UNIVERSITÉ DU QUÉBEC À MONTRÉAL

DYNAMIQUE D'UTILISATION DU CARBONE ORGANIQUE DE DIFFÉRENTES ORIGINES PAR LE BACTÉRIOPLANCTON D'EAU DOUCE

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AVANT-PROPOS

Le carbone organique dissous constitue le principal réservoir de C contenu dans les écosystèmes aquatiques d'eau douce, surclassant par un ordre de grandeur la quantité de carbone organique contenu dans les organismes aquatiques (Prairie 2002). En consommant, transformant et dégradant ce pool de carbone, les bactéries hétérotrophes aquatiques ont le potentiel d'influencer profondément tant le fonctionnement que le rôle des écosystèmes d'eau douce dans le cycle global du carbone. Cette thèse étudie la dynamique de la consommation bactérienne de carbone organique dissous et la réponse métabolique qui s'en suit en réponse à des changements dans l'environnement et en particulier, dans la composition et l'origine du pool de carbone organique.

Les résultats de cette thèse sont présentés dans quatre chapitres, dont deux chapitres ont été publiés sous forme d'article scientifique, un autre actuellement en cours de révision et un dernier en préparation pour soumission. Mon superviseur, Prof. Paul del Giorgio, a assuré les moyens financiers et logistiques pour mener à bien ces recherches, a participé au développement des idées et de l'approche expérimentale, conseillé sur les analyses et fournit ses commentaires sur les différents chapitres de cette thèse. Son nom apparaît donc comme coauteur à chacune des différentes publications. Certains travaux ont par ailleurs été réalisés en collaboration avec Prof. S. Leigh McCallister de la Virginia Commonwealth University (VCU) qui a participé au développement du système expérimental utilisé aux chapitres III et IV, ainsi qu'à l'utilisation des isotopes de carbone et à la rédaction des deux derniers chapitres de cette thèse. Son nom apparaît donc comme coauteur de ces chapitres.

Ce travail contribue grandement à l'avancement des connaissances en écologie microbienne aquatique et en biogéochimie du carbone:

1- Cette étude reconstruit la dynamique de la consommation bactérienne de C en milieu aquatique en incluant au sein d'un même cadre conceptuel les différents aspects (ex. consommation à court, long terme et globale) qui composent cette dynamique. Les résultats montrent que la dynamique de consommation à court et long terme décrit des patrons spécifiques aux différents écosystèmes et possèdent des voies de régulation qui leur sont propres de sorte qu'il est impossible de

reconstruire la dynamique de consommation globale à partir d'un de ces aspects en particulier. Cette étude réconcilie en partie les conclusions de certaines études antérieures basées sur des approches méthodologiques ciblant seulement un aspect de la biodisponibilité et de la dynamique de consommation.

2- Cette thèse représentente une des rares études testant directement en milieu naturel l'hypothèse que le carbone organique issu de la production phytoplanctonique est plus facilement consommable et présente un niveau nutritif plus élevé que le carbone dérivé de l'environnement terrestre. Les résultats montrent d'une part que le C algal est effectivement préférentiellement consommé par les bactéries aquatiques, mais contrairement aux suppositions courantes, ce substrat algal est principalement dirigé vers la voie respiratoire. D'autre part, le carbone terrestre ne semble pas soutenir uniquement la consommation à long terme, mais supporte aussi un niveau élevé de métabolisme à court terme et contribue fortement à la synthèse de biomasse.

3- Les résultats de cette thèse représentent une des premières démonstrations de l'existence d'un effet d'amorçage au niveau des voies métaboliques empruntées par différents pools de carbone i.e. la respiration d'un pool de carbone algal semble stimuler l'assimilation du carbone terrigène dans la biomasse bactérienne.

4- Contrairement à la multitude d'études récentes, cette thèse montre que la dynamique de consommation de différents pools de carbone organique dissous ne dépend pas seulement de la composition intrinsèque de ces pools, mais aussi de la forte interaction qui existe entre le métabolisme bactérien et le niveau de nutriment ambiant. Ainsi, le rôle des nutriments ne semble pas se limiter uniquement à une modulation de la consommation, mais aussi de la voie métabolique empruntée par le carbone consommé (ex. respiration, biosynthèse, excrétion).

5- Cette étude montre que les bactéries sont autant des sources que des puits de certains pools de matière organique et que la balance entre ces rôles dépend de l'état physiologique de la communauté bactérienne. À son tour, cet état physiologique bactérien semble être régi à la fois par l'origine de la matière organique et par le niveau de nutriment ambiant. De plus, cette étude suggère un rôle important du compartiment bactérien lacustre dans le stockage de C à long terme en lac via la

production de carbone réfractaire, rôle récemment postulé en milieu marin, mais peu démontré en eau douce.

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LISTE DES ABRÉVIATIONS

BA	« Bacterial abundance »
BA_{ST}	« Short-term bacterial abundance »
BCC	« Bacterial carbon consumption »
BCC_{ST}	« Short-term bacterial carbon consumption »
BGE	« Bacterial growth efficiency »
BGE_{LT}	« Long-term bacterial growth efficiency »
BBP	« Bacterial biomass production »
BP	« Bacterial production »
BP_{ST}	« Short-term bacterial production »
BP_{LT}	« Long-term bacterial production »
BR	« Bacterial respiration »
BR_{ST}	« Short-term bacterial respiration »
CDOM	« Chromophoric dissolved organic matter »
CHL *a*	« Chlorophyll *a* »
CO_2	« Carbon dioxide »
DIC	« Dissolved inorganic carbon »
DOC	« Dissolved organic carbon »
DOM	« Dissolved organic matter »
EEM	« Excitation-emission matrice »
FDOM	« Fluorescent dissolved organic matter »
LA	« Lake surface area »
LTCC	« Long-term carbon consumption »
LTL	« Long-term labile »
MD	« Mean depth »
PARAFAC	« Parallel factor analysis »
PCA	« Principal component analysis »
PCR	« Principal component regression »
POC	« Particulate organic carbon »
POM	« Particulate organic matter »

RECRES	« Respiratory carbon recovery system »
RMA	« Ranged major axis regression »
RQ	« Respiratory quotient »
SD	« Standard deviation »
SE	« Standard error »
$SP\text{-}BR_{ST}$	« Short-term cell-specific bacterial respiration »
$SP\text{-}BP_{ST}$	« Short-term cell-specific bacterial production »
STCC	« Short-term carbon consumption »
STL	« Short-term labile »
TP	« Total phosphorus »
TN	« Total nitrogen »
TR	« Total respiration »
WRT	« Theoretical water retention time »

LISTE DES SYMBOLES

A_{440}	« Water color determined at 440 nm »
Δ	« Rate of change »
$\delta^{13}C$	« ^{13}C:^{12}C atomic ratio »
k	« First-order decay constant »
n	« Sample size »
p	« Probability value»
R^2	« Coefficient of determination in least-square regression models »

RÉSUMÉ GÉNÉRAL

L'objectif principal de cette thèse est de dresser un portrait intégratif de la consommation bactérienne en carbone organique dissous, et de la réponse métabolique subséquente, en considérant les interactions potentielles entre les pools de DOC consommés, les voies métaboliques impliquées et l'environnement. Spécifiquement, les chapitres de cette thèse examinent comment la dynamique globale de la consommation bactérienne de C est médiée par la consommation de certains pools de DOC et leurs interactions en eau douce, à quel point différentes sources de DOC contribuent à la consommation bactérienne en C et interagissent pour déterminer la réponse métabolique des communautés bactériennes aquatiques, comment des changements dans l'environnement peuvent médier la consommation bactérienne et la métabolisation des différents pools de DOC et finalement, de quelle façon les communautés bactériennes peuvent à leur tour influencer la composition du DOC ambiant. Ces questions ont été explorées chez les communautés bactériennes hétérotrophes dans divers écosystèmes d'eau douce, différant en productivité et en apport de carbone terrigène, à l'aide d'essais biologiques et de la modélisation. Les objectifs spécifiques de ce travail étaient de (1) établir les patrons de consommation bactérienne à court terme, long terme et globale au sein de différents écosystèmes d'eau douce et leurs relations avec la composition et l'origine du DOC, (2) explorer les patrons de consommation et de production de différents pools de carbone et les liens potentiels entre ces patrons et le métabolisme bactérien, la composition du DOC et de la disponibilité des nutriments (3) décrire la dynamique de consommation des pools de DOC d'origine algale et terrestre en lacs, (4) explorer les stratégies d'utilisation et allocation métabolique du carbone algal et terrigène employées par les communautés bactériennes. Les résultats ont révélé un lien faible entre les différents aspects de la biodisponibilité du DOC i.e. la dynamique de consommation à court terme, long terme et globale et une régulation différentielle de ces aspects par des pools spécifiques de DOC. Le pool labile d'origine algale a été rapidement et préférentiellement consommé de manière non proportionnelle à sa contribution au pool de DOC total et sa taille a augmenté systématiquement avec la productivité du système. Le DOC d'origine terriège a pour sa part contribué significativement à la consommation à court terme, mais a aussi supporté la majorité de la consommation à long terme ou le métabolisme résiduel. Les résultats indiquent par ailleurs que la biodisponibilité du DOC terrigène n'est pas simplement dictée par sa composition propre, mais aussi par une forte interaction avec le phosphore ambiant. Les résultats démontrent de plus que non seulement les différents pools de DOC ont été différentiellement consommés, mais ont été également différentiellement alloués à la production de biomasse et à la respiration bactérienne. Contrairement aux suppositions courantes, le DOC algal a principalement été alloué à la respiration et non pas à la biosynthèse bactérienne, une stratégie qui semble avoir favorisé l'incorporation du C terrestre dans la biomasse. Finalement, nos résultats montrent que le métabolisme bactérien n'agit pas seulement comme puits de certains pools de C, mais aussi en tant que source et que la balance entre ces rôles varie en fonction de l'état physiologique des communautés bactériennes, qui est à son tour dépendant de l'interaction entre l'origine du substrat et la concentration en nutriment. Collectivement, les résultats de cette thèse suggèrent que la dynamique de consommation bactérienne de DOC et la transformation de ce pool de C par le métabolisme, que ce soit sous forme de CO_2, de nouvelle biomasse ou en une forme

différente de DOC, résultent de la présence et de l'interaction de différents pools de DOC présent et de l'interaction de ces pools avec les nutriments.

Mots clés: Consommation de carbone, métabolisme bactérien, carbone organique, apport terrestre, production algale, allochthonie, allocation des resources, isotope de carbone.

GENERAL ABSTRACT

The aim of this thesis is to provide an integrative view of the consumption and metabolic processing of dissolved organic carbon (DOC) by freshwater bacterial communities, with a focus on potential interactions between DOC pools of different origin, the metabolic pathways involved, and the environment. In particular, the various chapters of this thesis examine how the overall bacterial C consumption dynamics are mediated by the consumption of different DOC pools and their interactions in freshwaters, the extent to which different sources of DOC contribute to bacterial C consumption and further interact to shape the overall metabolic response of bacterial communities, how the environment mediates the bacterial consumption and processing of different DOC pools, and how bacteria may in turn exert feedback on bulk DOC by modifying its composition and properties. These issues were addressed in heterotrophic bacterial communities inhabiting freshwater ecosystems, which differ in productivity and terrigenous C inputs, using a bioassay and modeling approach. The specific objectives of this work were to (1) assess the patterns in short-term, long-term and overall DOC consumption across different freshwater ecosystems and how they relate to DOC composition and origin, (2) explore the patterns in bacterial consumption and production of different carbon pools, and the links they may have with key aspects of bacterial metabolism, DOC origin, and nutrient availability, (3) describe the biological consumption dynamics of algal and terrestrial DOC within the bulk DOC pool of lakes, and (4) explore the strategies of resource utilization and metabolic allocation of algal versus terrestrially derived C by lake bacterial communities. Results revealed a weak connection between the different facets of DOC bioavailability i.e. short-term, long-term and overall bacterial DOC consumption dynamics, and a differential regulation of these aspects by specific pools of bulk DOC. The labile algal DOC pool was rapidly and preferentially consumed irrespectively of its relative contribution to bulk DOC, and increase as a function of system productivity. Terrestrial DOC, on the other hand, contributed to significant fraction of initial DOC consumption, but supported mostly the long-term, residual metabolism. The bioavailability of terrestrial was not simply a function of its intrinsic properties, but also strongly enhanced by phosphorus concentration. The results further show that not only these DOC pools were differentially consumed, but were also selectively allocated to biomass or respiration: Contrary to current assumptions, algal DOC was mainly diverted towards respiratory pathways, a pattern that appeared to facilitate the incorporation of terrestrial C into biomass. Finally, our results show that bacterial C processing does not act as a simple sink of specific DOC pools, but also as a source, and that the balance between these roles varies as a function of the physiological state of bacterial cells, in turn determined by the interplay between substrate origin and ambient nutrients. Collectively, our results suggest a close interaction between nutrient and specific pools of bulk DOC which ultimately shapes the overall bacterial C consumption dynamics and the final outcome of bacterial C processing in freshwaters, whether these pools are being mineralized into CO_2, repackaged into living biomass, or converted into a different form of dissolved organic C.

Key words: C consumption, bacterial metabolism, organic carbon, terrestrial inputs, algal production, allochthony, resource allocation, carbon isotope.

INTRODUCTION GÉNÉRALE

Le concept de « boucle microbienne » introduit au début des années 80 marqua un tournant majeur dans la reconnaissance du rôle que jouent les bactéries hétérotrophes dans le fonctionnement des écosystèmes aquatiques (Pomeroy 1974, Williams 1981, Azam et al. 1984). Les pionniers de l'écologie microbienne montrèrent que ces microorganismes, dont la biomasse domine dans l'ensemble des écosystèmes aquatiques (Whitman, Coleman et Wiebe, 1998), font partie intégrante du réseau alimentaire aquatique en réintroduisant une portion significative de la production primaire perdue sous forme de matière organique dissoute dans la chaîne alimentaire classique. De plus, par la respiration d'une fraction importante de cette matière organique, les bactéries représentent une source majeure de CO_2, influençant le bilan métabolique des écosystèmes aquatiques et jouant un rôle biogéochimique de premier plan (del Giorgio et Williams, 2005).

Contrairement à l'écosystème océanique, dans lequel le concept de boucle microbienne a été développé, les milieux aquatiques d'eau douce sont directement connectés à l'environnement terrestre et reçoivent une quantité importante de matière organique terrigène pouvant soutenir le métabolisme des communautés bactériennes qui la consomment (Wetzel, 1995). Bien que ce phénomène ait été documenté il y a maintenant quelques décennies, l'utilisation qu'en font les bactéries n'a reçu une attention particulière qu'au cours des dernières années (Karlsson, Jansson et Jonsson, 2007 ; Kritzberg *et al.*, 2004 ; McCallister et del Giorgio, 2008). Ces études ont montré l'influence prépondérante des apports en C allochtones sur la productivité des communautés bactériennes pouvant dans certains cas surpasser la production primaire aquatique dans les systèmes peu productifs (Jansson *et al.*, 2000). De plus, de multiples études ont démontré que la respiration du DOC terrigène contribue à maintenir l'hétérotrophie nette observée dans la majorité des écosystèmes d'eau douce (Duarte et Prairie, 2005 ; Karlsson, Jansson et Jonsson, 2007 ; McCallister et del Giorgio, 2008). Cependant, on en connait encore très peu sur la cinétique de la consommation bactérienne des pools de DOC issus des différentes sources de C dans le milieu aquatique, ainsi que sur l'utilisation différentielle ou sélective qu'en font les bactéries pour maintenir diverses activités cellulaires, comme la croissance, la respiration et l'excrétion de métabolites. Ce manque de connaissances limite notre compréhension du rôle clé joué par

le compartiment bactérien dans le fonctionnement de l'environnement aquatique autant au niveau écologique que biogéochimique.

Cette thèse dresse un portrait intégratif de la consommation bactérienne en carbone organique dissous en considérant particulièrement la dynamique de consommation bactérienne de C et la réponse métabolique qui s'en suit face à des changements dans la composition, l'origine du pool de DOC consommé ainsi que dans l'environnement. Plus particulièrement, cette thèse a comme objectifs (1) d'établir les patrons de consommation bactérienne à court terme, long terme et globale au sein de différents écosystèmes d'eau douce et leurs relations avec la composition et l'origine du DOC, (2) d'explorer les patrons de consommation et de production de différents pools de carbone et les liens potentiels entre ces patrons et le métabolisme bactérien, la composition du DOC et de la disponibilité des nutriments, (3) de décrire la dynamique de consommation des pools de DOC d'origine algale et terrestre en lacs, (4) d'explorer les stratégies d'utilisation et allocation métabolique du carbone algal et terrigène employées par les communautés bactériennes. Les sections qui suivent présentent une revue de la littérature courante reliée à ces différents objectifs, ainsi que l'approche conceptuelle et méthodologique utilisée pour les atteindre.

ÉTAT DES CONNAISSANCES

Carbone organique dissous : origine, composition et rôle

Le carbone organique présent dans les milieux aquatiques représente non seulement un des principaux réservoirs de carbone de la biosphère, mais également une de ses composantes les plus dynamiques (Hedges 1992), affectant le cycle du carbone autant à l'échelle régionale qu'à l'échelle globale. La majeure fraction (>90%) du pool de carbone organique aquatique se retrouve généralement sous forme dissoute, communément définie comme étant la fraction passant par un filtre de 0.2 ou 0.45 μm. À l'échelle de l'écosystème, la quantité de carbone contenue dans le carbone organique dissous (DOC) dépasse par plus d'un ordre de grandeur la quantité contenue dans la biomasse bactérienne et des autres organismes aquatiques (ex. phytoplancton, zooplancton, poisson) (Prairie, 2008). Ainsi, tout processus qui puisse altérer ou transformer ce pool de carbone a de ce fait le potentiel

d'influencer significativement le fonctionnement de l'écosystème aquatique tant au niveau biogéochimique qu'écologique.

Le DOC joue une multitude de rôles affectant autant la physique, la chimie que la biologie des écosystèmes aquatiques. De par ses propriétés optiques, le DOC absorbe une fraction significative des rayons lumineux entrant dans la colonne d'eau. Cette diminution de la lumière disponible limite la photosynthèse (Morris *et al.*, 1995), protège les microorganismes des rayons ultraviolets toxiques (Maranger, del Giorgio et Bird, 2002 ; Sieracki et Sieburth, 1986) et affecte la régulation thermique de la colonne d'eau (Schindler *et al.*, 1990 ; Snucins et Gunn, 2000). En réagissant avec la lumière, la fraction colorée du pool de DOC produit divers radicaux libres et peroxydes d'hydrogène ayant le potentiel de briser certaines molécules organiques complexes, affectant ainsi la composition du pool de DOC (Scully *et al.*, 1995 ; Vione *et al.*, 2006). Le DOC peut aussi diminuer la disponibilité des nutriments inorganiques ou encore des métaux lourds en formant des complexes organiques (Kirchman, 2003 ; Perdue, 1998). Finalement, le DOC constitue la principale source de carbone et d'énergie soutenant les communautés bactériennes hétérotrophes et constitue ainsi la base alimentaire de la boucle microbienne (Azam *et al.*, 1983).

Les sources de DOC en environnement aquatique sont diverses et peuvent être séparées en deux grandes catégories: les sources qui produisent le DOC à l'intérieur même de l'écosystème aquatique (sources autochtones) et les sources faisant partie intégrante de l'environnement terrestre (sources allochtones). Le DOC d'origine autochtone est principalement issu de la production phytoplanctonique en zone pélagique, bien que dans certains lacs peu profonds les macrophytes et les algues benthiques contribuent de manière considérable à la production de carbone organique autochtone (Demarty et Prairie, 2009 ; Rooney et Kalff, 2003). Une partie du relargage du DOC issu de la production primaire dans le milieu aquatique se produit lors de la mort cellulaire ou par l'exsudation active et passive de différents composés carbonés représentant jusqu'à 60% de la production primaire phytoplanctonique (Søndergaard, Riemann et Jørgensen, 1985 ; Vegter et De Visscher, 1984).

Divers monomères et polymères de faible poids moléculaire comme des protéines, acides aminés, sucres simples ou encore des acides carboxyliques domine généralement (>70% dans certaines conditions) le pool de DOC d'origine autochtone (Marsalek et

Rojickova, 1996 ; Søndergaard et Schierup, 1982). Cependant, une fraction de ce pool de DOC autochtone comprend aussi des molécules plus complexes et de plus grand poids moléculaire comme certains polysaccharides qui peuvent persister dans l'environnement aquatique. La contribution relative de ces diverses molécules à la biomasse totale varie sensiblement en fonction de l'espèce ou des populations impliquées ainsi que de l'état physiologique des cellules (Biddanda et Benner, 1997 ; Morris, 1981).

De par leur situation géographique, les écosystèmes d'eau douce reçoivent une grande quantité de DOC en provenance de l'environnement terrestre avoisinant. Ces apports allochtones peuvent dépasser la quantité de C produits à l'intérieur de l'écosystème et constituer la majeure partie du pool de DOC en eau douce (Cole *et al.*, 2002 ; Jonsson *et al.*, 2001). Dans l'environnement terrestre, le DOC se forme lors du passage de l'eau à travers la canopée arboricole et de sa percolation subséquente dans les sols. Le DOC d'origine terrestre inclut donc les molécules organiques en provenance de la litière végétale, des exsudats de racines et des métabolites secondaires des microorganismes habitant les sols. Contrairement au pool de DOC issu de la production algale, une faible portion seulement du pool de DOC allochtone est représentée par les molécules de faible poids moléculaire. Les substances humiques comprenant les acides humiques et fulviques eux-mêmes composés de divers acides hydrophobiques et hydrophiliques, de phénols et autres molécules aromatiques dominent plutôt ce pool de DOC (Aitkenhead-Peterson, McDowell et Neff, 2003). En fait, dans certains lacs et rivières dits « humiques», ces substances peuvent composer jusqu'à 90% du pool total de DOC (Kronberg, 1999).

Biodisponibilité et dynamique de consommation du DOC

D'un point de vue biologique, le pool de DOC peut être divisé en un pool labile et un pool réfractaire, le pool labile étant défini comme la quantité de DOC utilisée par le bactérioplancton durant une période variant généralement entre quelques jours et quelques semaines et le pool réfractaire comme représentant le DOC non consommé (del Giorgio et Davis, 2003). La proportion de DOC labile ainsi définie est généralement faible (15%) en eau douce, impliquant que la majeure partie du DOC n'est pas accessible aux communautés bactériennes, du moins sur des échelles temporelles comparables au temps de résidence moyen de la plupart des écosystèmes lacustres. Deux études compilant les données existantes

de biodisponibilité du DOC récoltées au sein de divers écosystèmes aquatiques (ex. lacs, rivières, marais, estuaires et océan) ont cependant observé des variations significatives autant entre écosystèmes qu'au sein d'un même écosystème (del Giorgio et Davis, 2003 ; Søndergaard et Middelboe, 1995). Ces variations ne sont généralement qu'en partie expliquées par la concentration totale de DOC du système (del Giorgio et Davis, 2003), suggérant que la composition et l'origine du DOC, et non sa concentration, constituent un facteur clé dans la régulation de la labilité et de la consommation bactérienne en carbone.

Comme mentionné à la section précédente, le pool de DOC présente un caractère particulièrement hétérogène en termes de composition, mais également en termes de réactivité ou d'accessibilité à la consommation bactérienne. Cette hétérogénéité est particulièrement apparente dans les expériences de dégradation biologique du DOC : les bactéries utilisent d'abord les composantes plus labiles en laissant les molécules plus récalcitrantes dans le milieu (Mateles et Chian, 1969). En résulte alors une courbe de consommation diminuant dans le temps et pouvant être modélisée selon un continuum de réactivité (Koehler *et al.*, 2012 ; Vähätalo, Aarnos et Mäntyniemi, 2010). La dynamique de consommation du DOC ambiant reflète ainsi directement la contribution relative des divers pools de C qui le composent et qui diffèrent en termes de biodisponibilité et de dynamique de dégradation (Middelburg, 1989). Cette thèse soutient que cette vision dynamique de la consommation devrait être considérée à l'heure d'identifier les facteurs qui régulent la labilité du DOC, puisqu'il est probable que les différents pools ou différents aspects de la consommation (ex. consommation à court ou long terme) répondent différemment à des changements dans la composition ou la source de C.

Cette approche à la consommation a cependant été peu explorée dans les études antérieures qui ont souvent utilisé des méthodologies ciblant des pools ou des parties différentes de la dynamique de consommation (consommation à court ou long terme). Par exemple, l'approche classique utilisée pour déterminer la quantité de DOC disponible à la consommation bactérienne consiste à (1) isoler le pool de DOC en filtrant l'eau recueillie sur le terrain sur filtres fins (pores d'une taille variant entre 0.2-0.45 µm), (2) réintroduire la flore bactérienne dans le milieu de culture en prenant soin de filtrer l'inoculum sur filtre plus grossier (0.7-1 µm), l'idée étant de limiter la présence des prédateurs bactériens afin de s'assurer de mesurer des taux de consommation maximaux et comparables et finalement, (3)

suivre la décroissance des concentrations de DOC sur une période variant entre quelques jours et quelques semaines (del Giorgio et Davis, 2003). Cependant, les méthodes et appareils analytiques actuellement employés ne permettent pas de détecter de faibles variations dans la concentration de DOC à court terme et par conséquent, ce n'est souvent qu'après quelques jours d'incubation qu'un changement significatif de concentration peut être observé. Ainsi, cette approche sous-estime fort probablement la consommation bactérienne in situ puisque la partie du DOC disponible qui soutient la consommation initiale est probablement ignorée et cible plutôt un pool de DOC étant défini comme semi-labile.

D'autres approches ont été employées pour estimer la consommation bactérienne en C et ces approches requièrent notamment moins de temps d'incubation. Par exemple, la détermination de la production bactérienne suivant l'incorporation d'un substrat radioactif (Bergström et Jansson, 2000 ; Meyer, 1994) ou des mesures de respiration bactérienne à l'aide de la spectrométrie en masse (del Giorgio, Pace et Fischer, 2006b ; Giorgio et Bouvier, 2002) ont été utilisés pour estimer la consommation bactérienne sur quelques heures et ont par conséquent permis de fournir une vision plus juste de la consommation in situ. Lorsque comparées à l'approche classique, il en résulte cependant une surestimation des taux de consommation pouvant aller jusqu'à un ordre de grandeur (del Giorgio et Pace, 2008). Il est toutefois possible de réconcilier ces différentes mesures de la consommation bactérienne de C en considérant qu'elles résultent en fait de l'expression de différents aspects de la consommation plutôt que de l'expression d'un simple biais méthodologique (voir discussion, chapitre I). Une étude récente a d'ailleurs démontré que la consommation à court et long terme suit des dynamiques indépendantes au long de gradients environnementaux et que différents facteurs influencent leurs dynamiques respectives (del Giorgio et Pace, 2008). Cette approche dynamique de la consommation bactérienne est explicitement développée au chapitre I de cette thèse.

