahllöf, I. 2002. Molecular community analysis of microbial diversity. *Environmental Biotechnology* 13: 213-217.

del Giorgio, P.A., Cole, J.J., et Cimbleris, A. 1997. Respiration rates in bacteria exceed phytoplankton production in unproductive aquatic systems. *Nature* 385: 148-151.

del Giorgio, P.A., et Cole, J.J. 1998. Bacterial growth efficiency in natural aquatic system. *Annual Review in Ecology and Systematic* 29: 503-541.

del Giorgio, P.A., Bird, D.F., Prairie, Y.T., et Planas, D. 1996. Flow cytometric determination of bacterial abundance in lake plankton with the green nucleic acid stain SYTO 13. *Limnology and Oceanography* 41: 783-789.

del Giorgio, P.A., Prairie, Y.T., et Bird, D.F. 1997. Coupling between rates of bacterial production and the abundance of metabolically active bacteria in lakes, counted using CTC reduction and flow cytometry. *Microbial Ecology* 34: 144-154.

del Giorgio, P.A., et Bouvier, T.C. 2002. Linking the physiologic and phylogenetic successions in free-living bacterial communities along an estuarine salinity gradient. Limnology and Oceanography 47:471-486.

del Giorgio, P. A, et Williams, P. J. leB. 2005. *Respiration in aquatic ecosystems*. Oxford University Press.

del Giorgio, P. A., et Gasol, J. M. 2008. Physiological structure and single-cell activity in marine bacterioplankton. *In Microbial Ecology of the Oceans, Second Edition.* Kirchman, D. L. (ed), John Wiley & Sons, Inc. pp. 243–298.

DeLong, E. F., et Pace, R. F. 2001. Environmental diversity of bacteria and archea. *Systematic Biology* 50: 470–478.

Donavaro, R, G.M. Luna, A. Dell'Anno, et B. Pietrangeli. 2006. Comparison of two fingerprinting techniques, terminal restriction fragment length polymorphism and automated ribosomal intergenic spacer analysis, for determination of bacterial diversity in aquatic environments. *Applied and Environmental Microbiology* 72:5982-5989.

Ducklow, H. 2000. Bacterial production and biomass in the oceans, in: Kirchmann, D.L. (Ed.) *Microbial ecology of the oceans. Wiley Series in Ecological and Applied Microbiology,* : pp. 85-120.

Ducklow, H. 2008. Microbial services: challenges for microbial ecologists in a changing world. *Aquatic Microbial Ecology* 53: 13-19.

Dumont, M., Harmand, J., Rapaport, A., et Godon, J. J. 2009. Towards functional molecular fingerprints. *Environmental Microbiology* 11:1717-1727.

Eiler, A., Langenheder, S., Bertilsson, S., et Tranvik. L. J. 2003. Heterotrophic bacterial growth efficiency and community structure at different natural organic carbon concentrations. *Applied and Environmental Microbiology* 69:3701-3709.

Fenchel, T., et Finlay, B. J. 2004. The ubiquity of small species: patterns of local and global diversity. *BioScience* 54: 777–784.

Fernàndez, A., Huang, S., Seston, S., Xing, J., Hickey, R., Criddle, C., et Tiedje, J. 1999. How stable is stable? Function versus community composition. *Applied and Environmental Microbiology* 65: 3697-3704.

Fierer, N. Morse, J. L., Berthrong, S. T., Bernhardt, E. S., et Jackson, R. B. 2007. Environmental control on landscape-scale biogeography of stream bacterial communities. *Ecology* 88: 2162-2173.

Findlay, S.E.G., Sinsabaugh, R. L., Fischer, D.T., and Franchini, P. 1998. Sources of dissolved organic carbon supporting planktonic bacterial production in the tidal freshwater Hudson River. *Ecosystems* 1: 227-239.

Findlay, S.E.G., et Sinsabaugh, R.L. 2003. Response of hyporheic biofilm metabolism and community structure to nitrogen amendments. *Aquatic Microbial Ecology* 33:127-133.

Findlay, S. E. G, R. L. Sinsabaugh, W. V. Sobczak, et M. Hoostal. 2003. Metabolic and structural response of hyporheic microbial communities to variations in supply of dissolved organic matter. *Limnology and Oceanography* 48:1608-1617.

Findlay, S. E. G, et Sinsabaugh, R. L. 2006. Large-scale variation in subsurface stream biofilms: A Cross-Regional Comparison of Metabolic Function and Community similarity. *Microbial Ecology* 52:491-500.