Transformation métabolique du DOC consommé

Une fois consommé, le DOC est transformé en divers produits métaboliques par le compartiment bactérien. Comme mentionnée en début d'introduction, la production de nouvelle biomasse à partir d'un pool dissous permet la réintroduction d'énergie et de carbone autrement indisponible à la chaîne alimentaire. Cette voie de transfert d'énergie est

particulièrement importante dans les systèmes improductifs, où la production bactérienne peut surpasser la quantité d'énergie générée par la production autochtone (Jansson *et al.*, 2000). De plus, comme la majeure partie du pool de DOC des écosystèmes lacustres est de nature terrigène, le compartiment bactérien a le potentiel d'agir comme chaînon principal dans le transfert du DOC allochtone vers la chaîne alimentaire aquatique. La conversion du DOC en CO_2 via la respiration est aussi un processus important dans le fonctionnement des écosystèmes aquatiques en influençant la dynamique des gaz dans la colonne d'eau et en influençant la balance métabolique de ces systèmes. Particulièrement, la respiration de carbone terrigène contribue à maintenir l'hétérotrophie nette observée dans la majorité des écosystèmes d'eau douce et influence ainsi le cycle du carbone à l'échelle régionale. Finalement, l'efficacité de croissance bactérienne, définie comme étant la quantité de C alloué à production bactérienne sur la quantité totale de C consommé (BGE=BP/(BP+BR), renseigne sur la voie métabolique empruntée par le DOC une fois consommé (del Giorgio et Cole, 1998).

Ainsi, à l'imagine de la dynamique de la consommation, le métabolisme bactérien peut aussi être divisé en fonction des différentes activités cellulaires impliquées. Cette ségrégation du métabolisme, et particulièrement l'idée que ces différentes voies métaboliques soient elles aussi indépendamment régulées, n'est pas fréquemment considérée dans les études évaluant l'utilisation du carbone par le compartiment bactérien. Par exemple, plusieurs études passées ont implicitement supposé que les mesures de production bactérienne ou de respiration reflètent autant la quantité que le type de DOC consommé (Ågren *et al.*, 2008 ; del Giorgio et Pace, 2008 ; Moran et Hodson, 1990). Or, l'efficacité de croissance bactérienne varie énormément au sein des écosystèmes aquatiques (del Giorgio et Cole, 1998) et également en fonction du type de substrat consommé (Berggren, Laudon et Jansson, 2007 ; Lennon et Pfaff, 2005), suggérant que la proportion de carbone alloué à la respiration et à la production n'est pas constante, mais plutôt variable. Ceci questionne la validité d'employer les mesures métaboliques comme indices représentatifs autant de la quantité que du type de C consommé et suggèrent la possibilité d'une allocation différentielle des pools de DOC consommé aux diverses voies métaboliques impliquées.

Le compartiment bactérien ne se réduit pas en un puits net de carbone organique dissous par la consommation de C, la production de biomasse ou la respiration. Il a été

démontré ces dernières années que ce compartiment agit aussi en tant que source de DOC en produisant diverses biomolécules (Ogawa *et al.*, 2001 ; Ortega-Retuerta *et al.*, 2009). En particulier, la production de matière récalcitrante par le compartiment bactérien a reçu une attention grandissante en raison de son rôle potentiel dans la séquestration du carbone en milieu aquatique (Jiao *et al.*, 2010 ; Yamashita et Tanoue, 2008). Certaines études ont par exemple montré la présence persistante dans les eaux naturelles de protéines membranaires comme les porines P (Tanoue *et al.*, 1995), certaines classes de lipides (Wakeham, Pease et Benner, 2003), ainsi que des peptidoglycanes issus de la capsule cellulaire (Benner et Kaiser, 2003 ; Schleifer et Kandler, 1972). Cette capacité des bactéries à générer une large gamme de molécules organiques a de plus été démontrée dans des expériences en laboratoire, où la production de différentes classes de composés, dont certains colorés, à partir de substrats simples comme le glucose a été observée (Kramer et Herndl, 2004 ; Ogawa *et al.*, 2001). Fait intéressant, certains de ces composés s'apparentent aux substances humiques couramment observés en milieu aquatique, suggérant une contribution bactérienne à ce pool de DOC qui persiste dans l'environnement aquatique (Kramer et Herndl, 2004 ; Shimotori, Omori et Hama, 2009 ; Yamashita et Tanoue, 2008). Les mécanismes impliqués dans cette production et les facteurs qui les régulent ne sont cependant pas encore bien connus, mais certaines études indiquent qu'une perte de matériel cellulaire lors de la division des cellules (Kawasaki et Benner, 2006) ou suivant la lyse virale (Middelboe et Lyck, 2002) ou la prédation protiste (Strom *et al.*, 1997) soit à l'origine du relargage de DOC. De plus, la transformation et l'excrétion directe de certains composés organiques ont aussi été proposées comme mécanisme de production de DOC (Gruber *et al.*, 2006 ; Ogawa *et al.*, 2001).

La production de matière organique ne se limite cependant pas à la génération de composés réfractaires, mais aussi à la production de composés généralement considérés biodisponibles comme certaines substances représentées par les protéiniques ou les acides aminés (Cammack *et al.*, 2004 ; Lønborg *et al.*, 2009 ; Parlanti *et al.*, 2000). Par exemple, la concentration d'un composé détecté par fluorescence et ressemblant au tryptophane a été utilisée comme facteur prédictif du niveau d'activité bactérienne en lacs (Cammack *et al.*, 2004). Cette même étude a par ailleurs montré une production nette de ce même composé par le métabolisme bactérien, soulevant une question conceptuelle importante à l'heure d'utiliser la composition du DOC comme indice potentiel de la biodisponibilité puisque ce même

indice peut aussi agir en tant que proxy du métabolisme. Ainsi, une partie des travaux menés dans cette thèse, explorant le lien entre la composition, la source de DOC et la dynamique de consommation, ont été dévolus à identifier les facteurs métaboliques et environnementaux qui influencent la balance entre la production et la consommation de certains pools de DOC.

Liens avec la composition et l'origine du DOC et l'environnement

Ces dernières années, de multiples travaux ont étudié le lien entre la composition du pool de DOC, sa biodisponibilité et le métabolisme avec résultats contradictoires et non concluants. Par exemple, certains travaux ont évoqué la consommation préférentielle des molécules de faible poids moléculaire puisque celles-ci sont directement accessibles, nécessitent peu ou pas de transformation enzymatique avant d'être assimilées et favorisent une croissance efficace des communautés bactériennes (Berggren *et al.*, 2010a ; Kirchman, 2003). Cependant, il a été démontré qu'une fraction de ces composés moléculaires persistent dans l'environnement (Amon et Benner, 1996). La présence de structures aromatiques dans le pool de DOC est généralement considérée comme étant un facteur freinant autant la consommation en carbone que la productivité bactérienne (Berggren, Laudon et Jansson, 2009 ; Marschner et Kalbitz, 2003), bien que certaines études aient démontré une utilisation de ces composés dans certains systèmes (Bano, Moran et Hodson, 1997). La composition atomique en C, N, H et O informant sur la qualité nutritive et l'état d'oxydation des molécules a aussi été utilisée pour prédire la biodisponibilité et l'efficacité de croissance avec un certain succès (Hunt, Parry et Hamilton-Taylor, 2000 ; Kroer, 1993 ; Sun *et al.*, 1997). Certains types de composés organiques comme les sucres simples et les acides aminés ont été préférentiellement consommés dans certaines expériences (Rosenstock et Simon, 2001 ; Sundh, 1992 ; Weiss et Simon, 1999), mais quelques études ont aussi démontré des niveaux variables d'utilisation (Benner, 2003).

La source produisant les différentes molécules organiques retrouvées dans le milieu aquatique a depuis longtemps été proposée comme facteur explicatif de variabilité de la labilité du DOC et du métabolisme (Sepers, 1977). Bien que très peu d'études aient directement testé cette hypothèse, une des suppositions largement répandues en écologie aquatique et en biogéochimie du carbone est que le DOC issu de la production algale présente un niveau de labilité beaucoup plus élevé que sa contrepartie terrestre (Bianchi, 2011 ;

Hobbie, 1988). Cette labilité élevée reposerait la structure moléculaire simple et de faible poids du DOC algal et favoriserait la production de biomasse plutôt que la respiration. Il a toutefois été récemment démontré que des molécules de faible poids moléculaire originaire de l'environnement terrestre, bien que représentent qu'une faible proportion du pool de DOC total, puissent aussi être consommées rapidement et efficacement par les communautés bactériennes lacustres (Ågren *et al.*, 2008 ; Berggren *et al.*, 2010a). De plus, la « fraîcheur » relative du pool de DOC algal comparativement au pool terrestre, partiellement dégradé dans les sols avant d'être acheminé aux lacs, semblerait aussi favoriser une plus grande consommation et productivité bactérienne (Berggren, Laudon et Jansson, 2009 ; Raymond et Bauer, 2001). Or, de récentes études suggèrent que le DOC ayant séjourné longuement dans les sols avant d'être acheminé au milieu aquatique puisse aussi être significativement consommé (McCallister et del Giorgio 2012).

La diversité des approches à la consommation mentionnées précédemment et employées par ces études explique possiblement l'apparente contradiction de ces résultats en ciblant des aspects différents de la biodisponibilité ou de la dynamique de consommation. De manière importante, ces résultats soulignent qu'encore aujourd'hui, aucun indice strictement basé sur la composition ou l'origine du carbone ne peut prédire adéquatement la biodisponibilité du DOC et le métabolisme bactérien. Par exemple, la consommation à court terme dans la rivière Hudson était davantage reliée à des indices de productivité primaire comme le niveau de chlorophylle alors que la consommation à long terme était plutôt reliée aux apports allochtones dans une étude récente (del Giorgio et Pace, 2008). Cependant, bien que l'approche conceptuelle à la consommation évoquée précédemment peut fournir de l'information utile sur la dynamique de consommation de différents pools de C à court et long terme, elle ne permet pas d'identifier et de suivre directement la dynamique de consommation des différentes sources de DOC présentes dans le milieu aquatique et les voies métaboliques empruntées par celles-ci.

L'emploi des isotopes stables a gagné en popularité ces dernières années puisque cet outil permet de reconstituer la diète des organismes et de suivre le flot d'éléments et d'énergie entre consommateurs dans le réseau trophique aquatique (Carpenter *et al.*, 2005 ; Cole *et al.*, 2002 ; Pace *et al.*, 2004 ; Solomon *et al.*, 2011). Cette approche consiste à comparer le signal isotopique de l'organisme étudié à celui des sources nutritives potentielles

pour en déduire leur contribution relative à la diète de l'organisme en question. Bien que cet outil ait été utilisé abondamment pour identifier les sources de C soutenant la productivité des organismes aquatiques situés au haut de la chaîne alimentaire (ex. zooplankton) (Cole *et al.*, 2011 ; Karlsson *et al.*, 2012 ; Karlsson *et al.*, 2003 ; Mohamed et Taylor, 2009), très peu d'études ont utilisé cette méthode chez le bactérioplancton (Karlsson, Jansson et Jonsson, 2007 ; Kritzberg *et al.*, 2004 ; McCallister et del Giorgio, 2008). Basées sur la signature isotopique de la biomasse bactérienne ou sur le CO_2 issu de la respiration, ces quelques études supportent l'idée d'une consommation bactérienne autant du DOC d'origine algale que celui issu de l'environnement terrestre et suggèrent une consommation préférentielle du DOC d'origine autochtone. Cependant, le design expérimental employé dans ces études permet seulement d'identifier la contribution relative des sources de carbone terrestre et algal au pool de DOC initialement consommé et non de suivre l'évolution de la consommation relative de ces pools de différentes origines dans le temps. De plus, très peu d'études ont suivi en parallèle autant la source qui supporte la respiration que celle supportant la production de biomasse (Hullar *et al.*, 1996), limitant ainsi notre compréhension des stratégies d'allocation des ressources autochtones et allochtones employées par les communautés bactériennes, ainsi que les interactions entre les différents pools de DOC présents et consommés tant autant au niveau de la consommation que de la réponse métabolique.

Cette thèse prête une importance particulière au rôle potentiel de la composition et de l'origine du C dans la régulation de la dynamique de consommation de DOC et de la réponse métabolique qui s'en suit. Cependant, elle n'exclut pas la possibilité que d'autres facteurs environnementaux puissent moduler l'utilisation du DOC par les communautés bactériennes. En particulier, le rôle des nutriments, qui ont le potentiel de moduler autant la quantité de DOC consommé (Zweifel, 1999 ; Zweifel, Norrman et Hagstrom, 1993) que la réponse métabolique (del Giorgio et Newell, 2012 ; Smith et Prairie, 2004), a été exploré.

OBJECTIFS

La base conceptuelle de la thèse (Fig. 0.1) repose sur quatre questions fondamentales qui seront explorées par les différents chapitres de cette thèse:

1- Comment la dynamique globale de la consommation bactérienne de C est médiée par

certains pools de DOC et leurs interactions en eau douce? (chapitres I, III)

2- À quel point différentes sources de DOC contribuent à la consommation bactérienne en C et interagissent pour déterminer la réponse métabolique qui s'en suit des communautés bactériennes aquatiques? (chapitres I, II, III et IV)

3- Comment des changements dans l'environnement peuvent médier la consommation bactérienne et la métabolisation des différents pools de DOC? (chapitres II, III)

4- De quelle façon les communautés bactériennes peuvent à leur tour influencer la composition du DOC ambiant? (chapitre II)

APPROCHES ET MÉTHODES

Aspects métaboliques considérés

Le métabolisme cellulaire se résume invariablement à quelques grandes voies fondamentales: l'acquisition d'énergie et de matières structurantes, la synthèse de biomasse ou croissance, les activités respiratoires et l'excrétion de différents métabolites. Cette thèse couvre l'ensemble de ces processus chez le bactérioplancton et d'en explorer la dynamique et la régulation par l'environnement, la source de C consommé et la quantité et la qualité du DOC. Les chapitres I et III présentent en détails les processus impliqués dans la consommation en C. À cette fin, cette consommation a été divisée en processus à court et long terme exprimés autant en termes de taux que de quantité de C consommé ou encore, en terme de sources de carbone consommées. Le chapitre II explore les facteurs qui influencent la consommation et la production de divers composés organiques par les communautés bactériennes. Dans le but de relier cette dynamique de production-consommation à divers paramètres métaboliques autant mesurés à court qu'à long terme, nous avons mesuré l'abondance, la respiration, la production et l'efficacité de croissance de la communauté bactérienne, ainsi que la respiration et la production au niveau cellulaire c.-à-d. la respiration et la production spécifique. Le chapitre IV traite de la consommation et de l'allocation différentielle de diverses sources de C et par conséquent autant la consommation totale en C que la respiration et la production de biomasse ont été suivis. Finalement, nous avons comparé l'efficacité des sources de carbone algales et terrestres à supporter la croissance bactérienne dans ce même chapitre.

Collection des données

L'effort d'échantillonnage a été concentré au sein d'un même bassin versant comprenant divers types d'habitats aquatiques (lacs, marais, rivières). Ce bassin est situé en Estrie (45.24°N, 72.12°W) dans la région limitrophe des basses terres du Saint-Laurent et de la région montagneuse des Appalaches, et est uniformément recouvert d'une forêt naturelle mixte. Cette stratégie d'échantillonnage vise à restreindre l'influence régionale dans les caractéristiques du bassin versant et des écosystèmes aquatiques qui s'y trouvent (c.-à-d. type de forêts et de sols, roche mère, climat, hydrologie, communauté phytoplanctonique et bactérienne) et ainsi, de limiter autant les variations dans le type de matière organique terrestre exporté vers le réseau aquatique que celui produit à l'intérieur de l'environnement aquatique. L'échantillonnage des différents plans d'eau s'est limité à recueillir une quantité d'eau suffisante (200L) pour procéder aux différentes incubations métaboliques et aux analyses biologiques (concentration en chlorophylle a, abondance bactérienne) et chimiques (concentrations en nutriments et carbone organique dissous, propriétés isotopiques et optiques de la matière organique).

Un des objectifs poursuivit au chapitre I est de décrire les variations écosystémiques de divers aspects de la consommation de C et conséquemment, un ensemble de 8 lacs, 6 rivières et 4 marais ont été échantillonnés à trois reprises (juin, juillet et août) durant la saison estivale. Dans les chapitres qui explorent plus explicitement les relations entre l'origine du DOC et certains aspects ciblés du métabolisme (consommation ou excrétion de carbone, allocation à la respiration ou à la croissance ; chapitre II, III et IV), l'échantillonnage s'est limité aux lacs et quelques rivières du bassin versant à l'étude. Ce choix s'explique d'une part par le fait que ces écosystèmes constituent un gradient autant en termes de productivité que d'apport en C allochtone et d'autre part, par la présence prépondérante de lits de macrophytes en zone marécageuse. Cette présence constitue un problème non négligeable dans l'identification des sources de carbone supportant le métabolisme : la signature isotopique des macrophytes se confond avec celle du carbone terrestre rendant impossible l'estimation de la contribution relative des sources algale et terrestre au pool de DOC et au métabolisme à l'aide de modèles de mélange à un isotope et deux composantes fixes (sources). Il est à noter qu'un des affluents du lac Fraser (Figure 1.2, site 13), une petite rivière forestière longue de

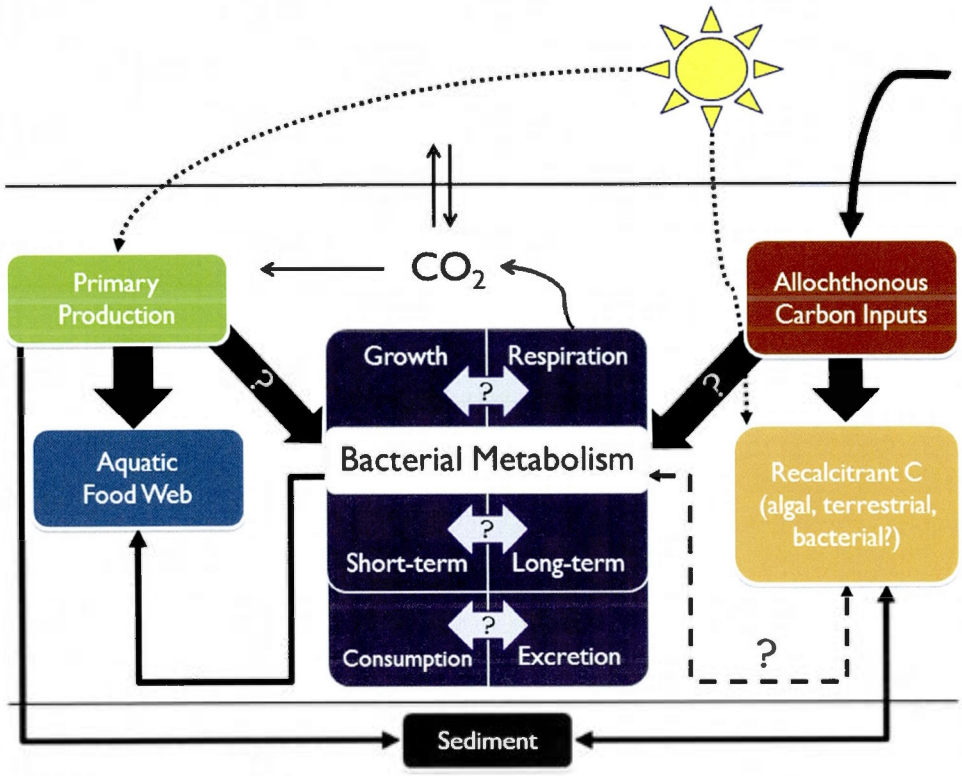

Figure 0.1 Les aspects du métabolisme bactérien considérés dans cette thèse et leurs liens potentiels avec divers pools de carbone organique dissous. Les points d'interrogation dénotent les interactions à l'étude. Schéma modifié du livre de del Giorgio et Williams (2005).

quelques kilomètres et recouverte par la végétation environnante, est considéré comme étant un système dominé par les apports allochtones et par conséquent, la signature isotopique du DOC acheminé par cette rivière a servi à valider la signature généralement attribuée au DOC terrestre de -27.0‰.

Deux types de systèmes expérimentaux ont permis de suivre l'ensemble des paramètres métaboliques à l'étude dans cette thèse. Dans un premier temps, l'eau recueillie aux différents sites échantillonnés a été filtrée sur 3 µm afin d'isoler la communauté bactérienne des autres composants planctoniques (phyto et zooplancton, protistes et autres flagellés) et d'autre part, de limiter la perte ou la lyse des cellules bactériennes résultant en une perte nette de métabolisme durant le processus de filtration (Ferguson, Buckley et Palumbo, 1984 ; Gasol et Morán, 1999 ; Goldman et Dennett, 1985). Le filtrat a ensuite été transféré dans un système à flux continu et hermétique à l'air consistant en un conteneur cubique de 4L en plastique souple placé en amont et raccordé à un erlenmeyer de 4L auquel est rattaché un port de sortie (del Giorgio, Pace et Fischer, 2006a); ce système permet la collection rapide d'échantillons sans changer les conditions ambiantes, particulièrement les concentrations en oxygène. Nous avons suivit la décroissance de la concentration en oxygène sur 48h dans ces incubations à l'aide d'un spectromètre de masse afin de dériver la consommation de C à court terme (ou respiration bactérienne, BR). Parallèlement, le changement des concentrations de DOC a été mesuré sur une période de 21 ou 28 jours par oxydation au persulfate de sodium humide avec un analyseur à carbone organique dissous, permettant d'estimer la consommation à long terme (ΔDOC) et une comparaison directe des taux de consommation à court et long terme. Aux chapitres II et IV, nous avons aussi déterminé la production bactérienne (BP) à l'aide d'une méthode basée sur l'incorporation de leucine tritiée et déterminé l'abondance des cellules (BA) par cytométrie en flux à ces différentes échelles de temps. De ces mesures ont été dérivées l'efficacité de croissance bactérienne (BGE) définie comme la fraction de carbone consommé alloué à la croissance (BP/BP+BR), la consommation bactérienne totale en carbone (BCC) calculée comme la somme de BP et BR, et la production et respiration spécifique (SP-BP et SP-BR) estimée en divisant BP ou BR par l'abondance moyenne. Finalement, des échantillons ont été récoltés durant les 28 jours d'incubation pour une série de sites choisis afin de procéder à un balayage complet du spectre de fluorescence de la matière organique dissoute et d'étudier de quelle

façon le métabolisme bactérien influence la qualité de cette matière.

Aux chapitres III et IV, un second système expérimental décrit en détail dans McCallister, Guillemette et del Giorgio (2006) a permis de recueillir le CO_2 respiratoire d'origine bactérienne aux fins d'analyse isotopique permettant la détermination des sources de C supportant la respiration et la production bactérienne. Diverses étapes ont été nécessaires pour l'obtention d'une masse de CO_2 respiratoire suffisante et exempte de contaminants avec ce système: 1) une seconde filtration sur 0.2 µm (capsule Gelman) a été menée avec l'eau échantillonnée et filtrée sur 3 µm (20L) afin de conserver uniquement le pool de DOC, 2) une acidification et un bullage intensif du filtrat avec de l'hélium ultra pur pour enlever le carbone inorganique présent, 3) une neutralisation et une oxygénation du milieu de culture et 4) une inoculation de ce milieu avec la flore initiale afin de démarrer l'incubation. Au chapitre IV, nous avons récolté le CO_2 respiratoire au bout de 5 jours d'incubation suite à une seconde acidification et un bullage à l'hélium. À la fin de cette même incubation, nous avons récolté la biomasse bactérienne pour en déterminer la signature isotopique. Au chapitre III, nous avons modifié la méthode de McCallister, Guillemette et del Giorgio (2006): avant de démarrer la récolte du CO_2 respiratoire au jour 6 d'incubation, un échantillon d'eau (1L) contenant des cellules bactériennes vivantes a été conservé. Une fois la récolte du CO_2 terminée, nous avons reneutralisé et réoxygéné le milieu de culture, et réinjecté cet inoculum pour relancer l'incubation pour une période de 7 jours. Ce scénario s'est répété une seconde fois permettant ainsi de suivre le changement dans la signature isotopique durant trois semaines et ainsi d'observer ou non l'existence d'une consommation préférentielle d'une source de carbone donnée par le compartiment bactérien.

Il est à noter que l'ensemble des incubations métaboliques a été conduit en conditions contrôlées c.-à-d. dans le noir et à 20°C, permettant d'une part d'éliminer tout ajout de matière organique par la production primaire durant l'incubation et d'autre part, de générer des mesures de métabolisme comparables au sein d'une même étude ou entre les divers chapitres de cette thèse. Cette température d'incubation se situe dans un intervalle de ± 3 degrés de celle observée dans les différents écosystèmes aquatiques échantillonnés durant la période estivale et est donc considérée comme réaliste et représentative des conditions ambiantes.

Approches utilisées

Les mesures simultanées de consommation à court et long terme ont non seulement permis d'explorer leur dynamique respective au sein et à travers les différents écosystèmes aquatiques visités au chapitre I, mais a aussi permis d'étudier en détail de quelle façon ces aspects de la consommation étaient reliés entre eux. Pour ce faire, nous avons d'abord combiné ces mesures pour recréer l'historique de la consommation bactérienne en C sur 28 jours et appliqué une version simple du modèle de dégradation de Westrich et Berner (1984) afin de dériver une constante de dégradation de premier ordre (k). Cette constante renseigne sur la forme du patron global de consommation et sur l'évolution dans le temps du taux de consommation initial (court terme): une forte valeur de k implique une forte inflexion de la courbe de consommation et par conséquent, un changement rapide d'un taux initial élevé vers un taux final beaucoup plus faible. Inversement, une faible valeur de k suggère un taux de consommation relativement constant dans le temps suggérant une composition plus homogène en termes de réactivé du pool de DOC. La dynamique de cette constante, ainsi que les facteurs qui la contrôlent sont explorés au chapitre I.

Les chapitres II, III et IV utilisent un modèle de mélange basé sur l'isotope stable du carbone (^{13}C) pour estimer la contribution relative des sources de carbone terrestre et algal au pool de DOC du système étudié, à la respiration bactérienne et à la synthèse de biomasse. Ce modèle contient deux composantes fixes ayant chacune une signature isotopique typique du carbone terrestre ou algal auxquelles est comparée la signature isotopique de l'aspect métabolique étudié. Le signal du DOC d'origine terrestre est généralement estimé à une valeur de -27.0 ‰ dans la littérature (Boschker et Middelburg, 2002) et nous avons attribué cette valeur à la composante terrestre du modèle de mélange. Tel que discuté précédemment, nous avons toutefois déterminé la signature isotopique du DOC présent dans un des affluents du lac Fraser largement influencé par les apports allochtones pour valider cette approche. La détermination de la signature isotopique algale en eau douce représente un réel défi causé principalement par le fait que le phytoplancton représente une fraction mineure de la matière particulaire présente dans l'eau, souvent dominée par les détritus allochtones, rendant difficile l'isolation des cellules algales aux fins d'analyse. Cette problématique n'est pas inhérente à cette thèse et plusieurs études ont par le passé employées différentes approches

pour estimer la signature algale (analyse lipidique, attribution d'un fractionnement algal à la signature du CO_2 ou carbone inorganique ambiant, ou encore analyse de la matière particulaire ou de culture pure d'algue) avec des résultats plus ou moins réalistes dépendant du type d'approches choisies (Marty et Planas, 2008). Dans cette thèse, nous avons utilisé une méthode employée avec succès en lacs consistant à estimer la signature algale à partir de celle du zooplancton (Karlsson, Jansson et Jonsson, 2007 ; Marty et Planas, 2008 ; McCallister et del Giorgio, 2008). Cette approche présente l'avantage d'être peu coûteuse et rapide puisqu'elle consiste simplement à recueillir un nombre limité d'organismes (~100) avant l'analyse en spectrométrie de masse. Cependant, de nouvelles études suggèrent qu'une fraction du zooplancton est supportée par le carbone terrestre (Berggren *et al.*, 2010b ; Cole *et al.*, 2011) induisant un biais potentiel dans l'estimation isotopique de la composante autochtone du modèle de mélange. Pour pallier ce problème, nous avons supposé un contenu en carbone terrestre de 16 % dans la biomasse du zooplancton, soit la teneur moyenne observée par Mohamed et Taylor (2009) dans des lacs tempérés en Ontario. Bien que l'emploi d'une telle supposition représente une limite de cette méthode, ce biais potentiel devrait se réduire à une sous ou une sur estimation systématique de la contribution absolue de C algal ou terrestre par le modèle de mélange et par conséquent, devrait avoir très peu d'effet sur les patrons entre la source de C consommé et les aspects du métabolisme étudiés décrient dans les différents chapitres. Une analyse de sensibilité supportant cette dernière affirmation a été conduite au chapitre IV.

La caractérisation du substrat soutenant le métabolisme bactérien c.-à-d. pool de DOC ne s'est pas limitée à l'approche isotopique et au modèle de mélange décrit précédemment. Aux chapitres I et II, l'emploi de techniques en fluorescence a permis non seulement de décrire la composition de la matière organique des lacs, rivières et marais échantillonnés et d'étudier l'influence de cette composition sur la consommation bactérienne en C (Chapitre I et II), mais aussi de déterminer le type de molécules organiques produit par les communautés bactériennes dans ces systèmes (Chapitre II). Nous avons utilisé l'approche de Stedmon, Markager et Bro (2003b) consistant à effectuer un balayage exhaustif du spectre de fluorescence des échantillons de DOM afin de construire une série de matrices spectrales c.-à-d. contenant l'intensité de fluorescence à chaque longueur d'onde d'excitation (240-400 nm) et d'émission (280-560 nm) considérée. Une analyse en facteur parallèle (PARAFAC ;

Stedmon et Bro 2008) a permis de révéler la présence de molécules fluorescentes ayant des propriétés chimiques distinctes et semblables aux protéines et acides aminés, et aux acides fulviques et humiques. Au chapitre II, nous avons converti la production bactérienne de FDOM en unités de carbone afin de quantifier la production de matière récalcitrante en lacs. Pour ce faire, nous avons utilisé l'approche et les relations développées par Mayer, Schick et Loder (1999) et Cumberland et Baker (2007b) entre la concentration en C et la fluorescence pour des substances étalons protéiniques et humiques, respectivement.

Dans ce même chapitre II étudiant la production et la consommation de matière organique fluorescente (FDOM), le lien entre cette production/consommation et les divers aspects du métabolisme mesurés en laboratoire (BP, BR, BA, BCC, BGE, SP-BR, SP-BP) a été exploré. Un problème inhérent à cette approche provient de l'utilisation de variables indépendantes potentiellement autocorrélées, ici les divers paramètres du métabolisme, dans un modèle multivarié pour prédire la dynamique de production ou consommation de FDOM. Pour pallier à ce problème d'autocorrélation, nous avons d'abord procédé à une analyse en composantes principales (PCA) et utilisé les composantes principales ou axes comme nouvelles variables prédictives dans une analyse de régression multiple; cette procédure statistique est dénommée régression en composantes principales (PCR) (Jolliffe, 1982).

STRUCTURE DE LA THÈSE

L'objectif principal de cette thèse est de dresser un portrait intégratif de la consommation bactérienne en carbone organique dissous, et de la réponse métabolique subséquente, en considérant les interactions potentielles entre les pools de DOC consommés, les voies métaboliques impliquées et l'environnement. Pour atteindre cet objectif, les résultats de cette thèse sont présentés dans quatre chapitres dont certains publiés ou d'autres soumis pour publication:

Chapitre I. Guillemette, F. and del Giorgio, P.A. 2011. Reconstructing the various facets of dissolved organic carbon bioavailability in freshwater ecosystems. *Limnology and Oceanography* 56: 734-748.

Ce chapitre explore la dynamique de divers aspects de la consommation bactérienne de carbone organique dissous, soit la consommation à court et long terme et la taille des pools de carbone labiles supportant le métabolisme bactérien au sein de différents écosystèmes aquatiques d'eau douce (lacs, marais, rivières). La combinaison des mesures de métabolisme à court et long terme a permis de reconstruire la courbe de consommation complète et de dériver une constante de dégradation globale afin d'explorer les liens existants entre la consommation à court et long terme. Ce chapitre aborde par ailleurs la régulation de ces divers aspects de la consommation par la qualité du DOC et par différentes variables environnementales.

Chapitre II. Guillemette, F. and del Giorgio, P.A. 2012. Simultaneous production and consumption of fluorescent dissolved organic matter by lake bacterioplankton. *Environmental Microbiology* 14: 1432-1443.

Le chapitre II explore la consommation et la production bactérienne de divers composés organiques (acides humiques, fulviques et protéines) identifiés à l'aide d'outils en fluorescence et plus particulièrement, les conditions sous lesquelles le métabolisme bactérien agit en tant que puits ou source de ces diverses composantes du pool de DOC. Dans un premier temps, les taux de productions et de consommation des divers composés identifiés sont reliés à divers aspects du métabolisme (respiration et production bactérienne, efficacité de croissance, consommation totale de C) à l'aide d'une analyse de régression en composante principale. Par la suite, le lien existant entre la production/consommation de matière organique fluorescente et l'origine de cette matière est exploré par l'utilisation de l'isotope stable du carbone.

Chapitre III. Guillemette, F., S. L. McCallister and del Giorgio, P.A. Differentiating the degradation dynamics of algal and terrestrial carbon within complex natural dissolved organic carbon in temperate lakes. En préparation pour *Journal of Geophysical Research - Biogeosciences*.

Ce chapitre explore les liens potentiells entre la consommation bactérienne à court et long terme et la source de carbone consommé, soit le carbone algal produit au sein des lacs à l'étude ou le carbone d'origine terrestre acheminé vers ces écosystèmes aquatiques. L'isotope stable du carbone est employé afin de suivre le changement dans la source de carbone supportant le métabolisme bactérien sur une période de 21 jours. Ce chapitre démontre en outre que les bactéries aquatiques utilisent certaines sources de carbone préférentiellement et que la proportion relative des sources autochtones et allochtones, ainsi que leur interaction avec les nutriments, influence les patrons de consommation à court et long terme.

Chapitre IV. Guillemette, F., S. L. McCallister and del Giorgio, P.A. The selective consumption and differential metabolic allocation of terrestrial and algal C define allochthony in lake bacterioplankton. Soumis à *Ecology Letters.*

Le chapitre IV examine l'importance relative du carbone algal et terrestre dans le soutien de la production, de la respiration et de la consommation bactérienne totale en C. Ce chapitre montre qu'une consommation et une allocation métabolique sélective s'opèrent durant l'utilisation du DOC par le compartiment bactérien des lacs tempérés de sorte qu'il est impossible de prédire la voie métabolique empruntée par chacune des sources en se basant seulement sur leur contribution au substrat initial c.-à-d. le pool de DOC. De plus, un mécanisme de facilitation métabolique par lequel le carbone d'origine terrestre est préférentiellement incorporé dans la biomasse bactérienne suite à la respiration de carbone algale est démontré. Les implications de ce mécanisme de facilitation et de la sélectivité métabolique au niveau de la biogéochimie et du support des réseaux trophiques aquatiques sont aussi abordées dans ce chapitre.

CHAPITRE I

RECONSTRUCTING THE VARIOUS FACETS OF DISSOLVED ORGANIC CARBON BIOAVAILABILITY IN FRESHWATER ECOSYSTEMS

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AUTHOR CONTRIBUTIONS:

F. Guillemette 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

Nous avons exploré de multiples aspects de la consommation bactérienne en carbone organique dissous (DOC), soit le taux de consommation à court terme (STCC; <2 jours), dérivée des mesures de respiration bactérienne, et à long terme (LTCC) mesurée sur 28 jours à l'aide de bioessais en laboratoire, dans divers types d'écosystèmes aquatiques d'eau douce du sud du Québec incluant plusieurs lacs, rivières et marais situés au sein d'un même bassin versant. De plus, nous avons combiné les mesures de consommation à court et long terme pour estimer la proportion de DOC consommé et calculé une constante de dégradation de premier ordre (*k*). En moyenne, les taux de consommation à court terme étaient plus élevés de 25% en comparaison aux taux mesurés sur 28 jours, ces deux aspects de la consommation atteignant leurs niveaux les plus et moins élevés en lacs et marais, respectivement. La consommation à court et long terme était corrélée aux concentrations de DOC à travers l'ensemble des écosystèmes étudiés; cependant en lacs, la consommation à court terme était reliée aux concentrations en chlorophylle alors que la consommation à long terme était reliée aux apports en carbone allochthone. La constante de dégradation n'a affiché aucune variation spécifique au type d'écosystème étudié, mais était cependant reliée négativement aux concentrations de chlorophylle à travers ces systèmes. Une analyse en fluorescence de la matière organique dissoute (DOM) a révélée qu'autant la quantié de carbone consomée à court et long terme que la constante de dégradation étaient reliées à différentes propriétés de la DOM. Nous concluons que la consommation à court et long terme, ainsi que la constante de dégradation représentent des aspects complémentaire de la biodisponibilité du DOC qui sont non seulement régulés de manière différente, mais remplissent des rôles distincts dans le fonctionnement des écosystèmes aquatiques.

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

1.2 ABSTRACT

We explored various aspects of freshwater dissolved organic carbon (DOC) lability by comparing short-term (<2 day) bacterial C consumption (STCC; derived from bacterial respiration measurements) with long-term (28 days) C consumption (LTCC) in DOC bioassays in lakes, rivers, and marshes located within the same complex drainage basin in southern Québec. We also combined STCC and LTCC measurements to estimate the proportion of DOC removed, and to derive a first-order decay constant (k). STCC rates were on average 25% higher than LTCC, and both parameters showed distinct patterns, reaching their lowest and highest values in lakes and marshes, respectively. STCC and LTCC were correlated to DOC concentration across these freshwater ecosystems, whereas in lakes, STCC was positively correlated to chlorophyll and LTCC to terrestrial C inputs. k showed no ecosystem-specific patterns, but was negatively correlated to chlorophyll across systems. The size of the DOC pools supporting STCC and LTCC, as well as k, were related to distinct components of the DOC pool, as revealed by a parallel factor analysis (PARAFAC) of fluorescent dissolved organic matter excitation-emission spectra. Short- and long-term lability and C consumption, and the resulting k, are shown to be complementary facets of DOC bioavailability, which may play very different roles on aquatic ecosystem functioning.

KEY WORDS: bacterioplankton, DOC consumption, fluorescence, PARAFAC, freshwater, decay constant.

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1.3 INTRODUCTION

Freshwater ecosystems, including lakes, streams, and marshes, process large amounts of organic carbon that is exported from the terrestrial environment, in addition to that generated in the aquatic system itself. Inland waters do not act as a passive pipe transporting this organic material towards the sea, but are sites of intense biogeochemical activity(Cole *et al.*, 2007). The flow of dissolved organic carbon (DOC) from the terrestrial environments to inland waters and finally to the ocean margins is influenced by processes that remove DOC, such as sedimentation or degradation of the organic matter by a combination of abiotic and biological processes (Cole *et al.*, 2007 ; Holmes *et al.*, 2008). In particular, the consumption of DOC by bacteria represents one of the major sinks of DOC in the biosphere (Cole *et al.*, 2007 ; Raymond et Bauer, 2001), and there has thus been much interest in exploring factors that govern this DOC consumption. It has now been well established that bacterial consumption of DOC is influenced by a number of environmental factors including temperature, nutrient availability, and ultraviolet (UV) radiation (Anesio *et al.*, 2005 ; Marschner et Kalbitz, 2003 ; Zweifel, Norrman et Hagstrom, 1993).

The intrinsic chemical properties of the DOC also exert a major influence on the availability of DOC to bacteria (Berggren *et al.*, 2010a ; Fellman *et al.*, 2009c ; Roehm, Giesler et Karlsson, 2009), and in this regard, it is clear that bacterial DOC consumption is intimately linked to the concept of DOC lability. DOC in natural systems is composed of a complex mixture of organic compounds of different origins (Benner, 2003), and this chemical heterogeneity results in variations in reactivity within the bulk DOC. It is common to find mention in the literature of 2 or 3 discrete pools of DOC, defined on the basis of their degree of reactivity (i.e., time frame of consumption), often referred to as 'labile', 'semi-labile', and 'recalcitrant' (Kirchman *et al.*, 1993 ; Kragh et Søndergaard, 2004 ; Middelburg *et al.*, 1993).

However, there are several problems associated with the concept of DOC lability. The term is generally used to denote the proportion of DOC removed by bacteria over a certain period of time, but it has also been used as a synonym of total amount of DOC removed (Davis et Benner, 2007 ; Lønborg et Søndergaard, 2009) and of rate of C removal (Cherrier, Bauer et Druffel, 1996 ; del Giorgio et Pace, 2008); these are far from being

synonyms and in fact refer to very different properties of the DOC. Also, while the concept of discrete pools can be useful from a modeling point of view, DOC most likely comprises a continuum of reactivity (Amon et Benner, 1996 ; Boudreau et Ruddick, 1991 ; Middelburg, 1989). In addition, there is ambiguity from an experimental point of view. The term 'lability' is operational, since the apparent labile DOC, whether defined as a proportion, absolute amount or rate, depends greatly on experimental conditions (such as temperature, nutrient limitation, nature of the inoculum), and most important, on the experimental time scales. This becomes critical for study comparisons for example, since microbial consumption of DOC, and the associated DOC lability, have been quantified using very different approaches.

In this regard, some studies have taken a whole-ecosystem mass balance approach to determine DOC losses using inflow and outflow data. Most studies, however, have used an in vitro approach where the change in DOC is followed over dark incubations (del Giorgio et Davis, 2003 ; Søndergaard, M. et Middelboe, 1995). This experimental approach has been extensively used in soil waters (Fellman *et al.*, 2009c ; Fellman *et al.*, 2008), lakes (Amon et Benner, 1996 ; Sondergaard, 1984 ; Tranvik, 1988), rivers (del Giorgio et Pace, 2008 ; Holmes *et al.*, 2008), estuaries (Hopkinson *et al.*, 1998 ; Raymond et Bauer, 2000), and oceans (Carlson et Ducklow, 1996 ; Moran et Hodson, 1994 ; Zweifel, 1999). DOC consumption has also been determined in lakes and marine systems by following changes in dissolved inorganic carbon (DIC) concentration (Langenheder, Sobek et Tranvik, 2006 ; Obernosterer et Herndl, 2000). The changes in DOC or DIC that occur in most samples are small relative to the sensitivity of current analytical techniques and, thus, DOC consumption bioassays typically last weeks to months. Partly because of these limitations, plug-flow biofilm reactors (or bioreactors) were developed in order to measure the bioavailable C fraction within a few hours instead of days (Søndergaard et Worm, 2001 ; Volk, Volk et Kaplan, 1997). Others have used more sensitive measurements of bacterial metabolism as proxies for DOC consumption, including bacterial growth (Middelboe et Lundsgaard, 2003 ; Middelboe et Søndergaard, 1993 ; Sondergaard, Hansen et Markager, 1995), production (Ågren *et al.*, 2008 ; Berggren, Laudon et Jansson, 2009 ; Bergström et Jansson, 2000), and respiration (del Giorgio et Pace, 2008 ; McCallister et del Giorgio, 2008). These metabolic measurements are also carried out at much shorter time frames, in the order of hours to days.

When long- and short-term approaches have been carried out in parallel, they have yielded very different apparent levels of DOC lability. For example, del Giorgio et Pace (2008) found that short-term (6-8 h) bacterial consumption rates in the Hudson River were one order of magnitude higher than the rates of DOC utilization in long-term (3 weeks) incubations system. This apparent discrepancy is not surprising, since the short-term metabolic measures target a highly reactive pool that turns over very rapidly, whereas the longer-term measures target a DOC pool that is decreasingly reactive (del Giorgio et Davis, 2003). In this regards, Carlson (2002) argued that the turnover of the semi-labile pool in the ocean cannot be predicted from instantaneous measurements of bacterial production (determined by ^{3}H-leucine or thymidine incorporation) as this approach most likely does not capture bacterial consumption of more recalcitrant carbon compounds. Thus, the discrepancies in apparent DOC reactivity are in fact to be expected, and we propose that DOC bioavailability and its associated patterns of consumption cannot be fully described or understood by targeting only a portion of the reactivity spectrum, or any individual aspect of DOC lability.

In this study, we have quantified both short-term bacterial respiration rates, measured as O_2 consumption over 48 hours, and long-term DOC consumption, measured as declines in DOC over several weeks along a water flow-path as it traverses different types of freshwater ecosystems (lakes, rivers, marshes) within a complex watershed in Southern Québec. We also combined these short- and long-term measurements to estimate the proportion of DOC removed, and to reconstruct and model the complete dynamics of DOC consumption over a period of 4 weeks. We assess the patterns in short-term, long-term and overall DOC consumption across these freshwater ecosystems, and explore the factors that may regulate DOC bioavailability over these different time scales, including optical properties of the organic pool using parallel factor analysis (PARAFAC) of excitation–emission fluorescence spectroscopy, and a suite of environmental variables.

1.4 METHODS

1.4.1 Conceptual approach

In this paper we use the term bioavailability to denote the potential for DOC to be consumed by aquatic bacteria. This term is synonymous to biodegradability and bioreactivity, which are often used in the literature (Marschner et Kalbitz, 2003 ; Obernosterer et Herndl, 2000 ; Wickland, Neff et Aiken, 2007). Figure 1.1 shows a conceptual diagram that integrates the different dimensions of DOC bioavailability: In this scheme we make a clear distinction between the amount and/or proportion of DOC that can be removed by bacteria, which we refer to as 'labile DOC', and the rates at which this DOC is removed, which we refer to as 'bacterial C consumption rates' (BCC). The total labile pool is the proportion of DOC removed within a given time, 28 days of incubation in the case of our experiments, and we refer to the remaining DOC as the 'residual pool'. The total labile pool can be further divided into a short-term labile (STL) pool, corresponding to the proportion of DOC removed during the initial phases of the incubation (first two days in our case), and the long-term labile (LTL) pool, which is the proportion of DOC removed over the reminder of the incubation (28 days in this study). The removal of DOC is mediated by bacterial C consumption rates, and these can be broadly divided into rates at the initial phases of the incubation, which we refer to as short-term carbon consumption (STCC), and the average rates during the remainder of the incubation, which we refer to as long-term carbon consumption (LTCC). C consumption rates typically decline over the length of the incubation, and this decline can be modeled using a first-order decay model that contains a decay constant (k), and an estimate of the size of the labile pool. The resulting k represents the shape of the overall DOC decline, and provides information on how the initial consumption rates evolve in time, and therefore, on the nature of the DOC reactivity: A high k value implies a strong inflexion in the DOC vs. time curve, suggesting the rapid exhaustion of a highly-reactive pool and a rapid transition to refractory carbon characterized by much lower consumption rates. A low k value, on the other hand, implies a much less marked inflexion, and relatively constant rates of DOC consumption with time, suggesting a more homogenous composition of the labile pool.

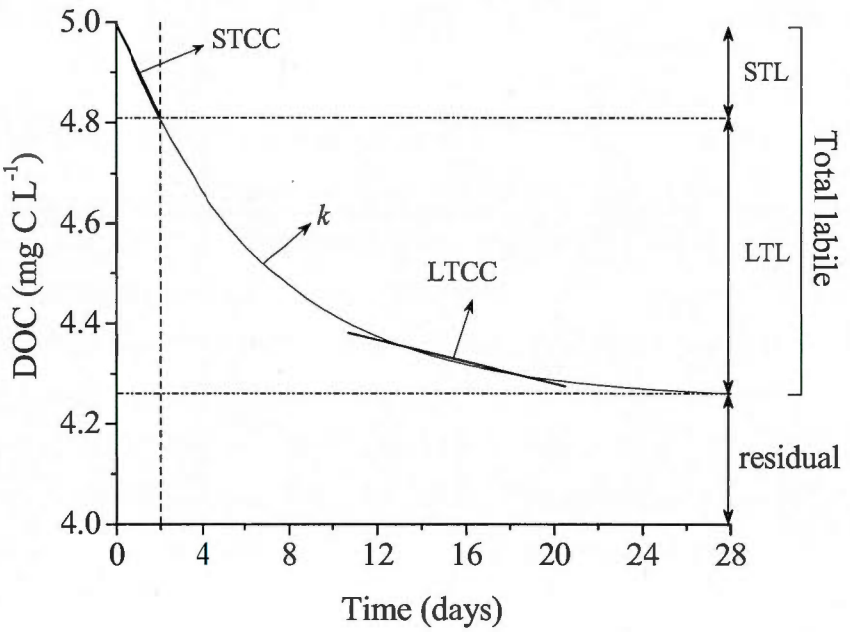

Figure 1.1 Conceptual schematic of the various facets of DOC bioavailability considered in this study. The total labile pool is the proportion of DOC removed within 28 days of incubation, and the remaining pool is considered as residual. The total labile pool can be further divided into short-term labile (STL), ~~corresponding the proportion of DOC~~ consumed during the first two days, and long-term labile (LTL) given by the proportion of DOC removed between day 2 and day 28. The short-term bacterial carbon consumption rate (STCC) is given by the initial slope of the consumption curve, and the long-term rate (LTCC) is estimated from the slope between day ~8 and day ~23. The *k* constant informs on the overall decay rate of bacterial carbon consumption.

We emphasize that the different facets of DOC bioavailability presented in Fig. 1.1 may not necessarily be derived or predicted from each other, and that consideration of only a subset of these parameters may lead to biased or incorrect conclusion regarding DOC bioavailability. For example, one could conclude that two DOC samples have the same level of bioavailability based on the fact that bacteria can extract the same amount (or proportion) of C over the incubation period (Fig. 1.1, total labile). However, this conclusion ignores potential differences in the rate of BCC sustained by these labile C pools: Most of the labile carbon could be removed within a few hours in one sample (high STCC and low LTCC), or within a few days to weeks in another (lower STCC and higher LTCC rates). Measurements of BCC on both short- and long-term are thus important not only to derive the *k* constant, but also to correctly interpret patterns in DOC bioavailability. The *k* constant is itself the direct result of the interplay between STCC and LTCC. For example, high STCC rates coupled with very low LTCC rates may yield high *k* values (i.e., early inflexion in the consumption curve), as opposed to time courses characterized by low *k*, that is, by a linear decline. However, samples that have similarly low *k* values may still have very different rates of C consumption, and thus, the bioavailability of a DOC sample should not be based solely on its *k* value. In the following sections, we present an experimental approach that accounts for the various features of DOC bioavailability identified in our scheme, and their possible ways of regulation.

1.4.2 Study site and sampling scheme

We sampled the surface waters of eight lakes, six rivers, and four marshes located within the same drainage basin and interconnected with one another along a water flow path (Fig. 1.2) in June, July, and August during summer 2005. The drainage basin is located in the Eastern Townships region of Southern Québec, Canada, about 100 km east of Montreal (45.24°N, 72.12°W). The watersheds are dominated by temperate mixed forest and low habitation density, and are underlined by the sedimentary geology of the St. Lawrence lowlands. The sampled lakes present a moderate gradient in DOC (2-7 mg C L^{-1}) and chlorophyll *a* (Chl *a*) (1-6 μg L^{-1}) concentrations, and in mean water residence time, varying from days to years (Table 1.1). The epilimnia of all these lakes are generally oversatured with carbon dioxide and hence, are net sources of C to the atmosphere (del Giorgio et Peters, 1994 ; Prairie, Bird et Cole, 2002). Marshes sampled consist of small (0.003-0.01 km^2) and shallow

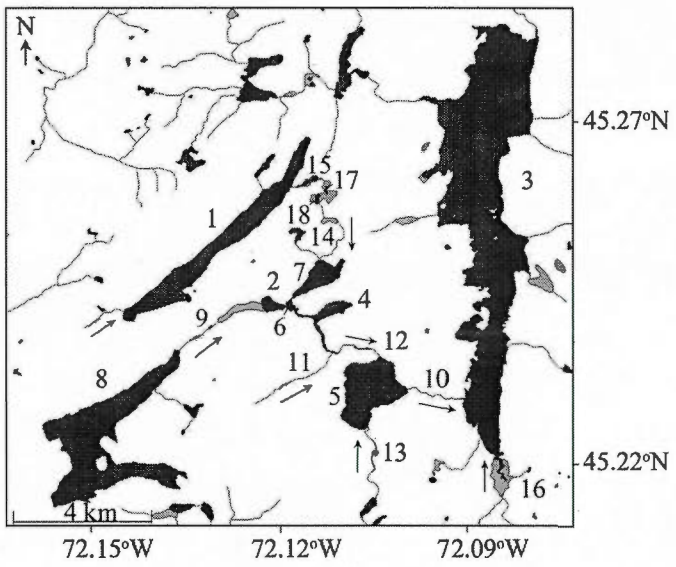

Figure 1.2 Map of the study watershed, showing the interconnected lakes (dark grey), rivers, and marshes (light grey) that were sampled. Black arrows show the direction of the water flow path, and the numbers represent the sampling sites (*see* Table 1 for site descriptions).