Fisher, M. M., Klug, J. L., Lauster, G., Newton, M., et Triplett, M.W. 2000. Effects of Resources and Trophic Interactions on Freshwater Bacterioplankton Diversity. *Microbial Ecology* 40: 125-138.

Franklin, R. B., et A. L. Mills. 2003. Multi-scale variation in spatial heterogeneity for microbial community structure in an eastern Virginia agricultural field. *FEMS Microbiology Ecology* 44: 335-346.

Franklin, R. B., et Mills, A. L. 2006. Structural and Functional Responses of a Sewage Microbial Community to Dilution-Induced Reductions in Diversity. *Microbial Ecology* 52: 280-288.

Fuhrman, J.A., et Azam, F. 1982. Thymidine incorporation as a measure of bacterioplankton production in marine surface waters: evaluation and field results. *Marine Biology* 66:109-120.

Fuhrman, J. A. 2002. Community structure and function in prokaryotic marine plankton. *Antonie van Leeuwenhoek* 81: 521–527.

Fuhrman, J. A., Hewson, I., Schwalbach, M. S., Steele, J. A., Brown, M. V., and Naeem, S. 2006. Annually reoccurring bacterial communities are predictable from ocean conditions. *Proceedings National Academy Science U. S. A.* 103: 13104-13109.

Futuyma, D.J. et Moreno, G. 1988. The evolution of ecological specialization. *Annual Review in Ecology and Systematic* 19: 207–233.

Garland, J. L., et Mills, A. L 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level-sole-carbon-source-utilization. *Applied and Environmental Microbiology* 57: 2351-2359.

Garland, J. L. 1996. Analytical approaches to the characterization of samples of microbial communities using patterns of potential C source utilization. Soil Biology Biochemistry 28: 213-221.

Garland, J. L. 2000. Potential and limitations of BIOLOG for microbial community analysis. In: *Microbial Biosystems: New Frontiers* (Bell, C.R.B., Johnson-Green, P., Eds.), pp. 521–527. Atlantic Canada Society for Microbial Ecology, Halifax.

Garland, J. L., Mills A. L., et Young, J. S. 2001. Relative effectiveness of kinetic analysis vs single point readings for classifying environmental samples based on community-level physiological profiles (CLPP). *Soil Biology and Biochemistry* 33:1059-1066.

Gasol, J.M., Simons, A.M. et Kalff, J. 1995. Patterns in the top-down versus bottom-up regulation of heterotrophic nanoflagellates in temperate lakes. *Journal of Plankton Research* 17: 1879-1903.

Gasol, J.M., Zweifel, U.L., Peters, F., Fuhrman, J.A., et Hagstrom, A. 1999. Significance of size and nucleic acid content heterogeneity as measured by flow cytometry in natural planktonic bacteria. *Applied Environmental Microbiology* 65: 4475- 448.

Gasol, J. M., Comerma, M., García, J. C., Armengol, J., Casamayor, E. O., Kojecká, P., et Šimek, K. 2002. A transplant experiment to identify the factors controlling bacterial abundance, activity, production, and community composition in a eutrophic canyon-shaped reservoir. *Limnology and Oceanography* 47: 62-77.

Gevers, D, Cohan, F. M., Lawrence, J. G., Spratt, B. G., Coenye, T., Feil, E. J., *et al.* (2005) Re-evaluating prokaryotic species. *Nature Reviews Microbiology* 3: 733–739.

Giller, P.S., Hillebrand, H., Berninger, U.G., Gessner, M.O., Hawkins, S., Inchausti, P. *et al.* 2004. Biodiversity effects on ecosystem functioning: emerging issues and their experimental test in aquatic environments. *Oikos* 104: 423-436.

Glöckner, F. O., Fuchs, B., and Amann, R. 1999. Bacterioplankton compositions of lakes and oceans: a first comparison based on fluorescence *in situ* hybridization. *Applied and Environmental Microbiology* 65: 3721-3726.

Gray, N.D., Howarth, R., Pickup, R.W., Jones, J.G., et Head, I.M. 2000. Use of combined microautoradiography and fluorescence in situ hybridization to determine carbon metabolism in mixed natural communities of uncultured bacteria from the genus Achromatium. *Applied and Environmental Microbiology* 66:4518-4522.