(mean depth of 0.5 m) impoundments, mostly resulting from the presence of beaver dams. Macrophytes, namely *Potamogetton* spp. *and Sagittarium latifolia,* cover a significant portion of these marshes, and standing dead trees are present in two of them (Fig. 1.2; No. 17-18). In a few lakes (Stukely, Bran-de-Scie, Leclerc, and Desmonts), small beds of *Myriophyllum spicatum* were also observed in addition to the two species present in marshes. The sampled lakes and marshes are connected by several rivers varying in size and water flow that were also sampled, and in addition, two headwater streams that do not link lakes or marshes were visited (Fig. 1.2; No. 11-13).

In addition to in situ temperature and oxygen profiles, 20 L samples of surface water (<2 m) were collected in acid leached (10% HCl) nanopure-rinsed polycarbonate bottles at the deepest point of each lake using a diaphragm pump connected to an acid-washed (10% HCl) plastic hose. In rivers and marshes, water samples were taken at a depth of ~15 cm under the water surface to avoid collecting sediments. All the samples were kept cool in the dark and brought back to the laboratory within two hours of collection.

1.4.3 Metabolic experiments

In order to minimize methodological disparities between STCC and LTCC measurements, we used the same incubation setups and filtration method to conduct both short-term bacterial respiration experiments and long-term DOC lability bioassays. The protocol used to determine bacterial respiration (BR) has been described in detail in a previous study (del Giorgio, Pace et Fischer, 2006b). Briefly, in the laboratory, 2 L of unfiltered water were set aside for nutrients and chlorophyll analyses as well as DOC characterization (*see* below), the remainder was filtered to isolate the bacterial community from the other planktonic components in the bulk water. This filtration process may cause significant loss of bacterial cells or may provoke cell breakage resulting in an overestimation of bacterial respiration, production, and other metabolic parameters (Ferguson, Buckley et Palumbo, 1984 ; Gasol et Morán, 1999). In order to minimize bacterial loss we used pre-combusted (500°C) borosilicate glass filters (Pall AD) with a nominal pore size of 3 μm. The large pore size allows most bacteria to go through (average of 92% ± 4), but also allows passage of some picoeukaryotes, especially flagellates. We quantified the abundance of small flagellates in unfiltered and filtered water samples by filtering 15-20 mL on black 0.2 μm Nuclepore filters, staining with 4,6-diamidino-2-phenylindole (DAPI) (5 μg mL^{-1}), and

Table 1.1

Biological and chemical characteristic of the lakes, rivers, and marshes sampled in this study. DOC is dissolved organic carbon (<0.2 μm), TP is total phosphorus, TN is total nitrogen, and A_{440} is DOC absorbance measured at 440 nm. Mean values of the three sampling dates are shown along with standard deviation in brackets. Numbers refer to sampling site positions within the studied watershed (*see* Fig. 1).

Water body	No.	DOC ($mg\ L^{-1}$)	TP ($\mu g\ L^{-1}$)	TN ($mg\ L^{-1}$)	Chl *a* ($\mu g\ L^{-1}$)	A_{440} (m^{-1})
Bowker	1*	2.46 (0.32)	1.0 (0.5)	0.12 (0.03)	1.7 (0.5)	0.38 (0.18)
Bran-de-Scie	2*	6.23 (0.11)	10.0 (2.8)	0.28 (0.05)	6.6 (3.0)	1.96 (0.40)
Brompton	3	6.54 (0.18)	6.4 (4.4)	0.27 (0.07)	5.0 (3.2)	1.73 (0.90)
Des Monts	4*	6.43 (0.77)	8.39 (4.6)	0.25 (0.06)	3.4 (1.6)	1.92 (0.11)
Fraser	5*	6.03 (0.08)	5.3 (1.7)	0.21 (0.01)	3.7 (0.7)	1.84 (0.20)
Leclerc	6*	5.18 (0.58)	6.0 (3.3)	0.20 (0.03)	—	1.67 (0.41)
Simoneau	7*	4.56 (0.15)	7.6 (3.0)	0.18 (0.02)	2.9 (0.3)	1.38 (0.50)
Stukely	8*	4.57 (0.10)	7.2 (2.7)	0.22 (0.05)	1.7 (0.8)	1.27 (0.30)
Bran-de-Scie stream	9*	5.03 (0.68)	13.1 (5.4)	0.39 (0.11)	—	1.50 (0.53)
Brompton Stream	10	5.86 (0.23)	6.3 (4.2)	0.26 (0.06)	3.2 (0.2)	2.73 (1.93)
Fraser Stream No. 1	11	4.67 (1.16)	8.7 (4.3)	0.41 (0.05)	—	1.04 (0.49)
Fraser Stream No. 2	12	6.02 (1.15)	9.0 (3.4)	0.27 (0.06)	1.1 (1.2)	1.77 (0.07)
Fraser Stream No. 3	13	10.22 (1.70)	21.6 (1.2)	0.45 (0.02)	3.9 (0.3)	3.68 (1.14)
Simoneau Stream	14*	5.30 (1.80)	8.9 (2.3)	0.25 (0.05)	3.3 (0.03)	2.42 (0.98)
Bowker Marsh	15	2.79 (0.33)	4.7 (1.9)	0.16 (0.01)	1.9 (0.6)	0.50 (0.13)
Brompton Marsh	16	11.47 (1.76)	14.6 (4.9)	0.43 (0.02)	—	4.43 (1.71)
Simoneau Marsh No. 1	17*	5.72 (1.91)	9.4 (2.5)	0.29 (0.07)	3.6 (3.9)	2.00 (0.40)
Simoneau Marsh No. 2	18*	5.13 (0.46)	7.1 (1.5)	0.26 (0.02)	—	1.84 (0.12)

* Sites selected for the spectrofluorometric characterization of the dissolved organic matter (DOM)

counting the cells by epifluorescence on an Olympus BX51 microscope (400X). Flagellate abundance in filtered samples was on average less than 10% of that in the ambient water.

Approximately 10 L of water from each site were gently pushed through pre-combusted (500°C) Pall AD (15 cm diameter) filters using a filtration tower (Millipore) coupled to a peristaltic pump by acid-washed silicone tubing. Filters were changed half-way to prevent pore clogging and further loss of bacterial biomass. The filtered water was used to fill an acid-washed 4 L Erlenmeyer flask and an acid-washed 4 L cubitainer bag. The cubitainer was placed on a stand and connected to the lower flask with acid-washed Tygon tubing so a siphon could be established by gravity. The Erlenmeyer flask was sealed with a white acid-washed silicon stopper fitted to a glass tube and connected to a Tygon tube closed with a Teflon pinch-valve that acted as a sampling port. All flow-through systems were placed in a large dark incubation chamber and kept at 20°C to standardize temperature for all metabolic experiments. The incubation temperature was within ± 3°C of the ambient temperature. In order to determine what the upper limit of metabolic activity in these samples and the potential effects of filtration, we also followed respiration in unfiltered water samples using the same flow-through systems.

We determined STCC rates as changes in oxygen concentration in the bottom flasks. Samples were taken at the time when the bottom flasks were first sealed, then at every 2 h for 6 h and finally at 24 h and 48 h (six time points total); at each time point, triplicate 7 mL glass tubes (Chemglass) were filled by opening the pinch-valve after having allowing 5 mL of water to flow in order to purge the system. All tubes were poisoned with 8 μL of saturated $HgCl_2$ solution and capped with a ground-glass stopper. All samples were kept immersed in cooler to prevent the ground-glass joint from leaking, and stored (< 1 week) at 10°C for the determination of oxygen concentration using a dual-inlet mass spectrometer. In brief, the method is based on the spectrometric determination of the ratio of argon to oxygen in the sample, after the gases in the sample have been allowed to diffuse through a permeable membrane into a high vacuum system connected to the mass spectrometer (Kana *et al.*, 1994). The oxygen concentration was estimated from this ratio using the solubility of argon corrected for salinity and temperature; the average standard error of oxygen concentration between triplicates was less than 2 $\mu g\ O_2\ L^{-1}$. The rates of oxygen consumption were derived from the slope of O_2 vs. time relationship fitted to a least-squares regression. Most of the time

courses were linear within the length of the incubations (48 h); ten time courses became clearly non-linear after time 24 h, and for these STCC was calculated over 24 h only. Oxygen consumption rates were converted to CO_2 production, in order to provide short-term estimates of organic carbon consumption, using a respiratory quotient of 1 (McCallister et del Giorgio, 2008).

LTCC was estimated by following the decline in DOC concentrations in the same samples that were used to determine short-term bacterial respiration. Sampling for DOC concentration measurements was done every two to four days for up to day 28, by collecting 40 mL of filtered water in two acid-washed and 500°C burned 40 mL vials (replicates) to which 40 μL of 5 mol L^{-1} sulphuric acid had been added, to attain a final pH of ~2. To minimize gas exchange between the cubitainer and the flask, duplicate samples of DOC were taken before the respiration setup was sealed (time 0) and after the last sample for oxygen determination 48 h so the overall water volume taken to determine bacterial respiration was always less than 1% of volume contained into the 4 L flask. DOC samples were kept refrigerated (4°C) to a maximum of 2 months after having been capped with Teflon lined septa cap (VWR). At day 2, respiration flow-through systems were dismantled and the filtered water from the respiration incubations was transferred into two acid-washed and burned (500°C) 500 mL culture glass bottles (unscrewed caps to prevent oxygen exhaustion), and samples were taken every 2-4 days to determine DOC concentration. LTCC was calculated from the slope of the DOC vs. time relationship fitted to a least-squares regression.

1.4.4 Modeling DOC consumption

In order to reconstruct the complete dynamics of DOC consumption we combined STCC and LTCC consumption rates to generate a single decay curve. A first-order decay model, based on the multi-G model (Westrich et Berner, 1984) with only one reactive member, was applied to this single decay curve. In all DOC consumption experiments there was a relatively large residual portion of the total DOC that was present at the end of the 28 d incubation. The equation for the first-order decay model that accounts for this residual pool is the following:

$$G_T(t) = G_{Lab}[\exp(-kt)] + G_{Res} \quad (1)$$

where G_T is the total DOC concentration at the beginning of the experiment; G_{Lab} and G_{Res} are the labile and the residual pools estimated by the model, respectively, k, the first-order decay constant, and t, the time of decomposition. Since the model did not provide realistic estimates of the labile and residual pool sizes in some cases (low k values; n=15), and that it could not account for the small labile pool used within two days, the size of the short- and long-term labile DOC pools was calculated as the difference between the initial DOC concentration and the concentration after day 2 for STL, and between day 2 and 28 for LTL.

1.4.5 Chemical analyses

Samples for total nutrients (phosphorus and nitrogen) were kept at 4°C in the dark prior to analyses. Phosphorus concentration in unfiltered samples was determined using the molybdenum-blue method after persulfate digestion. Nitrogen concentration was measured as nitrates after digestion with alkaline persulfate (Cattaneo et Prairie, 1995). Colorimetric analyses were carried on a Flow solution IV autoanalyzer (nitrogen) or on a UV-Visible Ultrospec 2100 spectrometer (Biochrom) (phosphorus). DIC and DOC were measured in filtered samples on an OI 1010 total inorganic and organic carbon analyzer that uses a wet persulfate oxidation method, and five point calibration curve using potassium hydrogen phthalate as standard. The analytical precision of the analyzer, based on 3 injections per sample, was ±0.003 μg C L^{-1} to 0.08 μg C L^{-1} for the low and high range of concentrations, respectively, and the detection limit lies at ~0.020 μg C L^{-1}. Finally, chlorophyll concentration was determined from ethanol extracts using the same spectrophotometer as for the phosphorus measurements.

1.4.6 DOC characterization

Absorption spectra were measured from 190 to 900 nm with a UV-Visible Ultrospec 2100 spectrometer (Biochrom) using a 2 cm quartz cuvette. The absorption coefficient (A_{440}) was calculated by dividing the optical absorbance at 440 nm by the path length in meters and multiplying by 2.303 (Cuthbert et del Giorgio, 1992). The spectrofluorometric characterization of the dissolved organic matter (DOM) pool was performed for 11 selected sites (*see* Table 1) in June and July following the protocol provided in Stedmon, Markager et Bro (2003a). Briefly, fluorescence excitation-emission matrices (EEM) were determined on a RF-5301PC spectrofluorometer (Shimadzu) with a 150-W xenon lamp at 2-nm excitation

wavelength intervals between 240 and 400 nm, and at emissions ranging between 280 and 560 nm with 5-nm increments. Samples were analyzed at 20°C ± 1 in a temperature-controlled cuvette chamber. Fluorescence spectra were corrected for the inner-filter effect, accounting for the absorption of both emission and excitation light by the DOM sample (McKnight *et al.*, 2001) and calibrated by normalizing to the area under the water Raman peak at excitation wavelength 350 nm of a NanoPure water sample run the same day. Finally, a Raman normalised NanoPure water EEM was removed from each spectrum in order to remove the Raman signal. This correction and normalization routine yield EEMs that are expressed as Raman units (R.U.; nm^{-1}).

EEMs were analyzed using the PARAFAC multivariate modeling technique, a trilinear decomposition method analogous to principal component analysis (Stedmon et al. 2003). PARAFAC decomposes the fluorescence spectra of DOM into independent fluorescence groups whose abundance can be related to differences in the composition and source material of the DOM matrix. The modeling of the fluorescence spectra was conducted on a dataset of 211 samples, including several samples from other lakes and previous degradation experiments not presented in this study, using the DOMFluo toolbox 1.7 for MATLAB as described in Stedmon et Bro (2008). However, we removed 4 EEMs from the dataset that were collected and analysed in June (No. 1, 5, 8, 17; Table 1) since the EEMs contained measurement errors. Consequently, the EEMs modeling was conducted on a dataset of 207 samples. The model was validated by split-half analysis and by further examining residuals to ensure no systematic signal was present. All parameters derived from the model were used to estimate the magnitude of the different fluorescence components present in our samples, expressed hereafter as the maximum fluorescence intensity in raman units (Fmax; R.U.).

1.4.7 Statistical analyses

Differences in terms of BCC and DOC lability between ecosystems were assessed using analysis of variance (ANOVA) in conjunction with a Tukey's pairwise differences test. A mixed stepwise routine (probability to enter or to leave the model was set to 0.05) prior to multiple-regression analysis was used to find the best predictive environmental variable(s) describing the variability of STCC, LTCC, and the k constant. While we included all variables presented in Table 1.1 for the cross-system analysis, we also considered lake size

and average water retention time in the stepwise routine for the lakes only analysis. Finally, linear regression models were used to evaluate the possible relationships between BCC or DOC lability and the fluorescence components. All statistical analyses were conducted with the JMP statistical software version 7.0 (SAS Institute).

1.5 RESULTS

1.5.1 Biochemical characterization of the watershed system sampled

There were large variations in DOC and other chemical variables along the water flow path and across the different systems sampled within this watershed. DOC and A_{440} were on average higher in rivers and marshes than in lakes, which may indicate a higher contribution of terrestrially-derived DOC to the total DOC pool in these systems (Table 1.1). Lakes showed the widest range of Chl *a* concentrations (mean values of 1.7-6.6 $\mu g\ L^{-1}$) with the highest values in meso-eutrophic lake Bran-de-Scie (6.6 $\mu g\ L^{-1}$; Table 1.1), where the contribution of autochthonous processes to the total DOC pool should be highest.

1.5.2 Patterns in short- and long-term DOC consumption

The metabolic experiments resulted in a dataset of 54 individual observations for the short-term aspects of DOC bioavailability, but an instrumental failure resulted in the loss of DOC samples needed for the estimation of the long-term DOC consumption from Simoneau marsh No. 2 in July (site No. 18, Table 1.1). Accordingly, the final dataset for LTCC, LTL, and *k* comprises 53 individual observations instead of 54.

STCC rates, as estimated in bacterial respiration experiments (filtered water), were significantly lower than total planktonic respiration (TR; unfiltered incubations) in lakes (42%; *t*- test, t=-3.89, df=46, p<0.001) whereas in rivers and marshes the difference between the unfiltered and filtered incubations was not significant (Fig. 1.3a). Rates of LTCC were on average 27% lower (*t*-test, t=-2.37, df=105, p<0.05) than STCC rates indicating that the more labile DOC pool was removed in the first few hours of incubation.

There were distinct between-ecosystem patterns in both STCC and LTCC (Fig. 1.3a). STCC rates were significantly higher in marshes than in lakes (ANOVA, F_{53}=5.20, p<0.01), rates of STCC in rivers being intermediate between lakes and marshes, whereas LTCC rates were higher in marshes than in both lakes and rivers (ANOVA, F_{52}=6.87, p<0.01). The proportion of DOC consumed during the short-term incubations was relatively constant between ecosystems (overall average 1.7% ±1%; Fig. 1.3b). However, there was a significant ecosystem differences in LTL, with marshes showing a larger proportion of DOC consumed over the month of incubation (ANOVA, F_{52}=13.4, p<0.0001; Fig. 1.3b).

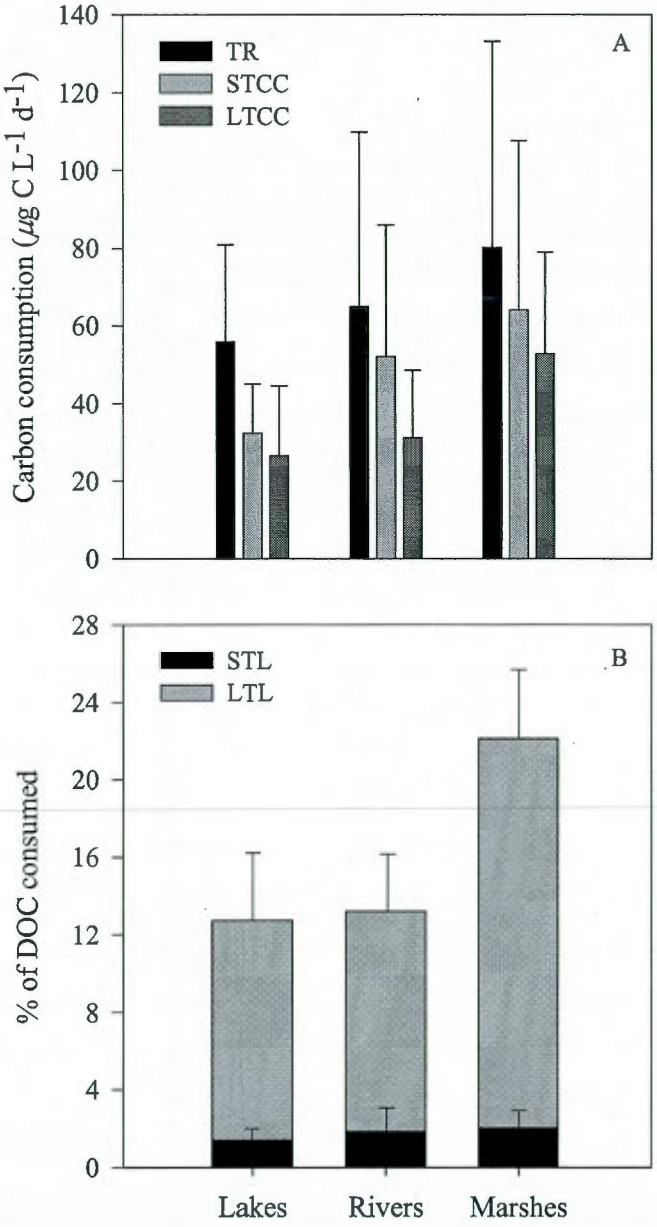

Figure 1.3 Average and range, by ecosystem type, in (A) total planktonic respiration (TR), short-term bacterial carbon consumption (STCC) (0-2 days), and long-term bacterial carbon consumption (LTCC) (2-28 days), and (B) the proportion of DOC consumed during both short- and long-term incubations. Each bar represents the average of the June, July, and August carbon consumption experiments, and the error bars correspond to standard deviation.

1.5.3 Linking short- to long-term DOC consumption

We first explored the relationship between STCC and LTCC over the entire dataset and found that the STCC was a poor predictor of the long-term carbon consumption (r^2=0.27, n=53, p<0.0001; Fig. 1.4a): there was roughly an order of magnitude of variation in LTCC for any given STCC estimate. Further, there was no significant relationship between STL and LTL (Fig. 1.4b). We integrated STCC and LTCC measurements within the same DOC time course (Fig. 1.5a), and fitted a one-reactant multi-G model as described earlier (*see* Methods). The model performed well over the different time courses, with quotients of determination ranging from 0.90 to 0.99 (mean of 0.98). The k constant varied by over 5 orders of magnitude between samples, ranging from 5.7 x 10^{-3} to 0.17 d^{-1} in lakes, from 2.3 x 10^{-5} to 0.09 d^{-1} in rivers, and from 3.8 10^{-3} to 0.12 d^{-1} in marshes. There was overlap in the k values between the different ecosystem categories, and although k was lower in marshes, the difference was not statistically significant (Fig. 1.5b).

1.5.4 Environmental regulation of carbon consumption

The mixed stepwise routine showed that STCC and LTCC were both positively correlated to DOC (r^2=0.62, n=18, p<0.0001, and r^2=0.58, n=18, p<0.001, respectively; Fig. 1.6a) across ecosystems. A more detailed analysis of factors influencing STCC and LTCC in lakes showed that the time-scale at which organic carbon is consumed may be regulated by different environmental factors: STCC was closely related to Chl a (r^2=0.72, n=7, p<0.05; Fig. 1.6b) while water residence time explained a significant portion of the variation in lake LTCC (r^2=0.69, n=8, p<0.05; Fig. 1.6c). The stepwise procedure further showed that Chl a was the only significant variable explaining variation in the k constant (r^2=0.73, n=13, p<0.0001; Fig. 1.7a).

1.5.5 Fluorescence characterization and links with carbon consumption

A total of five components could be validated following the PARAFAC analysis of the entire dataset of fluorescence spectra (n=207), and the model explained 98.7% of the variation. A visual inspection of the residual fluorescence spectra did not reveal systematic patterns in the fluorescence signal. All the components identified by the model have been previously described for aquatic systems (Table 1.2). Components 2 and 5 were identified as protein-like components since their fluorescence emission resembles that of free tyrosine and

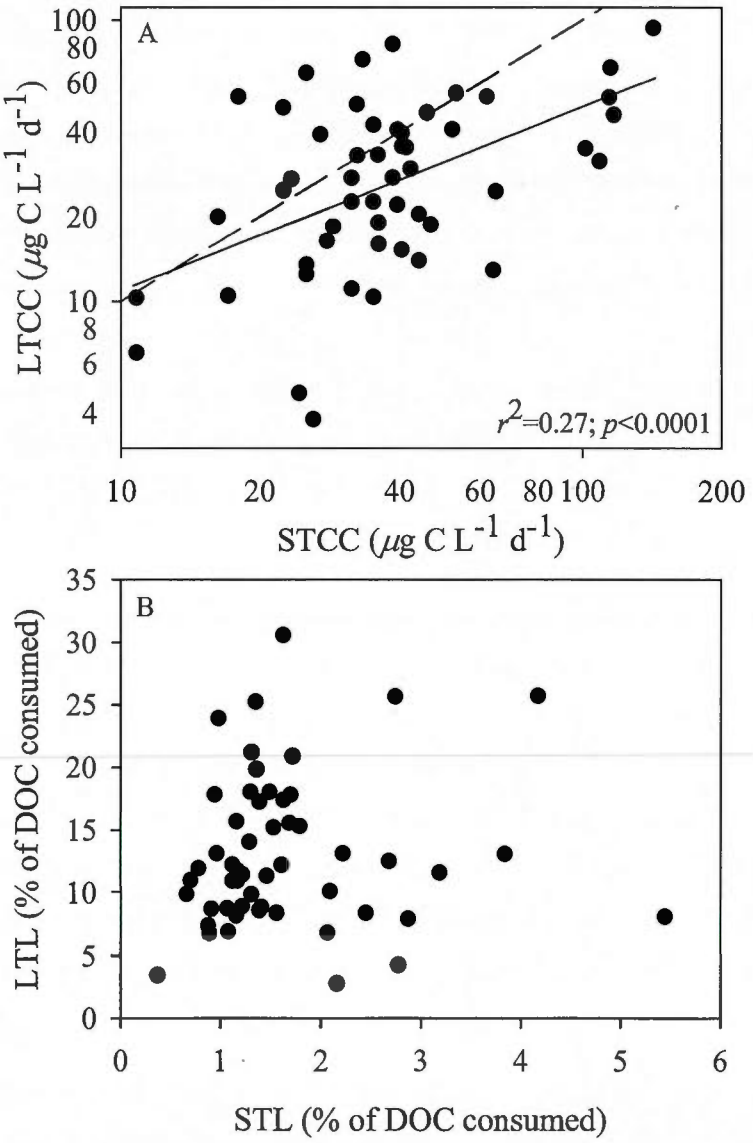

Figure 1.4 The relationship between (A) short-term bacterial carbon consumption rates (STCC) (0-2 days) and long-term bacterial carbon consumption rates (LTCC) (2-28 days), and (B) short- and long-term DOC lability (STL and LTL, respectively) determined for the same samples. The metabolic rates are log-transformed to attain normality and homoscedasticity. Data from the three sampling campaigns are shown.

tryptophan, respectively. We also identified fulvic-like and humic-like peaks (components 1, 3, 4), which are common feature in most freshwater environments (Stedmon et Markager, 2005a).

The different fluorescence components did not follow the same distribution among ecosystems: The fluorescence of component 1 was higher in marshes and rivers than in lakes, and followed the same distribution as the DOC concentration (Fig. 1.8). The component 2 was lowest in lakes and highest in rivers. The fluorescence of components 3 and 4 showed no pattern between ecosystems, whereas the fluorescence of component 5 was highest in lakes compared to rivers and marshes.