Gray, N. D., et Head, I. M. 2001. Linking genetic identity and function in communities of uncultured bacteria. *Environmental Microbiology* 3:481-492.

Green, J. L., Bohannan, B. J. M., et Whitaker, R. J. 2008. Microbial biogeography: From taxonomy to traits. *Science* 320:1039-1043.

Hagström, Å., Larsson, U., Hörstedt, P., et Normark, S. 1979. Frequency of dividing cells ,a new approach to the determination of bacterial growth rates in aquatic environments. *Applied and environmental microbiology* 37: 805-812.

Hahn, M. W. 2006. The microbial diversity of inland waters. *Current Opinion in Biotechnology* 17: 256–261.

Handelsman J. 2004. Metagenomics:Application of genomics to uncultured microorganisms. *Microbiology and Molecular Biology Reviews* 68: 669–685.

Hervás, A., Camarero, L., Reche, I., et Casamayor, E.O. 2009. Viability and potential for immigration of airborne bacteria from Africa that reach high mountain lakes in Europe. *Environmental Microbiology* 11: 1612-1623.

Hewson, I., et J. A. Fuhrman. 2004. Richness and diversity of bacterioplankton species along an estuarine gradient in Moreton Bay, Australia. *Applied and environmental microbiology* 70:3425–3433.

Hobbie, J. E., Dalley, R. J., et Jasper, S. 1977. Use of Nuclepore Filters for Counting Bacteria by Fluorescence Microscopy. *Applied and environmental microbiology* 33: 1225-1228.

Höfle, M. G., Haas, H., et Dominik, K. 1999. Seasonal Dynamics of Bacterioplankton Community Structure in a Eutrophic Lake as Determined by 5S rRNA Analysis. *Applied and Environmental Microbiology* 65: 3164-3174.

Höfle, M. G., Kirchman, D. L., Chrsiten, R., et Brettar, I. 2008. Molecular diversity of bacterioplankton: link to a predictive biogeochemistry of pelagic ecosystems. *Aquatic Microbial Ecology*. 53: 39-58.

Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., et al. 2005. Effects of Biodiversity on Ecosystem Functioning: A consensus of Current Knowledge. *Ecological Monographs* 75: 3–35.

Horner-Devine, M. C., Leibold, M. A., Smith, V. H., et Bohannan, B. J. M. 2003. Bacterial diversity patterns along a gradient of primary productivity. *Ecology Letters* 6: 613–622.

Horner-Devine, M. C., J. Green et B. J. M. Bohannan. 2006. Patterns in prokaryotic biodiversity Pages 19-39 *in* Prokaryotic diversity:mechanisms and significance. Logan, N.A., Lappin-Scott, H. M. and Oyston, P. C. F. (eds). Cambridge University Press. New York, NY, USA.

Horz, H. P., Barbrook, A., Field, C. B., et Bohannan, B. J. M. 2004. Ammonia-oxidizing bacteria respond to multifactorial global change. *Proceedings National Academy Science U. S. A.* 101: 15136-15141.

Jezbera, J., Hornák, K. et Šimek, K. 2005. Food selection by bacterivorous protists: insight from the analysis of the food vacuole content by means of fluorescence in situ hybridization. *FEMS Microbiology Ecology* 52: 351-363.

Jones, S. E., Newton, R. J., et McMahon, K. D. 2009. Evidence for structuring of bacterial community composition by organic carbon source in temperate lakes. *Environmental Microbiology* 11: 2463-2472.

Joux, F. et Lebaron, P. 2000. Use of fluorescent probes to assess physiological functions of bacteria at single cell level. *Microbies and Infection* 2: 1523-1535.

Judd, K.E., Crump, B.C., et Kling, G.W. 2006. Variations in dissolved organic matter controls bacterial production and community composition. *Ecology* 87: 2068-2079.

Kalff, J. 2002. Water movements. Pages 179-201 *in* Limnology. Prentice-Hall, Inc., Upper Saddle River, NJ, USA.

Kana, T., Darkangelo, C., Hunt, M.D., Oldham, J.D., Bennett, G.E., et Cornwell, J.C. 1994. Membrane inlet mass spectrometer for rapid high-precision determination of N_2, 0_2, and Ar in environmental water samples. *Analytical Chemistry* 66: 4166-4170.

Kent, A.D, Jones, S.E., Yannarell, A.C., Graham, J.H., Lauster, G.H., Kratz, T.K. et Triplett, E.W. 2004. Annual patterns in bacterioplankton community variability in a humic lake. *Microbial Ecology* 48: 550–560.

Kent, A. D., Yannarrell, A. C., Rusak, J. A., Triplett, A. W. et Mc Mahon, K. D. (2007) Synchrony in aquatic microbial community dynamics. *The ISME journal* 1: 28-47.

King, G. M. 2005. Ecophysiology of microbial respiration. In: del Giorgio P.A, Williams P.J.B. *Respiration in aquatic ecosystems*. Oxford University Press. Chap 2: 18-35.

Kirchman, D. L., K'Nees, E., et Hodson, R. 1985. Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems. *Applied and environmental microbiology* 49: 599-607.

Kirchman, D. L. 1993. Leucine incorporation as a measure of biomass production by heterotrophic bacteria. In *Handbook of Methods in Aquatic Microbial Ecology*. Kemp, P.F., Sherr, B.F., Sherr, E.B., and Cole, J.J. (eds). Danvers, USA: Lewis, pp. 509–512.

Kirchman, D. L. 2002. The ecology of *Cytophaga-Flavobacteria* in aquatic environments. FEMS Microbiology Ecology 39: 91-100.

Kirchman, D. L., Ditell, A. I., Findlay, S. E. G., et Fischer, D. 2004. Changes in bacterial activity and community structure in response to dissolved organic matter in the Hudson River, New York. *Aquatic Microbial Ecology* 35:243-257.

Kirchman, D. L., et Pedrós-Alió, C. 2007. Prediction for the future of microbial oceanography. *Oceanography* 20: 166-171.

Kritzberg, E.S., Langenheder, S. et Lindström, E.S. 2006. Influence of dissolved organic matter source on lake bacterioplankton structure and function-implications for seasonal dynamics of community composition. *FEMS Microbiology Ecology* 56:406-417.

Konopka, A., L. Oliver, et R. F. Turco, 1998. The use of carbon substrate utilization patterns in environmental and ecological microbiology. *Microbial Ecology* 35:103-115.

Kung, K.-J. S., Steenhuis, T S., Kladivko, E. J., Gish, T. J., Bubenzer, G., et Helling, C. S. 2000. Impact of Preferential Flow on the Transport of Adsorbing and Non-Adsorbing Tracers. *Soil Science Society of America Journal* 64:1290–1296.

Kuypers, M. M. M. et Jørgensen, B. B. 2007. The future of single-cell environmental microbiology. *Environmental Microbiology* 9: 6-7.

Langenheder, S. et Jürgens, K. 2001. Regulation of bacterial biomass and community structure by metazoan and protozoan predation. *Limnology and Oceanography* 46: 121–134.

Langenheder, S., Lindström, E. S., et Tranvik, L. J. 2005. Weak coupling between community composition and functionning of aquatic bacteria. *Limnology and Oceanography* 50:957-967.

Langenheder, S., Lindström, E.S. et Tranvik, L.J. 2006. Structure and function of bacterial communities emerging from different sources under identical conditions. *Applied and Environmental Microbiology* 72: 212-220.

Langenheder, S., et Prosser, J. I. 2008. Resource availability influences the diversity of a functional group of heterotrophic soil bacteria. *Environmental Microbiology* 10: 2245-2256.

Langenheder, S., et Ragnarsson, H. 2007. The role of environmental and spatial factors for the composition of aquatc bacterial communities. *Ecology* 88: 2154–2161.

Lebaron, P., Parthuisot, N., et Catala, P. 1998 Comparison of blue nucleic acid dyes for flow cytometric enumeration of bacteria in aquatic systems. *Applied and Environmental Microbiology* 64: 1725-1730.

Lebaron, P., Servais, P., Baudoux, A.C., Bourrain, M., Courties, C., et Parthuisot, N. 2002 Variations of bacterial-specific activity with cell size and nucleic acid content assessed by flow cytometry. *Aquatic Microbial Ecology* 28:131-140.

Leibold, M. A . et Norberg, J. 2004. Biodiversity in metacommunities: Plankton as complex adaptive systems? *Limnology and Oceanography* 49: 1278-1289.

Leibold, M. A, Holyoak, M., Mouquet, N., Amarasekare, P., Chase, J. M., Hoopes, M. F., *et al.* 2004. The metacommunity concept: a framework for multi-scale community ecology. *Ecology Letters* 7: 601–613.