We explored the links between the components of DOC bioavailability and the fluorescence characteristics of the DOM. For this we used the mean values for June and July for the 7 sites where data were available for the two months, and 4 individual measurements for July for sites # 1, 5, 8, 17. We found that only three facets (STL, LTL, and k) showed significant relationships, albeit with different peaks of fluorescence. The strongest relationships were found by expressing the fluorescence of a given component relatively to the ambient DOC concentration. STL was best predicted by the sum of protein-like components 2 and 5 (r^2=0.69, n=11, p<0.01; Fig. 1.9a), whereas LTL was best explained by the relative fluorescence of the protein-like component 2 (r^2=0.72, n=11, p<0.01; Fig. 1.9b). The k constant was only correlated (negatively) to the protein-like component 5 (r^2=0.76, n=11, p<0.01; Fig. 1.9c).

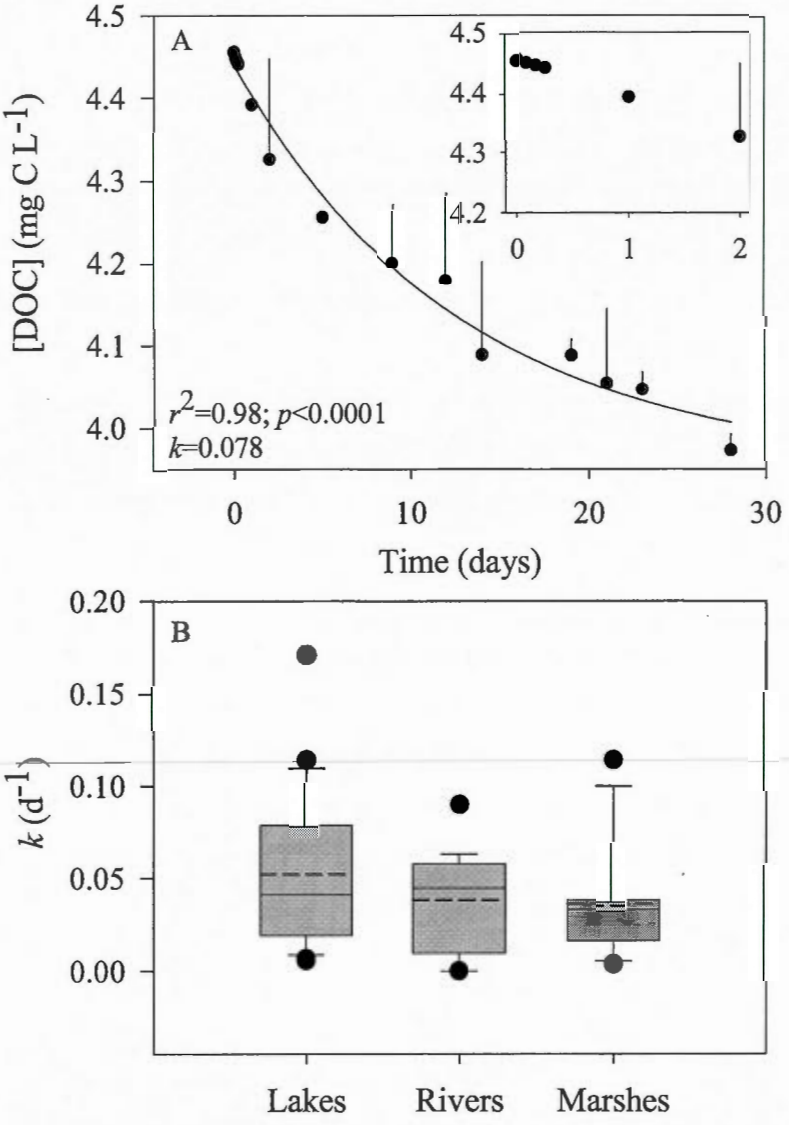

Figure 1.5 (A) Example of an integrated carbon consumption time course. The first portion (0-2 days) was derived from a short-term bacterial respiration experiment (inset), the second portion corresponds to DOC consumption in the long-term incubations of the same samples. A first-order decay model was then fitted to the data (black line) to derive a global first-order decay constant, *k*. The error bars correspond to standard deviation of individual measurements. (B) Box-and-whisker plots of the *k* constant by ecosystem type. The full and dashed lines show the median and the mean, respectively.

1.6 DISCUSSION

1.6.1 Methodological and conceptual considerations

In this study we used a dual approach to assess DOC bioavailability across ecosystems: Short-term BR experiments and long-term DOC consumption bioassays. While these two methods address the same general process, they clearly diverge in their estimates of carbon consumption, with rates in the short-term being on average 25% higher than in the long-term experiments. We argue that the difference between the two estimates of carbon consumption is related to the fact that these two methods do not target the same region of the DOC reactivity spectrum rather than methodological biases: Short-term BR targets a smaller, fast-cycling highly-labile carbon pool whereas long-term DOC bioassays assess loss rates of more recalcitrant carbon compounds. The short-term pool is difficult to detect based on DOC measurements: In six of our bioassays we followed the decrease in the DOC concentration over the initial 24 hours (every 2 h for 6 h), but could not detect any significant changes in DOC concentration in this timescale (data not shown). This observation is probably explained by the fact that a very small proportion of the bulk DOC pool is degraded within this short timescale (<2%, Fig. 1.3b), and that the total amount of DOC being removed lies within the detection limit of the current techniques (~20 μg C L^{-1} using wet persulfate oxidation in our laboratory).

We did however observe correspondence between the two methods at intermediate time-scales in a sample from Lake Fraser where we continued the BR measurements over 7 days. The BR rates and DOC consumption rates between days 2 and 7 were in good agreement (site No. 5, Table 1.1; 27.0 μg C L^{-1} d^{-1} and 25.2 μg C L^{-1} d^{-1} for BR and DOC lability experiments, respectively). In addition, there were a number of time courses where the short- and long-term rates of C consumption were similar (points close to the 1:1 line; Fig. 1.4a), thus generating quasi-linear patterns of consumption, and suggesting that the differences observed in most other samples are not intrinsic to the approaches.

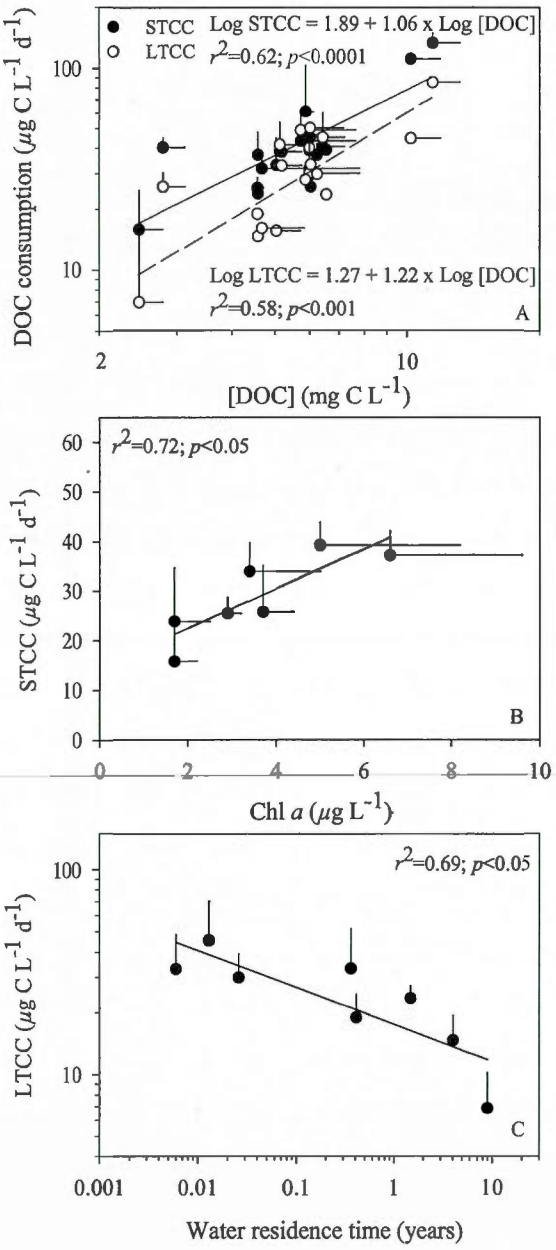

Figure 1.6 (A) The relationships between short-term carbon consumption rates (STCC) (0-2 days), long-term bacterial carbon consumption rates (LTCC) (2-28 days), and DOC concentration. DOC was the only selected variable after a mixed stepwise routine of variable selection (*see* text for details). Also shown are the best predictive relationships found for (B) STCC and (C) LTCC for lakes only upon the same routine. Each data point represents the mean of the June, July, and August carbon consumption experiments of a single sampling site, and the error bars denote standard deviation.

1.6.2 Relationships between the short- and the long-term components of bioavailability

One of the fundamental questions addressed in this study is whether short- and long-term lability and BCC can be predicted from one another, and if they are regulated in a similar manner by environmental factors. We found that while both STCC and LTCC were positively correlated to bulk DOC across these freshwater systems, only a small portion of the variation in LTCC could be explained by STCC. Moreover, there was no relationship between the proportions of C consumed over short- and long-terms. A similar observation was made in the Hudson River, where long-term consumption of the bulk DOC loaded upstream was clearly uncoupled from the short-term bacterial carbon processing along the river (del Giorgio et Pace, 2008). These authors further reported that short-term bacterial respiration was very sensitive to local features such as phytoplankton development and particle dynamics, whereas the long-term DOC consumption was more closely linked to external (i.e., terrestrial) inputs of organic carbon. We observed similar differences in the regulation of STCC and LTCC by environmental features in lakes: STCC was positively related to phytoplankton biomass, whereas LTCC was negatively related to the mean water residence time. This last variable has been shown to be negatively related to terrestrially-derived humic matter inputs to lakes (Hessen *et al.*, 1997 ; Rasmussen, Godbout et Schallenberg, 1989) suggesting that LTCC is likely positively regulated by terrestrial organic C inputs.

We found further evidence of the differential regulation of these short- and long-term facets of DOC bioavailability in the composition of the organic pool across all systems. The two protein-like components (C2, C5) identified by the PARAFAC analysis were positively related to the overall DOC lability, a relationship also observed by Fellman *et al.* (2008) ; Fellman *et al.* (2009a) in soil water and streams. Likewise, the tyrosine-like component 2 appeared to be positively related to both short- and long-term lability, whereas tryptophan-like component 5 appeared to enhance STL but not LTL. Stedmon et Markager (2005b) noted that the tyrosine-like component 2 remained unaltered upon microbial degradation within 7 days of incubation, and only started to decrease after 9 days of incubation, suggesting that this component may be consumed over longer time scales. Mayer et al. (1999) showed that tyrosine fluorescence reaches its highest values when in its monomer form or at low tryptophan concentration, indicating the presence of more degraded peptide material.

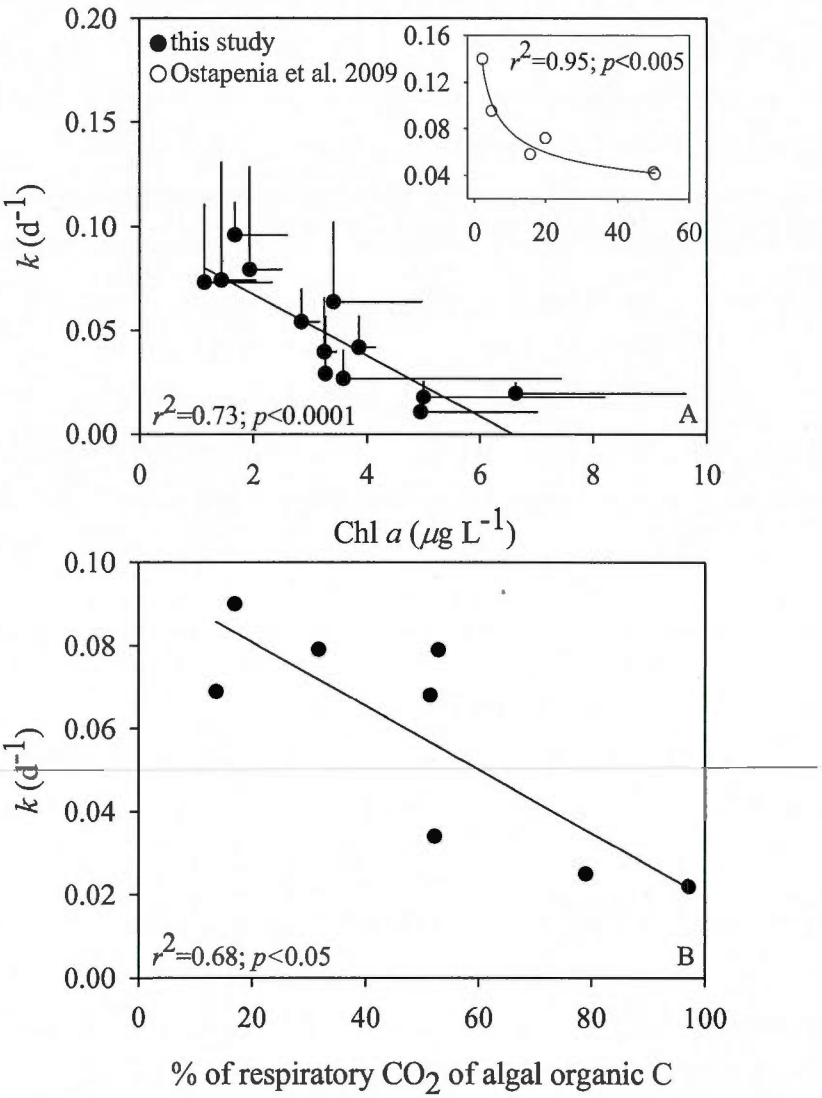

Figure 1.7 The first-order decay constant, k, as a function of Chl *a*. Also shown is the relationship found between the k constant and Chl *a* by Ostapenia, Parparov et Berman (2009) (inset). Each data point represents the mean of the June, July, and August carbon consumption experiments of a single sampling site, and the error bars denote standard deviation. (B) The first-order decay constant, k, as a function of the % of algal organic carbon (OC) supporting bacterial respiration. Estimations of the % algal OC respired were obtained from the study of McCallister et del Giorgio (2008). The k constant values were derived from carbon consumption experiments ran in parallel to the experiments conducted with the respiratory carbon recovery system (ReCReS) for the determination of the % of algal C supporting bacterial respiration.

Consequently, they hypothesized that high tryptophan fluorescence may indicate the dominance of unaltered proteins, probably of recent origin. In addition to our own results, the above evidences suggest that the tryptophan-like component 5 is preferentially consumed by bacteria over the tyrosine-like component 2, but that both proteinaceous components play a central role in determining the overall DOC lability.

In this regard, none of the fulvic-like or humic-like components appeared to play a role in shaping the overall DOC bioavailability, despite the fact that these fractions overwhelmingly dominated the DOM pool in all of these systems. Our results suggest that a small proteinaceous fraction play a major role in determining the overall DOC bioavailability and thus bacterial C metabolism. A similar observation was made by Berggren *et al.* (2010a), who reported that a small fraction of the terrestrial DOC pool, composed of simple compounds like amino and organic acids, supported much of the bacterial metabolism in boreal streams. This does not mean, however, that the humic and fulvic fractions are not consumed. Rather, we suggest that these fractions may fuel a low, but rather continuous level of bacterial activity, which becomes increasingly important as the other pools are exhausted, and which may have been under-represented in the time frame of our experimental incubations.

1.6.3 Ecosystemic patterns of carbon consumption

Previous cross-system comparisons have reached contrasting conclusions on how DOC bioavailability varies across landscapes. For example, Søndergaard et Middelboe (1995) synthesized published measurements of in vitro bioassays conducted in different aquatic ecosystems (lakes, rivers, and oceans), and concludd that carbon lability tends to be relatively invariant across natural aquatic systems (~14-19% of the total DOC pool; average of 15%). They noted that the percentage of labile DOC increases with DOC concentration across systems, and that for example, some high-DOC rivers could contain twice the amount in labile DOC than lakes. In contrast, del Giorgio et Davis (2003) did not find a relationship between DOC concentration and % of DOC removed, and further reported that the lability of river DOC was on average lower than that of lakes. These contrasting results are likely linked to the fact that these meta-analyses of published DOC consumption data combine very

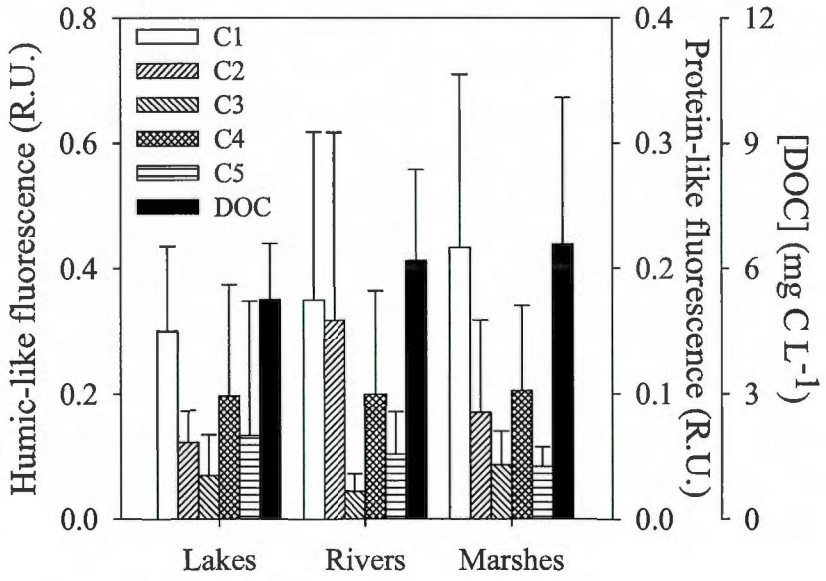

Figure 1.8 Ecosystem distribution of the fluorescence components identified by the parallel factor analysis (PARAFAC). Each bar corresponds to the mean fluorescence of a given component for June and July, and the error bar, to standard deviation. Also shown is the distribution of the concentration in DOC.

Table 1.2

The peak position of the five fluorescence components identified by the PARAFAC analysis and their correspondence with previously identified components.

Component	Excitation maxima (nm)	Emission maxima (nm)	Components identified from previous studies	Description
1	350	450	Stedmon et Markager (2005a) Component 4	Fulvic-like fluorophore.
2	270	295	Stedmon et Markager (2005a) Component 8	Tyrosine-like fluorescence.
3	260	445	Coble (1996) Component A	Humic-like fluorophore.
4	305	400	Stedmon et Markager (2005b) Component 3	Humic-like fluorophore.
5	285	335	Stedmon et Markager (2005a) Component 7	Tryptophan-like fluorescence.

different approaches and time-scales, such that the resulting patterns may contain strong experimental biases.

Several studies have suggested that bacteria do consume terrestrially-derived DOC in lakes (Kritzberg *et al.*, 2004 ; McCallister et del Giorgio, 2008 ; Tranvik, 1988), and that terrestrial organic carbon significantly subsidizes bacterial metabolism in recipient systems (Berggren *et al.*, 2010b ; Cole *et al.*, 2007 ; Jansson *et al.*, 2007). Nevertheless it is often assumed that terrestrial carbon transported from the watershed to lakes via runoff and river inputs is recalcitrant in nature, due to features such as high humic and lignin content, or a greater oxidization state as the result of extensive degradation and aging during transport (Berggren, Laudon et Jansson, 2009 ; Raymond et Bauer, 2001 ; Sun *et al.*, 1997). Our observation of a relatively high bioavailability of river DOC contrasts markedly with this common assumption, and is not unique to this study: Holmes *et al.* (2008) showed that DOC in Artic rivers may be highly labile (from 2% up to 30%) as determined by long-term DOC degradation experiments on time scales comparable to ours (30 days). In agreement to Fellman *et al.* (2009c) ; Fellman *et al.* (2008), we found that rivers and streams could be a significant source of proteinaceous material, especially the tyrosine-like component 2 in our case, and that this protein-like DOM pool positively regulates DOC lability. This supports the notion that a significant fraction of allochthonous DOC may be bioavailable over ecologically-relevant time scales and, thus, greatly enhance DOC bioavailability in these terrestrially-influenced ecosystems.

Macrophytes have also been shown to contribute substantially to the DOC pool of recipient ecosystems through exudation of DOC (Bertilsson et Jones, 2003 ; Demarty et Prairie, 2009) or leaching of plant material (Lapierre et Frenette, 2009 ; Mann et Wetzel, 1996), and that this macrophyte-generated DOC may greatly influence water column metabolism (Rooney et Kalff, 2003 ; Søndergaard, 1983 ; Stets et Cotner, 2008). For example, Mann et Wetzel (1996) found that between 22% to 69% of the DOC produced by growing and senescent macrophytes could be used over 24 h, and Søndergaard (1983) showed that up to 30% of the DOC released by the angiosperm *Littorella uniflora* was consumed over longer time scales (10 days). Since macrophytes cover significant portions of the studied marshes, inputs of macrophyte-produced DOC may in part explain the relatively high DOC bioavailability found in these systems. It is also plausible that a significant amount

of this bioavailable DOC may have been directly exported to some of the rivers or lakes located downstream, thus contributing to the high level of BCC that we observed in these recipient systems.

1.6.4 Patterns in overall DOC consumption

The approach used in this study to assess the relationship between short- and long-term DOC consumption dynamics involved integrating both measurements into a single time course, and fitting a two-pool (labile and refractory) model to this time course of DOC consumption. The *k* degradation constants estimated by this model are well within the range of what others have found in aquatic ecosystems (Lønborg *et al.*, 2009 ; Ostapenia, Parparov et Berman, 2009 ; Stets et Cotner, 2008), and even in soil pore waters (Wickland, Neff et Aiken, 2007). While we found an ecosystem-specific pattern for both STCC and LTCC, no systematic pattern in the *k* constant was observed across the different types of ecosystem. This is interesting since both STCC and LTCC were involved in deriving the overall decay curve and estimating the *k* constant, and further suggests that even if STCC and LTCC vary systematically across ecosystems, they may not vary in a similar manner relative to one another within any system. The resulting overall pattern of consumption, reflected in the *k* constant, thus represents an emergent property of DOC that cannot be predicted on the basis of either of its components.

The factor that individually explained more of the observed variability in *k* across ecosystems was Chl *a*. Ostapenia, Parparov et Berman (2009) also found a negative relationship between the DOC *k* decay constant and Chl *a* in 6 lakes that span a wide range of trophic status (Chl *a* concentrations up to 50 $\mu g\ L^{-1}$), but their relation was non-linear with an asymptote approaching a *k* value of 0.04 d^{-1} (inset Fig. 1.7a). We did not find a similar pattern in our dataset probably because our Chl *a* concentrations were all below 8 $\mu g\ L^{-1}$. This result suggests a possible link between the shape of the consumption curve and the origin of the DOC being degraded. To test this idea, we revisited published measurements of the source of OC supporting bacterial respiration by McCallister et del Giorgio (2008) that were made for the same lakes and two of the streams sampled in this study, and found a negative relationship (r^2=0.68, n=8, p<0.05; Fig. 1.7b) between the *k* constant and the % algal OC supporting bacterial respiration (Fig. 1.7b). We also found that the *k* constant was most strongly related to tryptophan-like carbon compounds, and since the *k* constant was

negatively related to both Chl *a* and the % of algal DOC used for respiration by the bacterial community, we propose that this tryptophan-like material originates mainly from autochthonous aquatic production. This idea is supported by the fact that tryptophan-like material was present in higher concentration in lakes, which also contained higher Chl *a* concentrations, than in the other systems. This would thus suggest that a small, but very reactive fraction of the DOC pool, which appears to be of algal origin, may control in part the overall dynamics of C consumption as reflected in the *k* constant, and in turn, the degree of coupling between STCC and LTCC.

Ostapenia, Parparov et Berman (2009) hypothesized that the low *k* values observed at high Chl *a* concentration could be the result of dominance by cyanobacteria in more eutrophic systems, and further suggested that this may lead to relatively lower rates of decomposition and overall DOC lability. Our observations do not support this hypothesis, since Chl *a* concentrations in our study were relatively low, and there was no evidence for cyanobacterial blooms. Instead, we propose that as the concentration of highly-labile algal-derived DOC increases in the water column, not only STCC increases as a function of Chl *a*, but this rate remains more or less the same over longer time scales leading to lower *k* values (linear time courses). This idea is partly supported by the fact that we often observed low *k* values associated with high STCC and LTCC rates in lakes, such that the assumption that low *k* values imply low DOC bioavailability is not supported. In other words, a linear time course with an associated low *k* may still present a steep slope, and hence a high rate of C consumption. It should also be emphasized that it is not possible to predict the size of the labile pool from the *k* value over the different time courses, because similar *k* values could be associated with very different levels of DOC lability. Therefore, any conclusions concerning DOC bioavailability in natural systems should not be solely based on an estimate of the *k* value, but should also consider both the actual rates of BCC and the amount of DOC removed. In this respect, had they measured BCC rates, Ostapenia, Parparov et Berman (2009) might have also found that their low *k* values in more productive systems still corresponded to high rates of BCC or high overall bioavailability.

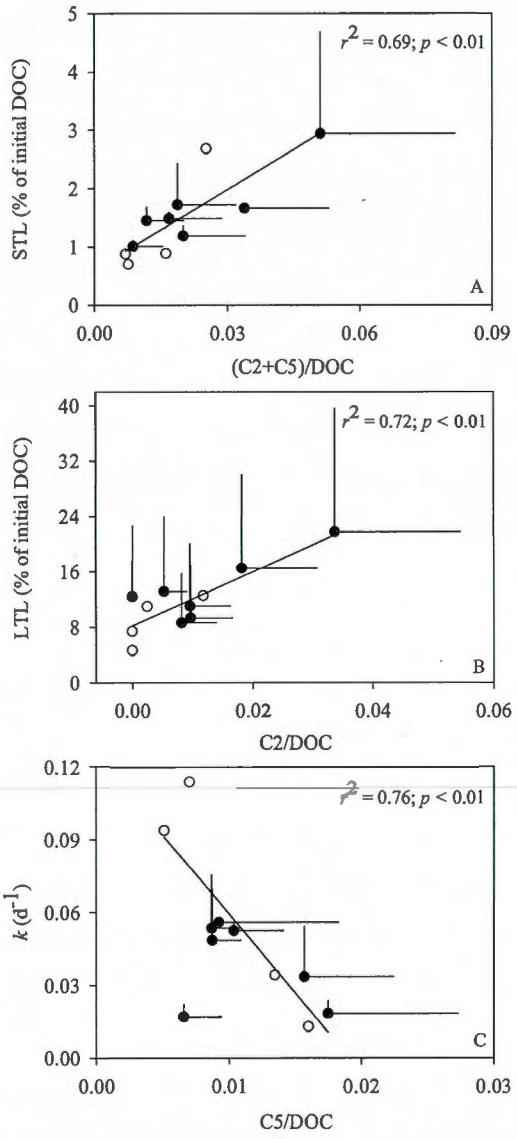

Figure 1.9 Regression models describing the relationship between (A) short-term lability (STL), (B) long-term lability (LTL), and (C) the first-order decay constant (k), and the fluorescence components identified by a parallel factor analysis (PARAFAC). Each black dot represents the mean of the June and July carbon consumption experiments for the 7 sampling sites where data were available for the two months, and error bars correspond to standard deviation. Open circles denote values for the 4 sites where data were available for July only (*see* Methods for details). The outlier was removed from the regression between the k constant and the fluorescence component 5 since it lies outside the 95% confidence interval (outlier included: r^2=0.45, n=11, p<0.05).