Lennon, J. T. et Cottingham K. L. 2008. Microbial productivity in variable resources environments. *Ecology,* 89: 1001-1014.

Levine, U. Y. et Crump, B. C. 2002. Bacterioplankton Community Composition in Flowing Waters of the Ipswich River Watershed. *The Biological Bulletin* 203: 251–252.

Lindström, E. S. 2000. Bacterioplankton community composition in five lakes differing in trophic status and humic content. *Microbial Ecology* 40: 104-113.

Lindström, E. S. et Leskinen, E. 2002. Do neighboring lakes share common taxa of bacterioplankton? Comparison of 16S-rDNA fingerprints and sequence from three geographic regions. *Microbial Ecology* 44: 1-9.

Lindström, E. S. et Bergström A. K. 2004. Influence of inlet bacteria on bacterioplankton assemblage composition in lakes of different hydraulic retention time. *Limnology and Oceanography* 49: 125-136.

Lindström, E. S., Forslund, M., Alesten, G. et Bergström, A. K. 2006. External control of bacterial community structure in lakes. *Limnology and Oceanography* 51: 339-342.

Lindström, E. S., Kamst-Van Agterveld, M. P., et Zwart, G. 2005. Distribution of typical freshwater bacterial groups is associated with pH, temperature, and lake water retention time. *Applied and Environmental Microbiology* 71: 8201-8206.

Logue, J. B., et Lindström, E. S. 2008. Biogeography of bacterioplankton in inland waters. *Freshwater Review* 1: 99–114.

Logue, J. B., Bürgmann, H., et Robinson, C. T. 2008. Progress in the ecological genetics and biodiversity of freshwater bacteria. *BioScience* 58: 103-113.

Logue, J. B., Lindström, E. S. 2010. Species sorting affects bacterioplankton community composition as determined by 16S rDNA and 16S rRNA fingerprints. *The ISME Journal* 3: 1-10.

Longnecker, K., Sherr, E.B. et Sherr, B.F. 2005. Activity and phylogenetic diversity of bacterial cells with high and low nucleic acid content and electron transport system activity in an upwelling ecosystem. *Applied Environmental Microbiology* 71: 7737-7749.

Loreau, M. 2000. Biodiversity and ecosystem functioning: recent theoretical advances. *Oikos*. 91:3-17.

Lozupone, C. A., et Knight, R. 2007. Global patterns in bacterial diversity. *Proceedings National Academy Science U. S. A.* 104:11436-11440.

Lundin, A. 2000. Use of firefly luciferase in ATP-related assays of biomass, enzymes and metabolites. *Methods in Enzymology* 305: 346-371.

MacIntyre, S., Sickman, J.O., Goldthwait, S.A., et Kling, G.W. 2006. Physical pathways of nutrient supply in a small, ultraoligotrophic arctic lake during summer stratification. *Limnology and Oceanography* 51:1107–1124.

Martiny, H. J. B., Bohannan, B. J.M., Brown, J. H., Colwell, R. K., Fuhrman, J. A., Green J. L. *et al.* 2006. Microbial biogeography: putting microorganisms on the map. *Nature Reviews* 4: 102-112.

Methé, B. A. et Zehr, J. P. 1999. Diversity of bacterial communities in Adirondack lakes: do species assemblages reflect lake water chemistry? *Hydrobiologia* 401: 77-96.

Meyer, A.F., Lipson, D.A., Martin, A.P., Schadt, C.W. and Schmidt, S.K. 2004. Molecular

and metabolic characterization of cold-tolerant alpine soil *Pseudomonas* sensu stricto. *Applied and Environmental Microbiology* 70:483-489.

Mills, A. L, J. S. Herman, G. M. Hornberger, and R. M. Ford. 2003. Functional redundancy promotes functional stability in diverse microbial bioreactor communities. *SAE Internatinal paper* number 2003-01-2509.

Morris, C.E., Bardin, M., Berge, O., Frey-Klett, P., Fromin, N., Girardin, H., *et al.* 2002. Microbial biodiversity: approaches to experimental design and hypothesis testing in primary scientific literature from 1975 to 1999. *Microbiology and Molecular Biology Reviews* 66:592–616.

Morita, R. Y. 1997. Bacteria in oligotrophic environments; starvation survival life style. In Bacteria in Oligotrophic Environments ; Starvation Survival Lifestyle, pp. 1–529. Edited by C. A. Reddy, A. M. Chakrabarty, A. L. Demain & J. M. Tiedje. New York: Chapman & Hall.

Mou, X., Sun, S., Edwards, R.A., Hodson, R.E. and Moran, M.A. 2008. Bacterial carbon processing by generalist species in the coastal ocean. *Nature* 451: 708-711.

Muyzer, G. et K. Smalla. 1998. Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. *Antonie van Leeuwenhoek* 73: 127-141.

Naeem, S. et Li, S. 1997. Biodiversity enhances ecosystem reliability. *Nature* 390: 507-509

Naeem, S., Loreau, M., et Inchausti, P. (2002) Biodiversity and ecosystem functioning: the emergence of a synthetic ecological framework. Pages 1–3 *in* S. Naeem, M. Loreau, and P. Inchausti, editor. Biodiversity and ecosystem functioning: synthesis and perspectives. Oxford University Press, Oxford, UK.

Nelson, C. E. 2009. Phenology of high-elevation pelagic bacteria: the roles of meteorologic variability, catchment inputs and thermal stratification in structuring communities. *The ISME journal* 3: 13-30.

Nishimura, Y., Kim, C., and Nagata, T. 2005. Vertical and seasonal variations of bacterioplankton subgroups with different nucleic acid contents: possible regulation by phosphorus. *Applied and Environmental Microbiology* 71: 5828-5836.

Norberg, J. 2004. Biodiversity and ecosystem functioning: A complex adaptive systems approach. *Limnology and Oceanography* 49: 1269-1277.

Ochman, H., Lerat, E., et Daubin, V. 2005. Examining bacterial species under the specter of gene transfer and exchange. *Proceedings National Academy Science U. S. A.* 103: 6595-6599.

Pace, M.L., and Prairie, Y.T. 2005. Respiration in lakes. In: del Giorgio P.A, Williams P.J.B. *Respiration in aquatic ecosystems*. Oxford University Press. Chap 7: 103-121.

Pedrós-Alió, C. 2006a Genomics andmarinemicrobial ecology. *International Microbiology* 9: 191–197.

Pedrós-Alió, C. 2006b Marine microbial diversity: can it be determined? *Trends in Microbiology* 14: 257-263.

Peres-Neto, P. R. et Jackson, D. A. 2001. How well do multivariate data sets match? The advantages of a Procrustean superimposition approach over the Mantel test. *Oecologia* 129: 169-178.

Petchey, O. L. et Gaston, K. J. 2006. Functional diversity: Back to basics and looking forward. *Ecology Letters* 9: 741-758.

Pomeroy, L. R. 1974. The Ocean's Food Web, A Changing Paradigm. *BioScience* 24: 499-504.

Pomcroy, L. R., LcB. Williams P. J., Azam, F., et Hobbic, J. E. 2007. The microbial loop. *Oceanoraphy* 20: 28-33.

Porter, K.G., et Feig, Y.S. 1980. The use of DAPI for identifying and counting aquatic micofauna. *Limnology and Oceanography* 25: 943-948.

Posch, T., Šimek, K., Vrba, J., Pernthaler, J., Nedoma, J., Sattler, B., *et al.* 1999 Predator-induced changes of bacterial size-structure and productivity studied on an experimental microbial community. *Aquatic Microbial Ecology* 18: 235–246.

Preston-Mafham, J., Boddy, L., et Randerson, P. F. 2002. Analysis of microbial community functional divcrsity using sole-carbon-source utilisation profiles - a critique *FEMS Microbiology Ecology* 42:1-14.

Reche, I., Pullido-Vinella, E., Morales-Baquero, R., et Casamayor, E. O. 2005. Does ecosystem size determines aquatic bacterial richness? *Ecology* 86: 1715-1722.

Reed, H. E. et Martiny, J. B. H. 2007. Testing the functional significance ofmicrobial composition in natural communities. *FEMS Microbiology Ecology* 62:161-170.

Reinthaler, T., Winter, C., et Herndl, G. J. 2005. Relationship between bacterioplankton richness, respiration, and production in the southern North Sea. *Applied and Environmental Microbiology* 71:2260-2266.

Robinson, C., et Williams, P. J. leB. 2005. Respiration and its measurement in surface marine waters. In: del Giorgio P.A, Williams P.J.B. *Respiration in aquatic ecosystems*. Oxford University Press. Chap 9: 147-180.