1.6.5 The biogeochemical and ecological implications of STCC, LTCC, and *k*

From a biogeochemical point of view, the time-scales of DOC consumption are important to understand the fate of C as it moves across the landscape, and its potential to generate greenhouse gases and to fuel linked biogeochemical processes at times and places that may be very different from those where the DOC originated (Cole *et al.*, 2007 ; Holmes *et al.*, 2008). From an ecological point of view, the consumption of DOC represents the entry point of energy into the microbial food web, and fuels a number of processes that are of key importance to the functioning of aquatic systems (Jansson *et al.*, 2007 ; Kritzberg *et al.*, 2004). Consequently, there has been a wide interest in assessing the regulating factors of DOC bioavailability. Studies have shown that the bulk C consumption by bacteria is modulated not only by the rate of supply of DOC, but also by its source and composition (Ågren *et al.*, 2008 ; Berggren *et al.*, 2010a ; Marschner et Kalbitz, 2003). In this study, we have further shown that the different aspects of DOC bioavailability (short vs. long-term consumption and lability) are likely to be regulated independently, and respond differentially to changes in the ambient DOC pool or to the environment. It is also likely that these different aspects of DOC bioavailability play different roles in terms of ecological and biogeochemical processes.

The shape of the DOC consumption dynamics, reflected in the *k* constant, and in the relative sizes of the short- and long-term labile pools, determines the potential of this C to fuel bacterial metabolism at different temporal and spatial scales. For example, in running waters and connecting systems, such as streams, rivers, and marshes, the shape of the DOC consumption dynamics will determine the amount of bacterial metabolism that this DOC may generate in the receiving systems downstream, thus influencing key aspects of ecosystem function such as trophic interactions, ecosystem respiration, and gas exchange (Cole *et al.*, 2007 ; Jansson *et al.*, 2007). In systems with longer water residence times, such as lakes, the *k* and the relative sizes of the short- and long-term labile pools will determine the capacity of the DOC pool to support microbial metabolism at different time scales within the system, which will have major implications, among others, on the magnitude of winter (under-ice) and hypolimnetic metabolism in these lakes, and the resulting trophic and gas dynamics. Furthermore, the shape of the DOC consumption curve has implications to ecosystem stability as well, since the relative importance of short- and long-term consumption will

influence the capacity of the DOC to act as a buffer to temporal variations in the input of new DOC into the system. We have shown that these key properties of the DOC are not necessarily correlated with total DOC concentration, or to any single DOC source. The consequence is that climate- or land use-driven shifts in DOC export from catchments, or aquatic DOC production, may have vastly different effects in terms of the ecosystem functioning of the receiving aquatic systems depending not only on the total amount of DOC involved, but on its patterns of biological availability.

CHAPITRE II

SIMULTANEOUS CONSUMPTION AND PRODUCTION OF FLUORESCENT DISSOLVED ORGANIC MATTER BY LAKE BACTERIOPLANKTON.

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AUTHOR CONTRIBUTIONS:

F. Guillemette 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

Des études récentes suggèrent que le bactérioplancton influence fondamentalement la composition du pool de matière organique dans les écosystèmes aquatiques, non seulement par la consommation, mais aussi par la production de composés organiques spécifiques; ce processus demeure cependant très peu compris. Nous avons conduit une série de bioessais en laboratoire afin de suivre la dynamique de consommation et de production de cinq pools de matière organique dissoute (DOM) fluorescente provenant de sept lacs et deux rivières du sud-est du Québec, Canada, et d'établir les liens potentiels entre cette dynamique et des aspects clés du métabolisme bactérien, l'origine de la DOM et la disponibilité des nutriments. Nous avons observé une décroissance de 3 à 15 % de la concentration en carbone organique dissous durant les différentes incubations tandis que les différents pools de DOM fluorescents ont affiché une dynamique très différente: deux classes de matière humique se sont accumulées dans l'ensemble des expériences, et ce, en fonction de l'efficacité de croissance bactérienne, qui elle-même était régulée positivement par la teneur en phosphore des eaux à l'étude. En revanche, deux groupes de matière protéinique et un groupe de matière humique ont été soit consommés, soit produits au cours de la période d'incubation. Nous avons observé que la production ou la consommation nette de ces composés organiques variait en fonction de l'origine de la matière organique (estimée à partir du $\delta^{13}C$ de la DOM) et l'activité microbienne totale. Nos résultats suggèrent que le bactérioplancton lacustre joue un rôle autant de producteur que de consommateur de DOM, influençant la composition du pool de DOM et son devenir dans les écosystèmes aquatiques, et qu'ultimement, ce rôle est déterminé par l'interaction entre l'origine de la DOM et la disponibilité des nutriments dans le milieu ambiant.

MOTS CLÉS: Bactérie, Métabolisme, Matière organique dissoute fluorescente, PARAFAC, Lac, Rivière.

2.2 ABSTRACT

Recent evidence suggests a key role of bacterioplankton in shaping the composition of the dissolved organic matter (DOM) pool in aquatic systems, not only through consumption but also through production of specific compounds, but the latter process is still not well understood. We used a bioassay approach to assess the patterns in bacterial production and consumption of five fluorescent DOM pools in seven lakes and two streams in Southeastern Québec, Canada, and the links these patterns may have with key aspects of bacterial metabolism, DOM origin, and nutrients availability. Total dissolved organic C declined by 3 to 15% during these incubations, whereas the specific DOM pools had very different dynamics: Two humic-like fractions accumulated in all incubations, with rates of production increasing as a function of bacterial growth efficiency, which itself increased with phosphorus concentrations. In contrast, two protein-like fractions, and a third humic-like fraction either increased or declined over the course of the experiments. The net production or consumption of these pools appeared to be a function of the contribution of terrestrial C to bulk DOM (derived from $\delta^{13}C$ of the DOM) and of total bacterial activity. Our results suggest that lake bacterioplankton play a dual role in DOM dynamics, as consumers and also producers, and that the interplay between DOM origin and nutrient availability appears to determine the net outcome of bacterial DOM processing, thus influencing the bulk DOM composition and its fate in these aquatic systems.

KEY WORDS: Bacteria, Metabolism, Fluorescent dissolved organic matter, PARAFAC, Lake, River.

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2.3 INTRODUCTION

Dissolved organic matter (DOM) in aquatic ecosystems originates from multiple sources, including allochthonous DOM transported from catchments to aquatic environments that adds to the pools generated within aquatic systems by primary producers. Heterotrophic bacteria are the main consumers of this organic matter, and further convert it either into biomass through bacterial production (BP) or into CO2 as bacterial respiration (BR). In this regard, bacterial C consumption and the ensuing metabolism have historically been viewed as one of the largest global sinks of organic carbon (Ducklow *et al.*, 1986 ; Sherr, Sherr et Albright, 1987).

A less recognized role of bacteria is the fact that these microbial communities may also act as a significant source of DOM in aquatic ecosystems (Jiao *et al.*, 2010 ; Kawasaki et Benner, 2006 ; Ogawa *et al.*, 2001). Recent estimates suggest that between 14% to 31% of bacterial production could be lost from the cell and released to the surrounding water as DOM (Kawasaki et Benner, 2006). From an ecological standpoint, this release of DOM represents a loss of carbon, nutrients, and energy from the food web. The bacterial production of DOM also potentially has major biogeochemical implications: It was hypothesized early on that most of the organic compounds generated by bacteria are largely refractory, thus lowering the general bioavailability of the bulk DOM pool (Brophy et Carlson, 1989 ; Tranvik, 1993). In this regard, the production of refractory material has been recently suggested as a significant pathway for carbon sequestration in the ocean (Jiao *et al.*, 2010 ; Ogawa *et al.*, 2001).

Among the types of organic matter that may potentially be bacterially generated, it has been shown that bacteria may act as a significant source of chromophoric DOM (CDOM) in aquatic ecosystems (Kramer et Herndl, 2004 ; Nelson, Carlson et Steinberg, 2004 ; Rochelle-Newall et Fisher, 2002). Recent advances in spectrofluoroscopy have facilitated the characterization of these colored products, and has allowed the identification of various fluorescent dissolved organic matter (FDOM) fractions (Coble, 1996 ; Stedmon, Markager et Bro, 2003a). Using this technic, it has been demonstrated that bacteria can turn both artificial and natural substrates into material that resembles humic matter found in natural waters (Kramer et Herndl, 2004 ; Shimotori, Omori et Hama, 2009 ; Yamashita et Tanoue, 2004).

Moreover, Yamashita and Tanoue (2008) reported a close link between the production of humic-like fluorescence and heterotrophic activity in the ocean interior, further suggesting that bacterial activity may represent a significant pathway for the formation and accumulation of biologically inert fluorescent organic matter in marine systems.

The production of FDOM is not solely limited to humic-like substances, however, as the production of proteinaceous compounds by bacteria has also been observed in various aquatic ecosystems (Cammack *et al.*, 2004 ; Lønborg *et al.*, 2009 ; Parlanti *et al.*, 2000). Recently, these protein-like fractions have been proposed as proxies for DOM lability, as the proportion of organic C consumed by bacteria could be predicted by their relative contribution to total FDOM (Fellman *et al.*, 2008 ; Fellman *et al.*, 2009a ; Guillemette et del Giorgio, 2011). The current evidence would thus suggest that proteinaceous pools can be both produced and consumed by aquatic bacteria. Likewise, even if humic substances are generally viewed as biologically inert, this FDOM fraction has also been shown to be consumed by bacteria (Moran, Sheldon et Zepp, 2000 ; Romera-Castillo *et al.*, 2011), and in this respect, may still represent a significant source of carbon and energy to bacterial communities. It thus appears that several key components of the FDOM pool may represent both a substrate and a by-product of bacterial metabolism.

This duality in the role of bacteria regulating the size and composition of the DOM pool challenges our current interpretation of the standing stock of the different FDOM components, both as DOM properties predicting its bioavailability or as indexes of the level of bacterial activity. Quantifying this dual role, and its regulation, may improve our understanding of how bacteria channel C not only through food-webs or to the atmosphere, but also through the production of DOM that may ultimately influence long-term C transport, biological and photochemical processing, and burial in aquatic ecosystems.

In this regard, one of the major gaps in our current understanding of bacterial-DOM interactions are the conditions under which bacteria act as net consumers or net producers of specific DOM pools; in particular, it is unclear how the net outcome relates to bacterial metabolism, or to intrinsic characteristics of the DOM itself. To address these issues, we conducted a series of bioassays using natural lake and stream water, where we followed the dynamics of five fluorescent DOM pools over 21 days as well as various aspects of bacterial metabolism. We assessed the patterns in production and consumption of these different pools,

and the links they may have with key aspects of bacterial metabolism, DOM origin derived from isotopic measurements, and nutrient availability.

2.4 MATERIALS AND METHODS

2.4.1 Sampling site and sampling scheme

Seven temperate lakes and two streams from the same drainage basin located in Southeastern Québec, Canada (45.24°N, 72.12°W), were visited in summer 2004; three lakes (Bran-de-Scie, Des Monts, and Fraser) were revisited a month after the initial visit. The lakes and streams represent a moderate gradient in phosphorus, dissolved organic C (DOC) and color (Table 2.1), and the watersheds of these different systems have similar characteristics in terms of vegetation (dominance of temperate mixed-wood forest) and geology (St. Lawrence Lowlands). As such, lakes and streams mainly differ in terms of relative productivity and the relative importance of terrestrial DOM inputs.

We collected 40 L of lake or stream water in acid-washed, 20-L polycarbonate bottles using a plastic hose mounted to a peristaltic pump. Back in the lab, we used precombusted Millipore (Billerica, MA, USA) AE glass fiber filters (1.0 µm) to remove particulate organic matter (POM) from lake water. Filters were preserved in foil, dried at 45oC, acid fumed overnight with HCl, and dried again at 45°C for further isotopic analysis. About 10 L of 1.0 µm-filtered water were set aside for the determination of bacterial metabolism and abundance, and the remaining water was passed through a Gelman filter capsule (0.2 µm, Pall, Port Washington, NY, USA) to generate bacteria free water used for regrowth experiments.

In the field we also collected zooplankton samples for isotopic ^{13}C analysis, which were used as an estimate of the algal endmember in isotopic mixing models of DOM (see details below). We filtered >200 L of lake surface water through a 50-µm mesh size screen, and collected the zooplankton in glass bottles filled with deionized water, which were stored at 4°C overnight to void their gut contents. Over 100 individual Cladocerans, represented by the genus Daphnia (*Daphnia mendotae* and *Daphnia catawba*) and Copepods, dominated by *Diacyclops bicuspidatus*, *Mesocyclops edax* and *Leptodiaptomus minutus*, were hand-picked, and collected in smooth-walled tin capsules, acidified with 10% HCl, and dried overnight at 45°C before isotopic analysis.

Table 2.1

Chemical data for the seven temperate lakes and two streams located in southern Quebec sampled in this study along with the estimated contribution of terrigenous sources to bulk DOM derived from a two-source (algal and terrigenous) mixing model (see text for details). The algal end-member was estimated by using the zooplankton $\delta^{13}C$ values unless indicated otherwise. Average values are shown along with ± SD for dissolved phosphorus and dissolved organic C (DOC) concentrations, and for water color determined at 440 nm (A_{440}).

Water Body	Date	Dissolved P (ug L^{-1})	A_{440} (m^{-1})	DOC (mg L^{-1})	DOC $\delta^{13}C$ (‰)	Algal end member (‰)	Terrigenous end member (‰)	DOM Mass Balance (% terrigenous)
Bowker	14 Sep 2004	2.9 ± 0.7	0.14 ± 0.01	2.76 ± 0.24	-28.8	-31.2	-27	57
Bran-de-Scie inlet	21 Sep 2004	11.9 ± 1.3	2.14 ± 0.02	4.90 ± 0.02	-27.8	-34.1[1]	-27	89
Bran-de-scie 1	03 Aug 2004	4.4 ± 0.8	2.00 ± 0.01	6.14 ± 0.43	-27.0	-34.0	-27	100
Bran-de-scie 2	31 Aug 2004	6.2 ± 0.7	2.65 ± 0.00	7.51 ± 0.07	-27.8	-34.0	-27	89
Brome	20 May 2004	8.2 ± 1.1	0.81 ± 0.02	3.41 ± 0.04	—	-29.6	-27	—
Des Monts 1	13 Aug 2004	2.5 ± 1.0	2.35 ± 0.00	5.74 ± 0.02	-27.1	-31.8	-27	99
Des Monts 2	07 Sep 2004	5.4 ± 0.4	2.51 ± 0.07	7.45 ± 0.16	-27.8	-33.0	-27	86
Fraser inlet	21 Sep 2004	6.4 ± 0.1	2.90 ± 0.02	6.06 ± 0.13	-27.2	-37.3[1]	-27	98
Fraser	27 Sep 2004	7.5 ± 0.2	2.14 ± 0.05	6.93 ± 0.27	-28.0	-32.1	-27	80
Simoneau	27 Jul 2004	1.23 ± 1.2	1.15 ± 0.07	4.51 ± 0.04	-28.0	-31.2	-27	76
Stukely	14 Sep 2004	3.1 ± 0.4	1.17 ± 0.03	4.97 ± 0.33	-27.5	-30.6	-27	87

[1] Algal end-member derived from the $CO_{2(aq)}$ measurements and assuming a 14‰ algal fractionation according to McCallister and del Giorgio (2008).

2.4.2 Regrowth experiments

Regrowth experiments were conducted following the protocol detailed in del Giorgio et al. (2006c). Briefly, one 4 L acid-washed Erlenmeyer flasks and a 2 L cubitainer bag were first filled with 0.2 µm filtered water to which a bacterial inoculum (1.0 µm filtered water) had been added (1% of total volume) at the beginning of each experiment. The bags were placed on a stand and connected to the lower flasks by acid-washed Tygon tubing (Saint-Gobain, Courbevoie, France) to establish a syphon between the two by gravity, allowing water to flow out when sampling the lower Erlenmeyer. All incubations with these flow-through systems were conducted over 21 days in a dark, temperature controlled (20°C) chamber.

FDOM and DOC samples were collected at the beginning of the incubations and every 2-3 days by filling either one 20 mL (FDOM) or two 40 mL borosilicate vials (DOC) to which 40 µL of sulphuric acid were added (DOC only). Vials were kept in the dark at 4oC until analysis. At the beginning and at every second day until day 8, 10 mL samples were collected in six of the 12 incubations and fixed with particle-free glutaraldehyde (1% final concentration) to determine long-term bacterial abundance (BALT). Triplicate abundance measurements were obtained using a FAC-Scalibur flow cytometer (BD biosciences, Franklin Lakes, NJ, USA), after staining cells with a SYTO 13 (Invitrogen, Carlsbad, CA, USA) solution (del Giorgio *et al.*, 1996). A solution of Fluoresbrite Microspheres beads (1.0 µm, Polysciences, Warrington, PA, USA) served as internal standard, and was controlled with Truecount Absolute counting tubes (BD biosciences).

2.4.3 In situ bacterial parameters

We used the same flow-through systems as described above to determine various aspects of bacterial metabolism over the initial 6 h of incubation (which we term short-term metabolism). At time zero and at every 2 hours for 6 hours, a total of 40 mL of water were sampled from the flask and distributed as follows: First, triplicate of 7 mL glass tubes (Chemglass, Vineland, NJ, USA) were filled with water, poisoned with 8 µL saturated $HgCl_2$ solution, and stored immersed at sample temperature for oxygen analysis. Dissolved oxygen concentration was measured on a membrane-inlet mass spectrometer according to Kana *et al.* (1994). BR_{ST} was derived from the slope of O_2 concentration versus time relationship, and

further converted into CO_2 using a respiratory quotient (RQ) of 1. The remaining water was split for bacterial abundance (BA_{ST}) measurements using the same methodology as for BA_{LT}, and for the determination of bacterial production using the ^{3}H-leucine incorporation technique (Kirchman, 1993), following the protocol in del Giorgio et al. (2006).

2.4.4 Spectrofluoroscopy and PARAFAC analysis

Absorption scans (190 to 900 nm) of filtered water samples were generated using a 2 cm quartz cuvette read in an Ultrospec 2100 (Biochrom, Cambridge, UK) ultraviolet-visible spectrometer. Following the procedure of Stedmon et al. (2003a), fluorescence EEMs were created by scanning the water samples from regrowth incubations using a RF-5301PC spectrofluorometer (Shimadzu, Kyoto, Japan) at 5 nm excitation intervals between 240 and 400 nm, and at emission ranging from 280 to 560 nm with 5 nm increments. Fluorescence EEMs were corrected for the inner-filter effect (McKnight *et al.*, 2001) and normalized to Raman units (R.U.; nm^{-1}).

We determined the main fluorescence groups, or fluorophores, by means of a PARAFAC analysis using the DOMFluor toolbox 1.7 for MATLAB (Mathworks, Natick, MA, USA) following Stedmon and Bro (2008). The model was based on 207 EEMs, which included scans from several lake and stream samples presented elsewhere (Guillemette et del Giorgio, 2011). The model was validated by split-half analysis, and by a thorough examination of the model's residuals to ensure no systematic signal was present.

2.4.5 Isotopic and chemical analyses

The $\delta^{13}C$ isotopic signature of both zooplankton and POM was determined using a Finnigan MAT (Bremen, Germany) Delta^{plus} dual-inlet continuous flow isotope ratio mass spectrometer (IRMS) with on-line sample combustion. Dissolved inorganic carbon (DIC) and DOM samples for the determination of stable carbon isotope ratios were prepared as follows: Acid-washed (10% HCl) and pre-combusted (525°C for 4 h) 40 mL borosilicate vials were filled with bulk (DIC) or 0.2 µm filtered (DOM) water, poisoned with $HgCl_2$, and capped with a teflon lined septa cap (VWR, Radnor, PA, USA) doubled with a polytetrafluoroethylene (PTFE)/rubber septa (Chromatographic Specialties, Brockville, Canada). Isotopic analysis ($\delta^{13}C$) of both DIC and DOM were performed on a modified O.I.

(College Station, TX, USA) 1010 TIC TOC analyzer connected to a Finnigan MAT Delta^{Plus} IRMS with a Conflo III continuous flow interface as described in St-Jean (2003). Stable isotopes values are reported hereafter in standard δ notation as:

$$\delta^{13}C = [(R_{sample} / R_{standard}) - 1] \times 10^3 \quad (1)$$

where R is ^{13}C:^{12}C.

DOC concentrations were determined on an O.I. 1010 TIC TOC analyzer that uses a heated wet persulfate oxidation method (St-Jean, 2003). Dissolved phosphorus (DP) concentration was determined in 0.2 µm filtered water samples using the standard molybdenum blue method (Cattaneo et Prairie, 1995). Finally, chlorophyll *a* concentrations were measured spectrophotometrically from ethanols extracts.

2.4.6 Calculations and statistics

The rates of change in FDOM were calculated from the slope of FDOM versus time relationship for the different components. BGE_{ST} was calculated as $BP_{ST}/BR_{ST}+BP_{ST}$ using the 6 h average BP_{ST}, and short-term total bacterial carbon consumption (BCC_{ST}) as the sum of BR_{ST} and mean BP_{ST}. SP-BR_{ST} and SP-BP_{ST} were calculated by dividing BR_{ST} or BP_{ST} by mean BA_{ST}. BP_{LT} was estimated as the change in cell abundance during the growth phase in the long-term incubation, and assuming a conversion factor of 20 fg C cell^{-1} (Bratbak, 1985 ; Lee et Fuhrman, 1987). Long-term bacterial respiration BR_{LT} was estimated as DOC change versus time. BGE_{LT} was derived from BP_{LT} divided by $BR_{LT}+BP_{LT}$.

To overcome the problem of using collinear explanatory variables, i.e. bacterial metabolic parameters, we carried out a principal component analysis of bacterial metabolic parameters, and used the principal components obtained as regressors in a multiple regression analyses between the rates of change of the FDOM components and bacterial metabolism. This procedure is known as principal component regression analysis (Jolliffe, 1982). Significant predictors were selected following a mixed stepwise routine (probability for a variable to enter or to leave the model was set to 0.05). All bacterial metabolic parameters were log-transformed to attain normality before PCA analysis. To account for experimental error associated to our predictive variables in the different linear regression models presented at Fig. 2.3, 2.4, and 2.5b, we used ranged major axis regression (RMA) as recommended by

Legendre and Legendre (1998) for normally-distributed variables expressed in different units. All statistical analyses were performed using the JMP 7.0 statistical software (SAS Institute, Cary, NC, USA).

2.5 RESULTS

2.5.1 FDOM characterization and dynamics during batch experiments

Five distinct fluorescence components were identified by the PARAFAC analysis over the entire data set of excitation-emission matrices (EEMs; n=207), all of which having already been described in previous studies (Coble, 1996 ; Stedmon et Markager, 2005a): Two of these components (C2 and C5) have been associated to protein-like fluorophores, since their fluorescence resembles that of tyrosine (ex = 270 nm/em = 295 nm) and tryptophan (ex = 285 nm/em = 335 nm). The model also identified two humic-like fluorophores located at ex = 260 nm/em = 445 nm and ex = 305 nm/em = 400 nm for component C3 and C4, respectively, and one fulvic-like component (C1; ex = 350 nm/em = 450 nm). The five-component PARAFAC model explained 98.7% of the total variation in fluorescence. We therefore conclude that this model is adequate to describe the variability in the DOM fluorescence spectra of these Northern temperate lakes and streams.

Figure 2.1a shows an example of a long-term regrowth experiment where we followed the patterns of FDOM with time. Typically, the fluorescence components either increased or decreased linearly with time, and we thus used the slopes obtained from linear least squares regression models to derive rates of change in FDOM (ΔFDOM) for each component; all models were significant ($p<0.05$) with $R^2 >0.5$. In four cases however, we could not detect any significant change in fluorescence intensity over time, and thus the ΔFDOM of the corresponding components was set to zero. Across experiments, the five FDOM components showed different patterns in ΔFDOM: Humic-like components C1 and C4 increased in all incubations, whereas humic-like component C3 and the two protein-like components increased in some samples and declined in others (Fig. 2.1b). Overall, the production of humic-like compounds was in the order of 1-17% of the initial pools, whereas the relative change in the two proteinaceous fractions ranged from -66% to 88% of the initial pools.