Rosello-Mora, R., and Amann, R. 2001. The species concept for prokaryotes. *FEMS Microbiology Reviews* 25: 39-67

Salles, J., Poly, F., Schmit, B., et Le Roux, X. 2009. Community niche predicts the functioning of denitrifying bacterial assemblages. *Ecology* 90: 3324-3332.

Schäfer, H., L. Bernard, C. Courties, P. Lebaron, P. Servais, R. Pukall, E. et al. 2001. Microbial community dynamics in Mediterranean nutrient-enriched seawater mesocosms: changes in the genetic diversity of bacterial populations. *FEMS Microbiology Ecology* 34:243-253.

Schmidt, T. M. 2006. The maturing of microbial ecology. *International Microbiology* 9: 217-223.

Smith, E. M. et Prairie, Y. T. 2004. Bacterial metabolism and growth efficiency in lakes: The importance of phosphorus availability. *Limnology and Oceanography* 49: 137-147.

Schimel, J., Balser, T. C et Wallenstein, M. 2009. Microbial stress-response physiology and its implications for ecosystem function. *Ecology* 88: 1386-1394.

Schultz, G.E., et Ducklow, H. 2000. Changes in bacterioplankton metabolic capabilities along a salinity gradient in the York River estuary, Virginia, USA. *Aquatic Microbial Ecology* 22:163-174.

Schwartz, M. W., Brigham, C. A., Hoeksema, J. D., Lyons, K. G. Mills, M. H., et van Mantgem, P. J. 2000. Linking biodiversity to ecosystem function: implications for conservation ecology. *Oecologia* 122: 297-305.

Shannon, C.E. 1948. The mathematical theory of communication. *The Bell System Technical Journal.* 27:379-656.

Shapiro, H. M. 2000. Membrane potential estimation by flow cytometry. *Methods* 21: 271-279.

Sherr, E., et Sherr, B. 2008. Understanding role of microbes in marine pelagic food web: A brief history. *In Microbial Ecology of the Oceans, Second Edition.* Kirchman, D. L. (ed), John Wiley & Sons, Inc. pp. 27–44.

Shipley, B. 2002. *Cause and correlation in biology: A user's guide to path analysis, structural equations, and causal inference.* 2nd edn. Cambridge University Press. Cambridge.

Šimek, K., Vrba, J., Pernthaler, J., Posch, T., Hartman, P., Nedoma, J., et Psenner, R. 1997. Morphological and compositional shifts in an experimental bacterial community influenced

by protists with contrasting feeding modes. *Applied and Environmental Microbiology* 63: 587–595.

Šimek, K., Pernhaler, J., Weinbauer, M.G., Hornák, K., Dolan, J.R., Nedoma, J. *et al.* (2001). Changes in bacterial community composition and dynamics and viral mortality rates associated with enhanced flagellate grazing in a mesoeutrophic reservoir. *Applied and Environmental Microbiology* 67: 2723-2733.

Smith, E. M., et del Giorgio, P. A. 2003. Low fractions of active bacteria in natural aquatic communities? *Aquatic Microbial Ecology* 31: 203-208.

Smith, V. H. 2007. Microbial diversity-productivity relationships in aquatic ecosystems. *FEMS Microbiology Ecology* 62:181-186.

Søndergaard, M., et Middelboe, M. 1995. A cross-system analysis of labile dissolved organic carbon. *Marine Ecology Progress Series* 118: 283-294.

Stepanaukas, R., Moran, M. A., Bergamashi, B. A., et Hollibaugh, J. T. 2003. Covariance of bacterioplankton composition and environmental variables in a temperate delta system. *Aquatic Microbial Ecology* 31: 85-98.

Stevenson, L. H. 1977. A case for bacterial dormancy in aquatic systems. *Microbial Ecology* 4:127-133.

Spehn, E. M, Hector, A., Joshi, J., Scherer-Lorenzen, M., Schmid, B., Bazeley-White, E., Beierkuhnlein, C. *et al.* 2005. Ecosystem effects of biodiversity manipulations in european grasslands. *Ecological Monographs* 75:37–63.

Stepanauskas, R., et Sieracki, M.E. 2007. Matching phylogeny and metabolism in the uncultured marine bacteria, one cell at a time. *Proceedings National Academy Science U. S. A.*104: 9052–9057.

Szabó, K. É., Itor, P. O. B., Bertilsson, S., Tranvik, L., et Eiler, A. 2007. Importance of rare and abundant populations for the structure and functional potential of freshwater bacterial communities. *Aquatic Microbial Ecology* 47:1-10.