While the protein-like and humic-like compounds are very different in terms of chemical structure, and probably in terms of functionality and reactivity as well, the dynamics of humic-like component C3 were closely correlated to those of the two protein-like compounds across the different incubations (R=0.92, n=11, $p<0.0001$ and R=0.94, n=11,

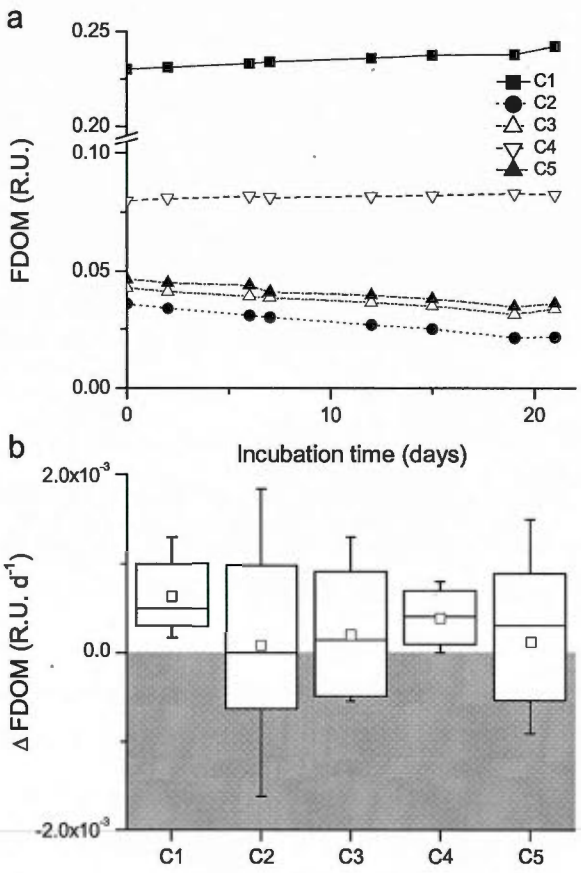

Figure 2.1 (a) Example of time course of the different fluorescent components identified in this study during the 21 days of incubation in the dark. Regression equations were used to derive rates of change in fluorescence of each component over time (C1: $y = 0.23 + 5.0 \times 10^{-4} x$, $R^2 = 0.95$, $P < 0.0001$; C2: $y = 0.35 - 6.9 \times 10^{-4} x$, $R^2 = 0.99$, $P < 0.0001$; C3: $y = 0.042 - 4.9 \times 10^{-4} x$, $R^2 = 0.94$, $P < 0.0001$; C4: $y = 0.081 + 1.0 \times 10^{-4} x$, $R^2 = 0.86$, $P < 0.01$; C5: $y = 0.046 - 5.4 \times 10^{-4} x$, $R^2 = 0.96$, $P < 0.0001$). (b) Box and whisker plot showing the variability in the rates of change of the five fluorescent components across the sampled lakes and streams. Whiskers show minimum and maximum ΔFDOM values, and open squares denote mean values. Shaded area corresponds to a net disappearance of FDOM.

$p<0.0001$, pairwise correlation with C2 and C5, respectively). This result points to a distinct behavior of this humic-like component C3 relative to the other two humic components, which were strongly correlated to each other ($R=0.75$, $n=11$, $p<0.01$), and we thus kept C3 separate but combined C1 + C4 for all subsequent analyses. Likewise, the two protein-like components were strongly correlated to each other ($R=0.92$, $n=11$, $p<0.0001$), and we thus combined them to simplify the analysis.

2.5.2 Bacterial community metabolism and FDOM dynamics

During the short (6h) incubations, short-term bacterial respiration (BR_{ST}) estimates ranged from 0.19 to 1.75 μg C L^{-1} h^{-1}, whereas short-term bacterial production (BP_{ST}) values ranged from 0.13 to 2.29 μg C L^{-1} h^{-1}. The calculated short-term bacterial growth efficiency (BGE_{ST}) showed a large variation across the sampled lakes and streams, ranging from 17.6% in oligotrophic lake Stukely to almost 70% in Bran-de-Scie inlet (Table 2.2). Short-term bacterial abundance (BA_{ST}) was much less variable across the sampled sites (coefficient of variation of 30%, 53% and 93% for BA_{ST}, BR_{ST} and BP_{ST}, respectively), and was not significantly correlated to either BR_{ST} or BP_{ST}. Cell-specific BR_{ST} (SP-BR_{ST}) and BP_{ST} (SP-BP_{ST}) estimates ranged from 0.73 to 9.14 fg C $cell^{-1}$ h^{-1} and from 0.36 to 6.63 fg C $cell^{-1}$ h^{-1}, respectively (Table 2.2). BP and BR in the six long-term (21d) regrowth incubations, hereafter designated as BP_{LT} and BR_{LT}, respectively, were on average lower than their short-term counterparts, with values ranging from 0.33 to 4.35 μg C L^{-1} d^{-1}, and from 3.4 to 17.6 μg C L^{-1} d^{-1}, respectively. Long-term bacterial growth efficiency (BGE_{LT}) was also on average lower than its short-term counterpart, with values ranging from 8.8 to 21.3 %. Overall bacterial DOC processing resulted in a 3 to 15% decrease of the initial DOC concentration across the different incubations over the 21 days of incubation.

The ordination of the short-term bacterial metabolic variables based on principal component analysis (PCA) is shown in Fig 2. The two first (and only significant) axes of the PCA explained 96.8% of the variability in the dataset. The variables representing overall community activity were all strongly correlated (positively) to axis 1, whereas the second component of the PCA discriminated between the dominant type of activity, BR_{ST} and BGE_{ST} being present in the upper and lower right quadrants, respectively. A second PCA performed on the long-term bacterial metabolic variables revealed a similar pattern, with all variables

Table 2.2

Bacterial metabolic parameters estimated in the short-term (6 hours) incubations, and in the long-term (8 days) regrowth experiments. BA_{ST} is short-term bacterial abundance, BR_{ST} is short-term bacterial respiration, BP_{ST} is short-term bacterial production, BCC_{ST} is short-term total bacterial C consumption, BGE_{ST} is short-term bacterial growth efficiency, and SP-BR_{ST} and SP-BP_{ST} stand for short-term cell-specific bacterial respiration and production, respectively. BP_{LT} and BGE_{LT} are the bacterial production and growth efficiency measured in long-term incubations, respectively, and Δ DOC is the mean rate of change in dissolved organic concentration derived from the slope of the DOC concentration vs. time relationship over 21 days. Mean values shown along with ± SD except for BR_{ST}, BP_{LT} and Δ DOC for which + SE is reported.

Water Body	BA_{ST} (cells x10^9 L^{-1})	BR_{ST} (μg L^{-1} h^{-1})	BP_{ST} (μg L^{-1} h^{-1})	BCC_{ST} (μg L^{-1} h^{-1})	BGE_{ST} (%)	SP-BR_{ST} (fg C $cell^{-1}$ h^{-1})	SP-BP_{ST} (fg C $cell^{-1}$ h^{-1})	BP_{LT} (μg L^{-1} d^{-1})	BGE_{LT} (%)	Δ DOC (μg L^{-1} d^{-1})
Bowker	1.84 ± 0.17	0.58 ± 0.13	0.13 ± 0.03	0.71 ± 0.30	18.3 ± 9.7	3.15 ± 0.04	0.71 ± 0.08	0.33 ± 0.06	8.8 ± 2.5	3.4 ± 1.5
Bran-de-Scie inlet	3.65 ± 0.44	0.74 ± 0.02	1.65 ± 0.38	2.39 ± 0.40	69.0 ± 4.5	2.03 ± 0.02	4.52 ± 0.05	—	—	21.9 ± 5.4
Bran-de-scie 1	2.06 ± 0.12	1.24 ± 0.10	1.13 ± 0.17	2.37 ± 0.27	47.7 ± 1.8	6.01 ± 0.01	5.49 ± 0.05	4.35 ± 1.34	19.8 ± 3.2	17.6 ± 2.0
Bran-de-scie 2	1.87 ± 0.43	1.71 ± 0.38	1.13 ± 0.36	2.84 ± 0.74	39.8 ± 2.5	9.14 ± 0.01	6.04 ± 0.06	—	—	10.9 ± 2.2
Brome	2.53 ± 0.15	0.52 ± nd[1]	0.43 ± 0.04	0.95 ± 0.04	45.3 ± 2.3	2.06 ± 0.01	1.70 ± 0.01	1.08 ± 0.25	17.8 ± 0.4	7.1 ± 1.9
Des Monts 1	2.14 ± 0.18	1.64 ± 0.09	0.86 ± 0.05	2.50 ± 0.14	34.4 ± 0.1	7.66 ± 0.02	4.02 ± 0.01	3.31 ± 0.50	18.7 ± 0.5	14.4 ± 1.7
Des Monts 2	1.74 ± 0.13	0.96 ± 0.26	0.60 ± 0.09	1.56 ± 0.35	38.5 ± 3.0	5.52 ± 0.11	3.45 ± 0.03	—	—	11.7 ± 1.2
Fraser inlet	3.45 ± 0.56	1.62 ± 0.44	2.29 ± 0.56	3.91 ± 1.00	58.6 ± 0.7	4.70 ± 0.05	6.64 ± 0.06	—	—	44.8 ± 2.1
Fraser	4.04 ± 0.78	0.59 ± 0.03	0.14 ± 0.04	0.73 ± 0.07	19.2 ± 3.7	1.44 ± 0.02	0.35 ± 0.01	3.06 ± 1.08	21.3 ± 3.2	11.3 ± 1.9
Simoneau	2.14 ± 0.29	0.75 ± 0.26	0.22 ± 0.01	0.97 ± 0.27	22.7 ±5.7	3.50 ± 0.08	1.03 ± 0.01	1.18 ± 0.09	12.9 ± 0.8	8.0 ± 1.2
Stukely	2.80 ± 0.17	0.84 ± 0.38	0.18 ± 0.04	1.02 ± 0.42	17.6 ± 4.0	3.00 ± 0.12	0.64 ± 0.01	—	—	5.5 ± 1.4

[1] nd, not determined

related to overall activity strongly positively correlated to the first axis (93.0% of explained variation), whereas the second axis related to variables that clearly differentiated catabolic and anabolic process (7.0 % of explained variation; Appendice A).

We carried out a first principal components regression analysis (PCR) using the short-term PCA components as potential predictors of ΔFDOM. This PCR analysis shows that the ΔFDOM of the protein-like components, and of the humic-like component C3 were negatively related to the first axis of the PCA (R^2=0.84, n=11, p<0.0001 and R^2=0.80, n=11, p<0.001, for the protein-like and humic-like C3, respectively; Fig. 2.3a-b). The PCR further showed a negative relationship between the ΔFDOM of humic-like C1 + C4, and the second axis of the PCA (R^2=0.78, n=11, p<0.001; Fig. 2.3c). The subsequent, non-significant PCA axes were not significantly related to ΔFDOM. A second PCR analysis performed with the long-term PCA components revealed similar relationships with ΔFDOM: the rates of change in protein-like fluorescence were negatively related to the first axis of the long-term PCA (R^2=0.66, n=6, p<0.05) whereas the ΔFDOM of the humic-like C1 + C4 was related to the second axis of the long-term PCA (R^2=0.85, n=6, p<0.01; Appendice B). Although we observed a similar negative trend between the ΔFDOM of humic-like C3 and the first principal component of both short- and long-term PCA, the relationship with the long-term principal component 1 was marginally significant (p=0.07).

2.5.3 Links between bacterial FDOM production/consumption, C origin and nutrients

We determined the relative contribution of terrigenous and algal sources to bulk DOM, using a two-source mixing model described by the following equations:

$$\delta^{13}C_{DOM} = f_1\delta^{13}C_{Terr} + f_2\delta^{13}C_{Algal} \quad (2)$$

$$f_1 + f_2 = 1 \quad (3)$$

where $\delta^{13}C_{DOM}$ corresponds to the isotopic signature of bulk DOM, and f_1 and f_2 are the relative contributions of terrigenous (C_{Terr}) and algal (C_{Algal}) sources to the DOM pool, respectively. The terrigenous end-member was set to the commonly accepted value of -27.0‰ (Boschker et Middelburg, 2002). The algal $\delta^{13}C$ end-point was constrained for the same set of lakes using several independent approaches by McCallister and del Giorgio (2008). These authors showed that the zooplankton isotopic signature yielded reliable estimates of algal $\delta^{13}C$, an approach also used by Karlsson *et al.* (2007). Isotopic values for

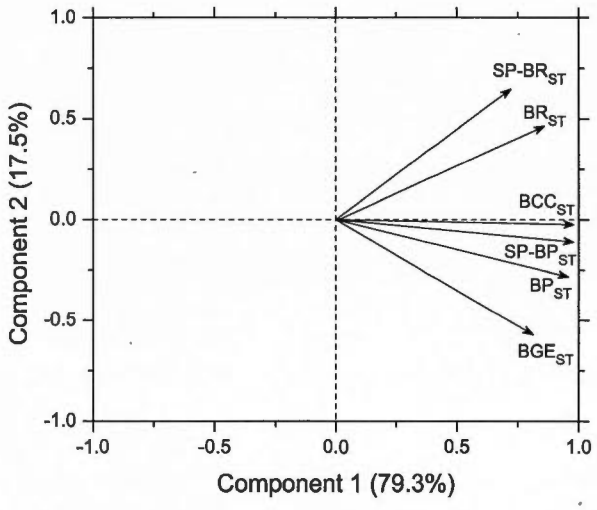

Figure 2.2 Correlations of the different short-term (6h) bacterial metabolic parameters with the first two axes of a principal component analysis. The percent of explained variation is shown in brackets. See Table 1 for a full description of acronyms.

zooplankton, which varied from -29.6 to -34.0‰, are shown in Table 2.1, and resolving equation 3 for the whole set of lakes yielded estimates of terrigenous contribution to bulk DOM ranging from ~57% to almost 100% (average of 87%). For comparative purposes, we also resolved the mixing model using either δ^{13}C-POC or $\delta^{13}C\text{-}CO_{2(aq)}$ as algal end-points, and these alternative estimates are shown in Appencide C. The average terrigenous contribution estimated by the three approaches agreed and were not significantly different from each other (ANOVA; F_{26}=0.23, p>0.05), and we used stream $CO_{2(aq)}$ δ^{13}C values as estimates of the algal end-member isotopic signature, since no zooplankton could be recovered in these systems. The exception was lake Bowker, where the three estimates differed substantially (57%, 30% and 14% for the zooplankton, POC and $CO_{2(aq)}$ based-estimates, respectively); we suspect a problem either with the measurements of δ^{13}C of $CO_{2(aq)}$ or with the fixed algal fractionation (14‰) that was assumed, and we thus used the zooplankton-based estimate for this lake as it yielded a more realistic estimate of allochthony.

There was a positive relationship between the % of DOM from terrigenous origin, and the rates of change of both protein-like components, and the humic-like component C3 (R^2=0.77, n=10, p<0.001, and R^2=0.70, n=10, p<0.01, for the protein-like components and humic-like component C3, respectively; Fig. 2.4a-b). In contrast, humic-like C1 + C4 were not related to the origin of the DOM, but instead varied positively with epilimnetic dissolved phosphorus concentration (R^2=0.77, n=11, p<0.01; Fig. 2.4c).

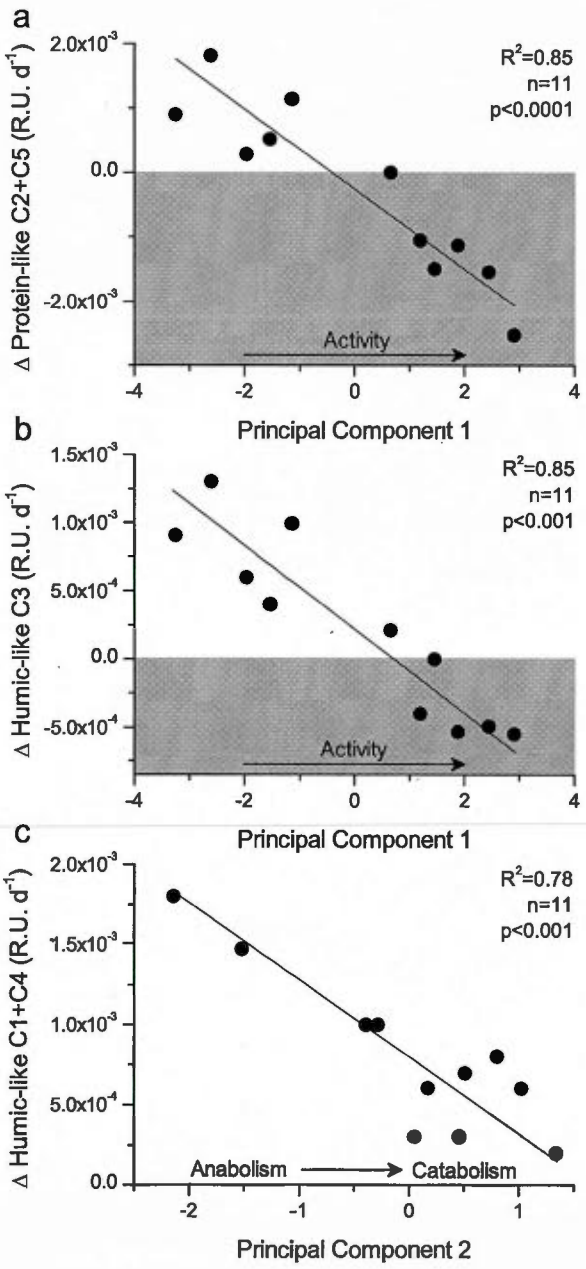

Figure 2.3 The relationships between the rates of change in protein-like components C2 and C5 (a), humic-like component C3 (b), and in humic-like components C1 and C4 (c), and the two first principal components of a PCA performed on the short-term bacterial metabolic dataset. Linear regression lines and parameter estimates were derived from ranged major axis regression models. Shaded areas denote a net disappearance of FDOM.

2.6 DISCUSSION

Our results clearly show that lake bacterioplankton play a dual role in DOM dynamics, as consumers and also producers, and that these roles are regulated very differently. Since algal cells and grazers were removed prior to the regrowth incubations, all experiments were carried in the dark, and a close link was found between ΔFDOM and bacterial metabolism in our study (Fig. 2.3), we attribute the observed FDOM dynamic to bacteria. The rates of ΔFDOM, expressed both as absolute (comparable Raman units) or relative (%) units, are well within the range of reported values for similar experiments carried in estuarine (Boyd et Osburn, 2004 ; Moran, Sheldon et Zepp, 2000 ; Søndergaard, Stedmon et Borch, 2003), marine (Shimotori, Omori et Hama, 2009 ; Stedmon et Markager, 2005b), freshwater (Cammack *et al.*, 2004 ; Søndergaard, Stedmon et Borch, 2003), and even in terrestrial systems (Wickland, Neff et Aiken, 2007).

Our results further suggest that the role of bacteria in FDOM dynamics is far from being straightforward, and in this regard, we identified two very distinct patterns: 1) Certain FDOM fractions were consistently produced during incubations in all samples tested; 2) other fractions were both produced or consumed (negative ΔFDOM values, Fig. 2.1b) across the different samples tested, suggesting that bacteria were simultaneously carrying out both processes, and that what we actually measure in these incubations is the net balance between the two. In addition, we were able to link these different patterns in FDOM dynamics to very different patterns of regulation, some involving bacterial metabolism, others probably involving intrinsic properties of the DOM itself.

There was a gradual shift from net production to net consumption of the two proteinaceous fractions and of the humic-like component C3, and this shift appeared to be correlated to measures of bacterial activity (i.e. bacterial C consumption) in our incubations (Fig. 2.3a). This negative relationship is in contrast with previous studies that reported a positive correlation between FDOM production and bacterial activity (Cammack *et al.*, 2004 ; Parlanti *et al.*, 2000). In our experiments, total bacterial activity (first axis of PCA, Fig. 2.5a) was positively correlated with the proportion of terrestrial C in the ambient DOM, and we suggest that the apparently diverging results between studies may be due to differences in the origin of the ambient DOM, rather than a direct effect of bacterial activity per se. On one

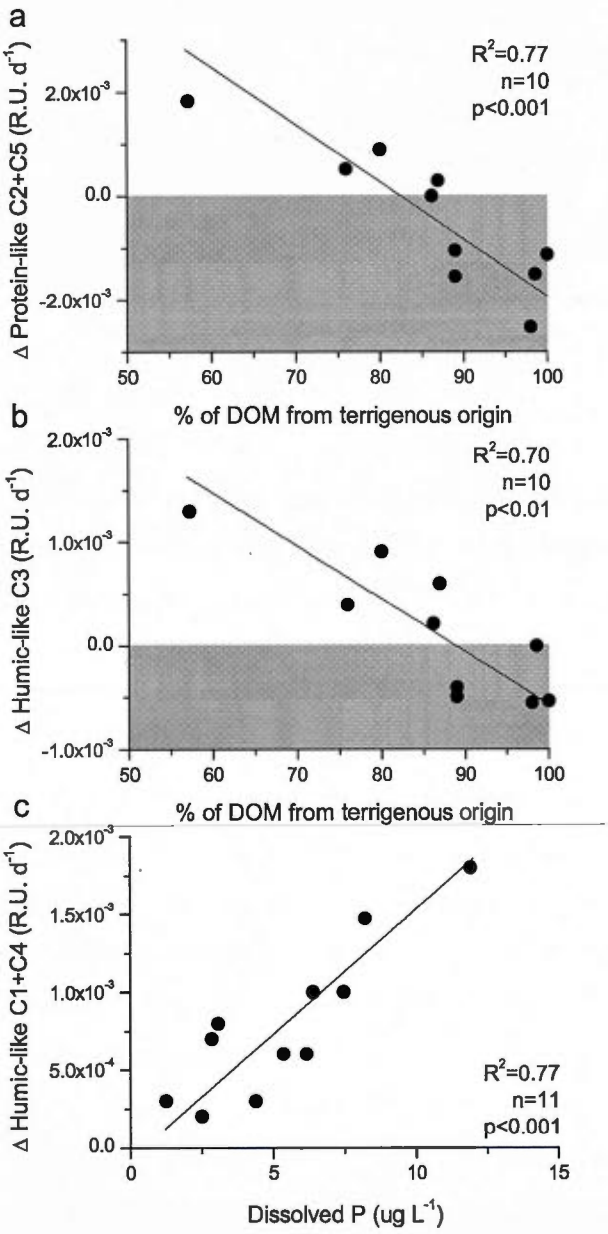

Figure 2.4 The relationships between the rates of change in protein-like components C2 and C5 (a), humic-like component C3 (b), and the % of bulk DOM originating from terrigenous soucres. (c) The humic-like components C1 and C4 as a function of dissolved P. Linear regression lines and parameter estimates were derived from ranged major axis regression models. Shaded areas denote a net disappearance of FDOM.

hand, high FDOM production rates have been previously associated to bacteria preferentially degrading algal-derived material in laboratory experiments (Rochelle-Newall et Fisher, 2002), and to systems where bacteria rely mostly on algal C (Yamashita et Tanoue, 2008). On the other hand, net consumption of protein-like fractions has been observed in soil (Wickland, Neff et Aiken, 2007), and in stream DOM degradation experiments (Fellman *et al.*, 2009b). Our results show a shift to net consumption of protein-like compounds with increasing allochthony of the bulk DOM pool, and thus serve to bridge these apparently contradictory reports, and further suggest that the dominant source of DOM consumed by bacteria may play a key role in determining the bacterially-mediated dynamics of FDOM, particularly in terms of proteinacious fractions.

Contrary to the patterns we observed for proteinacious and humic-like component C3, the two humic-like components C1 and C4 were consistently produced in all incubations, suggesting that the bacterially-mediated processing is fundamentally different for these two FDOM pools. This is further evidenced by the fact that whereas the dynamics of proteinaceous FDOM appeared to be linked to the composition of DOM and to total bacterial activity, as discussed above, the dynamics of humic-like fractions were clearly related not to total activity but to the balance between catabolic and anabolic processes (i.e. BGE). Several processes have been invoked to explain bacterial DOM formation, such as the release of cell material upon viral lysis, grazing and cell division, or the action of ectoenzymes and the direct exudation of various carbon compounds (Nagata, 2000). The relationship between humic-like FDOM production and BGE may reflect, for example, an increase in production of refractory material lost during cell division, such as D-amino acids (Kawasaki et Benner, 2006), which may be enhanced when anabolism is favored (i.e. high BGE). The DOM formation pathways described above, and the link they may have with BGE need to be further investigated.

Regardless of the nature of this link, we have shown that phosphorus plays a central role in regulating the balance between catabolism and anabolism in these lakes, and thus on the bacterially-mediated production of humic-like FDOM pools. Bacterial C metabolism had been shown to be influenced by phosphorus in this region (Cammack *et al.*, 2004 ; Smith et Prairie, 2004), and we add to those results by demonstrating that phosphorus may not only enhance growth efficiency and reduce cell-specific respiration (Fig. 2.5b), thus regulating the

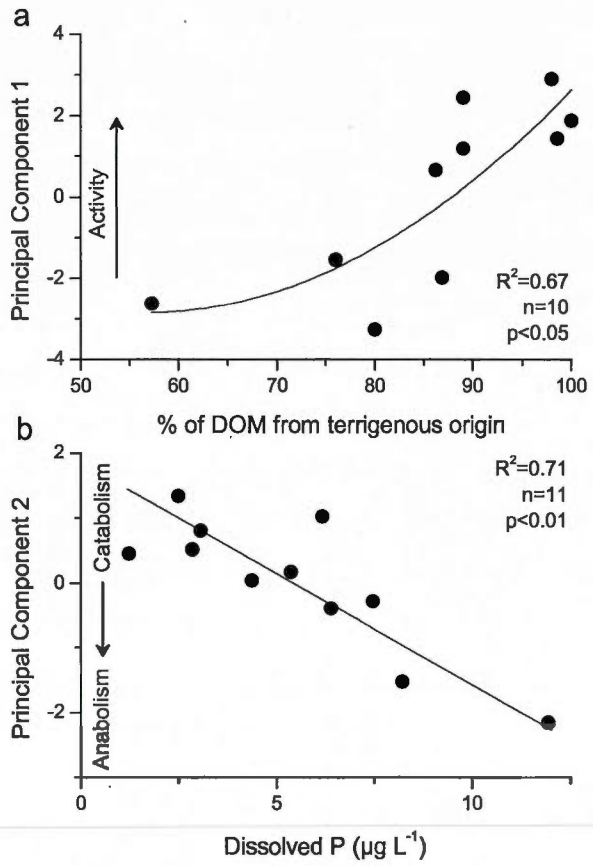

Figure 2.5 (a) The relationships between the first principal component of the PCA of short-term metabolic rates, and the % of DOM from terrigenous origin, and (b) the second principal component as a function of dissolved P. Linear regression line and parameter estimates were derived from a ranged major axis regression model.

amount of bacterial biomass that may be transferred to higher trophic levels, but also may control the rate of bacterially-mediated production of key DOM components (Fig. 2.3b). Recent evidence suggests that nitrogen may play an important role in regulating FDOM formation (Biers, Zepp et Moran, 2007), but to our knowledge no study to date has reported the potential importance of phosphorus.