Tilman, D. et Downing, J. A. 1994. Biodiversity and stability in grassland. *Nature* 367: 363-365.

Torsvik, V., et Øvreås, L. 2002. Microbial diversity and function in soil: from genes to ecosystems. *Current Opinion in Microbiology* 5: 240–245.

Trousselier, M., Courties, C., Lebaron, P., et Servais, P. 1999. Flow cytometric discrimination of bacterial populations on seawater based on SYTO 13 staining of nucleic acids. *FEMS Microbiology Ecology* 29: 319-330.

Urbach, E., Vergin, K. L., et Giovannoni, S. J. 1999. Immunochemical Detection and Isolation of DNA from Metabolically Active Bacteria. *Applied and Environmental Microbiology* 65: 1207-1223.

Van der Gucht, K., Cottenie, K., Muylaert, K., Vloemans, N., Cousin, S., Declerck, S., *et al.* 2007. The power of species sorting: Local factors drive bacterial community composition over a wide range of spatial scales. *Proceedings National Academy Science U. S. A.* 104: 20404-20409.

Venter, J.C., Remington, K., Heidelberg, J.F., Halpern, A.L., Rusch, D., Eisen, J.A. *et al.* (2004). Environmental genome shotgun sequencing of the Sargasso Sea. *Science* 304: 66-74.

Vitousek, P. M., D'Antonio, C. M., Loope, L. L., Rejmánek, M., et Westbrooks, R. 1997. Introduced species: A significant component of human-caused global change. *New Zealand Journal of Ecology* 21: 1-16.

Vrede, K. 2005. Nutrient and temperature limitation of bacterioplankton growth in temperate lakes. *Microbial Ecology* 49:245-256.

Weinbauer, M. G. 2004. Ecology of prokaryotic viruses. *FEMS Microbiology Reviews* 28: 127-181.

Weinbauer, M.G. and Rassoulzadegan, F. 2004. Are viruses driving microbial diversification and diversity? *Environmental Microbiology* 6: 1–11.

White, P., Kalff, J., Rasmussen, J. B., et Gasol, J. M. 1991. The Effect of Temperature and Algal Biomass on Bacterial Production and Specific Growth Rate in Freshwater and Marine Habitats. *Microbial Ecology* 21: 99-118.

Whitman, W. B. Coleman, D. C., et Wiebe, W. J. 1998. Procaryotes : The unseen majority. *Proceedings National Academy Science U. S. A* 95: 6578-6583.

Williams P. J. LeB. 1981. Microbial contribution to overall marine plankton metabolism: Direct measurements of respiration. *Oceanologica acta* 4: 359-364.

Woese, C. R. 1987. Bacterial evolution. *Microbiological Reviews* 51: 221-271.

Wohl, D.L., Arora, S. et Gladstone, J.R. 2004. Functional redundancy supports biodiversity and ecosystem function in a closed and constant environment. *Ecology,* 85:1534-1540.

Woodcock, S, Curtis, T. P., Head, I. M., Lunn, M., et Sloan, W. T. 2006. Taxa–area relationships for microbes: the unsampled and the unseen. *Ecology Letters* 9:805-812.

Woodward, G. 2009. Biodiversity, ecosystem functioning and food webs in fresh waters: assembling the jigsaw puzzle. *Freshwater Biology* 54: 2171–2187.

Yachi, S., et Loreau, M. 1999. Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis. *Proceedings National Academy Science U. S. A.* 96: 1463-1468.

Yannarell, A. C., et Triplett, E. W. 2004. Within- and between-lake variability in the composition of bacterioplankton communities: Investigations using multiple spatial scales. *Applied and Environmental Microbiology* 70 : 214-223.

Yannarell, A. C., et E. W. Triplett. 2005. Geographic and environmental sources of variation in lake bacterial community composition. *Applied Environmental Microbiology* 71:227-239.

Yannarell, A. C., Steppe, T. F., et Paerl, H. W. 2007. Disturbance and recovery of microbial community structure and function following Hurricane Frances. *Environmental Microbiology* 9:576-583.

Zwart, G., Crump, B.C., Kamst-van Agterveld, M.P., Hagen, F. et Han, S.K. 2002. Typical freshwater bacteria: an analysis of available 16S rRNA gene sequences from plankton of lakes and rivers. *Aquatic Microbial Ecology* 28:141-155.