Humic material in lakes has traditionally been considered of terrestrial origin (Steinberg et Muenster, 1985), however, in light of our results, we cannot rule out the possibility that a significant portion of the humic-like DOM that transits through inland waters, ultimately reaching the sea, may in fact originate from the aquatic environment itself. In order to assess the potential impact of bacterially-mediated DOM production on bulk DOM dynamics, we converted our Raman FDOM units into C units using the relationship between fluorescence and DOC developed by Cumberland and Baker (2007a) for an International Humic Standard Society (IHSS) sample (Suwanee River Natural Organic Matter), and in parallel, by using a bovin serum albumin (BSA) solution standard following Mayer et al. (1999); while both approaches target different fluorescent regions (i.e. humic-like and protein-like fluorescence, respectively), they essentially yielded the same conversion factor. If we only consider the average C production rate of the two humic fractions that occurred in all incubations, roughly 0.1% of the DOM pool per day was converted from likely an uncolored form into a colored, humic-like form. These bacterially-generated DOM pools may play a role in key lake processes, such as DOM flocculation (von Wachenfeldt, Bastviken et Tranvik, 2009) or photochemical DOM degradation (Molot et Dillon, 1997). However, our results suggest that they may also represent an important component of lake DOM export, especially in lakes with long water retention times. Regardless, these estimated DOM production rates imply that the amount of C that is mobilized by the bacterial compartment in lakes has been systematically underestimated in past studies, since the bacterial release of C is not captured in measures of BR and BP, and is thus unaccounted for in estimates of total bacterial C consumption (BCC) and BGE.

The role of bacteria not just as consumers but also as a source of DOM in the oceans was postulated over a decade ago (Nagata, 2000 ; Ogawa *et al.*, 2001), but it is only recently that the issue has re-emerged (Jiao *et al.*, 2010), driven by the realization that this pathways may significantly influence C storage in the oceans, in what has been termed the "microbial

carbon pump" (Jiao *et al.*, 2010 ; Ogawa *et al.*, 2001). Our observation of a net production of some apparently recalcitrant DOM pools in lakes and streams suggests that a comparable pathway may also occur in freshwaters, which could eventually influence the nature of the C delivered to the oceans. While we cannot directly attribute the observed patterns of FDOM formation to any cause in particular, our results suggest that the interplay between DOM origin, ambient nutrients, and bacterial metabolism may ultimately lead to very different DOM end-products. Regardless of the actual mechanisms operating in our incubations, our results strongly suggest a close nutrient-DOM interaction that may ultimately shape the final outcome of the bacterial processing of DOM. This interaction is critical, since climate and environmental change may lead to regional shifts not just in the amount and nature of organic C loaded to freshwaters (Monteith *et al.*, 2007 ; Tranvik *et al.*, 2009), but also to changes in the stoichiometry of nutrients and C delivery (Elser *et al.*, 2009 ; Hessen *et al.*, 2009).

CHAPITRE III

DIFFERENTIATING THE DEGRADATION DYNAMICS OF ALGAL AND TERRESTRIAL CARBON WITHIN COMPLEX NATURAL DISSOLVED ORGANIC MATTER IN TEMPERATE LAKES

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

3.1 RÉSUMÉ

Une des suppositions couramment véhiculées en écologie aquatique est que le carbone organique dissous (DOC) issu de la production algale est facilement biodégradable comparativement à son pendant terrestre; cependant, cette hypothèse a rarement été testée directement. Dans cette étude, nous avons suivi la production et la signature isotopique du CO_2 respiratoire bactérien sur une période de deux semaines dans des incubations en laboratoire et reconstruit et modélisé la dynamique de consommation du carbone organique d'origine algale et terrestre afin de tester cette hypothèse. Lors de l'expérimentation, la proportion de DOC algal a diminué de façon systématique dans le temps suggérant une consommation rapide de ce substrat. Les résultats issus du modèle de dégradation de premier ordre démontrent que le DOC d'origine algale a été utilisé dans une proportion et à un taux deux fois plus grand que le DOC d'origine terrestre. Cependant, une quantité trois fois plus grande de DOC terrigène a été consommée dans les différentes incubations, contribuant substantiellement à la consommation bactérienne en carbone à court terme et supportant l'essentielle de la consommation résiduelle à long terme. Il appert que la quantité absolue de carbone algal biodisponible augmente en fonction des concentrations de chlorophylle *a* et que la quantité de carbone terrestre consommable augmente en fonction des niveaux de phosphore en lac, suggérant que la dégradation du DOC algal et terrigène ne soit pas simplement fonction des propriétés propres à ces substrats, mais dépende aussi d'interactions avec les nutriments ambiants. Notre étude démontre que contrairement aux suppositions courantes, de grandes quantités de carbone terrigène sont consommées simultanément avec le DOC d'origine algale et qu'à cause d'interactions potentielles avec les nutriments, le DOC terrigène vraisemblablement supporte de hauts niveaux de métabolisme bactérien et de production de CO_2, et ce possiblement même dans les lacs plus productifs.

MOTS CLÉS: Bacterioplancton, Lacs, Isotopes, Consommation en carbone, Agale, Terrestre

3.2 ABSTRACT

It has often been hypothesized that the dissolved organic carbon (DOC) pool of algal origin in lakes is more bioavailable than its terrestrial counterpart, but this hypothesis has seldom been directly tested. Here we test this hypothesis by tracking the production and isotopic signature of bacterial respiratory CO_2 in 2-week lake water incubations, and using the resulting data to reconstruct and model the bacterial consumption dynamics of the algal and terrestrial DOC pools in ambient lake waters. The proportion of algal DOC decreased systematically over time in all experiments, suggesting a rapid consumption and depletion of this substrate. First-order decay modeling revealed that the algal DOC pool was used in proportions and at rates twice as high as the terrestrial DOC pool. On the other hand, the absolute amount of labile terrestrial DOC was on average three-time higher than labile algal DOC, and accounted for almost the entire long-term residual C metabolism, but also contributed significantly to short-term bacterial C consumption. The absolute amount of labile algal DOC increased with chlorophyll *a* concentrations, presumably associated to primary production, whereas total phosphorus appeared to enhance the amount of labile terrestrial DOC that bacteria could consume, suggesting that the degradation of these pools is not solely governed by their respective chemical properties, but also by strong interactions with nutrients. Our study shows that in contrast to current assumptions, there is a highly reactive pool of terrestrial DOC that is processed in parallel to algal DOC, and because of interactions with nutrients, terrestrial DOC likely support high levels of bacterial metabolism and CO_2 production even in more productive lakes.

KEY WORDS: Bacteria, Lake, Isotopes, Carbon consumption, Algal, Terrestrial

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3.3 INTRODUCTION

The organic C present in the bulk dissolved organic carbon (DOC) pool of lakes exceeds by over an order of magnitude the amount present in other detrital pools and in the biomass of aquatic organisms (Prairie, 2008). Consequently, any process that may induce even small variations in this pool of carbon and energy has the potential to greatly influence the functioning and the role of lakes both at the local and landscape level. The consumption and degradation of DOC by heterotrophic bacteria is one of the main processes influencing the bulk DOC pool of lakes with profound ecological and biogeochemical consequences. For example, DOC contributes to sustain the widespread net heterotrophy observed in many freshwater ecosystems (Duarte et Prairie, 2005 ; Karlsson, Jansson et Jonsson, 2007 ; McCallister et del Giorgio, 2008), and a portion of this otherwise unavailable C to higher trophic levels of aquatic food webs (Azam *et al.*, 1983 ; Berggren *et al.*, 2010b).

The bulk DOC pool of lakes is composed of a highly heterogeneous and complex mixture of organic compounds with different chemical attributes and availability to bacterial metabolism (Benner, 2003). Because bacteria tend to use the more labile components first while leaving the more recalcitrant molecules behind (Mateles et Chian, 1969 ; Middelburg, 1989), the overall bioavailability of the bulk DOC typically declines over time when isolated from new inputs, and this pattern can be modeled as a reactivity continuum (Koehler *et al.*, 2012 ; Vähätalo, Aarnos et Mäntyniemi, 2010). The degradation dynamics of the bulk DOC is thus the direct reflection of the contribution of various pools that coexist within this bulk DOC, and which differ in terms of bioavailability and degradation dynamics (Westrich et Berner, 1984). Identifying the intrinsic properties and dynamics of these major pools will undoubtedly improve our understanding of the controls on overall DOC decomposition in natural aquatic ecosystems.

There is both theoretical and empirical evidence that the consumption dynamics of specific DOC pools should differ based on their molecular size (Amon et Benner, 1996 ; Chrost et Faust, 1983), chemical composition (Amon, Fitznar et Benner, 2001 ; Weiss et Simon, 1999), and elemental stoichiometry (Hunt, Parry et Hamilton-Taylor, 2000 ; Sun *et al.*, 1997), which in turn, are presumably dependent on their respective origins (Benner, 2003). In lakes, DOC originates from local primary production (Bertilsson et Jones, 2003)

and is also imported from the terrestrial environment (Aitkenhead-Peterson, McDowell et Neff, 2003). In this regard, it has traditionally been assumed that algal DOC should be more readily consumed by bacteria than terrestrial DOC, owing to the presence of simple, low molecular weight carbon compounds that turn over very rapidly in the former (Chen et Wangersky, 1996 ; Sundh, 1992), and the presence of more complex and aromatic compounds (McKnight et Aiken, 1998) and its previous aging in soils in the latter (Berggren, Laudon et Jansson, 2009). These assumptions lead to a scenario wherein algal DOC should be preferentially degraded on short time scales, followed by a slower utilization of terrestrial DOC pool on longer time scales. Recent reports challenge this scenario by showing fast bacterial utilization of a highly labile pool of low molecular weight compounds of terrestrial origin in boreal streams (Ågren *et al.*, 2008 ; Berggren *et al.*, 2010a), leading to a second scenario wherein terrestrial DOC could fuel bacterial C consumption on both short and longer time scales.

In practice, however, tracking the bacterial consumption dynamics of specific pools within complex mixtures has been a major challenge, and the above scenarios are based on either a priori judgments on the basis of chemical attributes of each sources, or on experiments that have assessed C consumption of these pools in isolation. There have been a handful of studies that have attempted to trace the bacterial utilization of different DOC sources in lake water (Karlsson, Jansson et Jonsson, 2007 ; Kritzberg *et al.*, 2004 ; McCallister et del Giorgio, 2008), based on the isotopic signature of metabolic products (bacterial respiratory CO_2 or biomass), which have confirmed that bacteria consume both terrestrial and algal DOC when grown on natural lake DOC. These studies have provided important insight into the contribution of each source to bacterial metabolism, but do not allow to reconstruct the actual degradation dynamics of terrestrial and algal DOC. As a consequence, the actual consumption dynamics of these major C categories in natural complex mixtures of lake DOC remains to be described.

The aim of our study was to describe the degradation dynamics of algal and terrestrial DOC within natural bulk DOC pool across a diversity of northern lake types, and to link the resulting patterns of degradation to environmental factors. The main challenge associated to this question is to differentiate, at relevant time scales, the consumption of algal and terrestrial C that occurs simultaneously within a complex natural DOC mixture. In this

study we have used the approach developed by McCallister, Guillemette et del Giorgio (2006), based on tracking the production and isotopic signature of bacterially-produced respiratory CO_2, and have used this as a proxy to reconstruct the degradation of algal and terrestrial DOC over a period of 6, 13 and 20 days. Based on the respiratory CO_2 isotopic signature and on a coupled two-source (algal and terrestrial) mixing model, we then apportioned the amount of CO_2 originating from the degradation of algal and terrestrial DOC at each time interval. Finally, we reconstructed and modeled the complete degradation dynamics of these two pools as well as of the bulk DOC. Our study shows that algal and terrestrial DOC follow very different degradation dynamics in lake water: Algal DOC was essentially processed over short time scale whereas terrigenous material was degraded over both short and longer time scales, but followed slower overall degradation kinetics. In addition, phosphorus played a key role in modulating the amount of bioavailable terrestrial DOC, and more importantly the degradation kinetics of terrestrial DOC, suggesting that the biological degradation of these sources in lakes is not simply a function of their respective concentrations and intrinsic properties, but is also strongly influenced by nutrient dynamics.

3.4 METHODS

3.4.1 Study lakes and sampling scheme

We sampled five northern temperate lakes located in the Eastern townships region of south-eastern Québec (45.24°N, 72.12°W), Canada, between 2004 and 2007, and sampled an additional lake (Lac à la Truite) in 2006 located in the Laurentian region north of Montréal (46.01°N, 74.15°W). The watersheds of these lakes are characterized by temperate mixed-wood forest and low-density population, and are underlain by the sedimentary St. Lawrence Lowlands (Eastern townships) or by the Canadian Shield bedrock (Laurentian). The sampled water bodies represent a moderate gradient in lake productivity, both in terms of chlorophyll *a* (Chl *a*) and nutrient levels, and also of dissolved organic carbon (DOC) content and water color (Table 3.1). As a result, the likely influence of autochthonous and allochthonous C on these lakes differed (Guillemette et del Giorgio, 2012).

Integrated (<3 m) water samples (60 L) from the epilimnion of these lakes were collected using a plastic hose mounted to a diaphragm pump, stored in acid-washed (10% HCl) polycarbonate bottles, and kept cool in the dark upon processing (<3 hours). In the laboratory, 8 L of lake water were set aside for the determination of ambient nutrient and chlorophyll *a* concentrations. The remaining water was filtered through precombusted Pall AD glass fiber filters (3.0 µm; Port Washington, NY, USA) to isolate the bacterial communities from other planktonic components, and to serve as an inoculum for the different bacterial respiratory CO_2 recovery incubations described below. Pretests showed that 92.6 ± 4% of the initial bacterial abundance remained in the filtrate while keeping bacterial grazers (i.e. flagellates) to a minimum level (<10% initial (Guillemette et del Giorgio, 2011)). We further filtered 25L of this 3.0-µm filtered water through a Gelman filter capsule (Pall; 0.2 µm) to prepare the lake water media used in the incubation experiments. Samples for DOC analysis were taken from this filtered water and poisoned with 5N sulphuric acid.

In the field, zooplankton samples, which were used to approximate the isotopic signature of phytoplankton (*see* below), were collected in parallel to lake water samples, by pumping a large amount of lake water (200 L) through a 50-µm mesh size net. Organisms were stored at 4°C overnight to void their gut contents back in the laboratory. The following

Table 3.1

Limnological characteristics of the six Northern temperate lakes sampled[a]

Lake	Chl *a* ($\mu g\ L^{-1}$)	TP ($\mu g\ L^{-1}$)	DOC ($mg\ L^{-1}$)	DOC $\delta^{13}C$ (‰)	MD (m)	LA (km^2)	WRT (years)
Fraser 2004	1.5 ± nd	11.2 ± 0.8	6.90 ± nd	-28.0	8.6	1.6	0.4
Bran-de-Scie 2006	5.0 ± 1.1	15.0 ± 1.0	5.54 ± 0.10	-27.8	3.1	0.1	<0.1
Fraser 2006	6.7 ± 0.3	10.4 ± 1.0	5.06 ± 0.01	-27.3	8.6	1.6	0.4
À la Truite 2006	0.9 ± 0.1	2.5 ± 0.3	3.20 ± 0.01	-27.3	9.4	0.5	0.2
Stukely 2007	4.9 ± 1.7	6.3 ± 0.4	4.78 ± 0.01	-27.5	13.1	4.0	4.0
Simoneau 2007	2.9 ± 0.3	4.7 ± 0.1	4.37 ± 0.06	-28.0	9.3	0.5	0.4

[a]Abbreviations: Chl *a*, chlorophyll *a*; TP, total phosphorus; DOC, dissolved organic carbon; LA, lake surface area; WRT, theoretical water retention time; MD, mean depth; nd, not determined. Mean biological and chemical data reported with ±SD, $n = 2$.

day, we hand-picked over 100 individual Cladocerans, represented by the genus Daphnia (*Daphnia mendotae and Daphnia catawba*) and Copepods, dominated by *Diacyclops bicuspidatus, Mesocyclops edax* and *Leptodiaptomus minutus*, and the organisms were placed in smooth-walled tin capsules, fumed with 10% HCl, and dried overnight at 45°C pending isotopic analysis.

3.4.2 Bacterial respiratory CO_2 recovery experiments

We used a modified version of the procedure described in McCallister, Guillemette et del Giorgio (2006) to quantitatively recover the respiratory CO_2 produced by bacteria grown in 0.2-μm filtered lake water using the ReCReS system. The procedure involves an initial acidification and helium bubbling of the culture medium to remove background dissolved inorganic carbon (DIC) concentration (< 1% of initial), the neutralization and oxygenation of the acidified lake water to initial conditions, the inoculation with the natural lake bacterial assemblage and incubation of the regrowth medium in an airtight, 20 L glass system, and finally, the collection of the bacterially-produced CO_2 with dedicated cryogenic traps after several days of incubation by subsequent acidification and helium bubbling.

Our project involved recovering the CO_2 produced by bacteria at several points during a time course, so as to be able to reconstruct the DOC consumption dynamics. Rather than setting up several parallel systems with the same water and harvesting them for CO_2 at different times, we chose to reset the same system after the initial CO_2 harvest, so as to be able to recover the signature of the gas actually produced within that period rather than the cumulative signature, which would be more difficult to interpret. We repeated this reset procedure twice, such that we had three time points for CO_2 production and its isotopic signature for each sample, in addition to the initial time point. For these subsequent incubations, we collected 1 L of incubation medium before harvesting the CO_2, containing the bacteria grown in of the previous incubation, which we kept refrigerated to serve as an inoculum for the following incubation. Upon the complete collection of the respiratory CO_2, the incubation medium was re-neutralized and oxygenated before the injection of an inoculum consisting of a 30 mL concentrate of the 1 L incubation medium previously collected obtained using tangential flow ultra-filtration (Millipore, Billerica, MA, USA; 1000 kDa cartridge). These subsequent incubations were extended for at least 7 days before

performing a second respiratory CO_2 harvest, to account for declining rates of bacterial metabolism in the samples along the time course. The entire time course thus covered a period of approximately 18 to 20 days.

The traps containing bacterial respiratory CO_2 collected on day 6, 13 and 20 were mounted to a vacuum extraction line to isolate the gas from residual moisture, and the purified CO_2 was further quantified manometrically (Baratron, MKS Instruments, Andover, MA, USA; 0.5 μmol sensitivity) and transferred to break seals pending isotopic analysis. In addition, we followed the activity of the incubated bacterial communities over the course of the experiments by measuring bacterial production (BP) every 1-2 days using the ^{3}H-leucine incorporation technique as detailed in del Giorgio, Pace et Fischer (2006b).

3.4.3 Isotopic and chemical analyses

The $\delta^{13}C$ isotopic signature of zooplankton was determined using a Finnigan MAT (Bremen, Germany) Delta^{plus} dual-inlet continuous flow isotope ratio mass spectrometer (IRMS) with online sample combustion at the G.G. Hatch lab, Ottawa University (Ottawa, Canada). A subset of zooplankton samples were assessed for analytical precision and run in duplicate; the relative standard deviation was <0.3‰). Respiratory CO_2 breakseals were transferred into Exetainers, and analyzed for $\delta^{13}C$ with a continuous flow GasBench peripheral (Thermo Finnigan) interfaced to an Isotope Ratio Mass Spectrometer Delta XP (Thermo Finnigan) which has an analytical precision of 0.10 ‰ (G.G. Hatch Lab, University of Ottawa, Canada). DOC concentration and $\delta^{13}C$ determination was performed on a modified 1010 TIC TOC analyzer (O.I. Analytical, College Station, TX, USA) coupled to a Finnigan MAT DeltaPlus IRMS with a Conflo III continuous flow interface (Thermo Finnigan) as described in St-Jean (2003). Stable isotope values are reported hereafter in standard δ notation as:

$$\delta^{13}C = [(R_{sample} / R_{standard}) - 1] \times 10^3 \qquad \text{(eq. 1)}$$

where R is ^{13}C:^{12}C.

Total phosphorus concentrations were determined in unfiltered lake water using the standard blue molybdenum colorimetric methods (Cattaneo et Prairie, 1995). Ambient chlorophyll *a* concentrations were measured spectrophotometrically from ethanol extracts.

3.4.4 Algal and terrestrial C contribution to bacterial respiration

The relative contribution of algal and terrestrial C to bacterial respiratory CO_2 was estimated by a two-source mixing model:

$$\delta^{13}C_{CO_2} = f_1\delta^{13}C_{\text{Terrestrial}} + f_2\delta^{13}C_{\text{Algal}} \quad \text{(eq. 2)}$$

$$f_1 + f_2 = 1 \quad \text{(eq. 3)}$$

where $\delta^{13}C_{CO_2}$ corresponds to the isotopic signature of bacterial respiratory CO_2, and f_1 and f_2 are the relative contributions of terrestrial ($C_{Terrestrial}$) and algal (C_{Algal}) sources to respiratory CO_2, respectively. The terrestrial endmember was set to the commonly accepted value of -27.0‰ typically found for terrestrial C_3 plants (Boschker et Middelburg, 2002 ; Lajtha et Marshall, 1994). This assumption was validated by the isotopic signature of a forested stream entering lake Fraser ($\delta^{13}C$ of -27.2‰ ± 0.1). The algal $\delta^{13}C$ end-point was constrained using the isotopic signature of zooplankton according to recent studies (Karlsson, Jansson et Jonsson, 2007 ; Marty et Planas, 2008 ; McCallister et del Giorgio, 2008). We further assumed a 16% terrestrial C content in zooplankton biomass which corresponds to the mean zooplankton allochthony values reported for other Canadian shield lakes (N = 25) (Mohamed et Taylor, 2009).

3.4.5 Modeling algal and terrestrial C consumption

To assess the individual degradation dynamics of algal and terrestrial DOC, we first reconstructed the consumption curve of these different DOC pools as follows: We converted the amount of CO_2 recovered at the end of each time interval into DOC concentration units (mg C L^{-1}) by dividing the mass of CO_2 collected by the volume of water incubated, and by further subtracting the quantity of DOC consumed previously relative to the starting DOC concentration of each time step. This calculation was performed using the total amount of CO_2 recovered and bulk DOC concentrations to reconstruct the dynamic of the bulk DOC pool. We proceeded similarly to reconstruct the dynamics of the algal and terrestrial DOC pools, but we apportioned the amount of CO_2 produced and DOC concentration into algal and terrestrial C using the $\delta^{13}C$ isotopic signature of CO_2 and DOC (and their coupled mixed models), respectively. We then applied a first-order decay model, which includes both a

reactive and a non-reactive component as proposed by Westrich et Berner (1984), to derive a first-order decay constant from these reconstructed consumption curves. The model equation is the following:

$$G_T(t) = G_{\text{Labile}}[\exp(-kt)] + G_{\text{Residual}} \qquad \text{(eq. 4)}$$

where G_T is the initial DOC concentration; G_{Lab} and G_{Res} are the labile and the residual pools estimated by the model, respectively; k is the first-order decay constant; and t is the time of decomposition. While corresponding mathematically to a constant rate of decline in DOC concentration over time, the resulting k constant may provide useful information on how the initial consumption rates evolve in time, and therefore on the shape of the decay curve (Guillemette et del Giorgio, 2011). For example, a high k value denotes a strong inflexion in the decay curve suggesting the rapid transition from a highly labile DOC pool towards a more refractory pool. Inversely, a lower k value implies a much less marked inflexion, and therefore suggests a more homogenous composition of the labile DOC pool. We used a similar conceptual interpretation of the modeled k value to that of Guillemette et del Giorgio (2011) in this study.

3.4.6 Statistics

Mean differences between corresponding model parameters (i.e. k, labile and refractory pools) calculated for algal and terrestrial DOC pools were assessed using Student's T-test. We explored potential relationships between model output parameters and environmental variables using simple linear regression models. All statistical analyses were considered significant if $P < 0.05$, and were performed using the JMP 10 statistical software (SAS Institute, Cary, NC, USA).

3.5 RESULTS

3.5.1 Patterns in algal and terrestrial DOC degradation dynamics

Bacterial DOC degradation resulted in an average production of 42 ± 14 μM of CO_2 during the first cycle of incubation, declining to an average of 19 ± 16 μM in the final time point (Table 3.2). Bacterial production measurements carried out during the incubations confirmed that there was a roughly 2-day lag phase at the beginning of each new experiment (hereby defined as <10% of maximum BP, Appendice D). This pattern has been observed in the past, and attributed to the lack of dissolved inorganic C in the incubation system, which may inhibit some metabolic pathways involving the anaplerotic B-carboxylation reaction (McCallister, Guillemette et del Giorgio, 2006 ; Overbeck, 1979). In this regard, we removed 2 days from each time interval upon which respiratory CO_2 was collected to better reflect the actual period of time that is relevant to the consumption dynamics of DOC in our incubations. In all subsequent sections, including the modeling of DOC consumption, we use these corrected time intervals, i.e. 4, 9, 13 days.

The isotopic analysis of the recovered gas indicated a marked change in the $\delta^{13}C$ isotopic signature of the respiratory CO_2 collected over time. For the initial incubation, the $\delta^{13}C$ of respiratory CO_2 ranged from -32.1 to -27.9‰ across experiments (Table 3.2). The isotopic signature of respiratory CO_2 was neither as depleted as the algal $\delta^{13}C$ nor as enriched as the terrestrial isotopic signature suggesting that at the beginning of the incubations, bacteria were consuming a mix of algal and terrestrial C (Table 3.2). Results from the mixing model indicated that between 18% and 47% of bacterial respiration (and thus of DOC) involved algal C at the initial stage (Figure 3.1). The proportion of algal C consumed did not remain constant over time, however, as the isotopic signature of respiratory CO_2 systematically shifted towards more enriched $\delta^{13}C$ values as the incubations progressed (Table 3.2). There was a rapid and consistent decline in the % algal C respired over time, and by the final time point the DOC respired appeared to be almost entirely terrestrial, regardless of the initial proportion of algal DOC consumed (Figure 3.1).

The shifts in algal and terrestrial DOC consumed, estimated from the patterns in respiratory CO_2 described above, were in all cases well described ($R^2 > 0.99$) by a first-order decay model, and the resulting model parameters were remarkably different between these

Table 3.2

Bacterial respiratory CO_2 $\delta^{13}C$ isotopic signature measured in the different lake water incubations over short- and long-term[a]

Lake	Algal endmember (‰)[b]	Terrestrial endmember (‰)	Bacterial respiratory CO_2 $\delta^{13}C$			Mass CO_2 recovered (µM)		
			4d	9d	14d	4d	9d	14d
Fraser 2004	-33.1	-27.0	-28.4	nd	-27.3	48	nd	48
Bran-de-Scie 2006	-32.0	-27.0	-29.3	-28.4	-27.4	61	40	23
Fraser 2006	-33.1	-27.0	-32.1	-28.9	-28.3	48	23	11
À la Truite 2006	-31.8	-27.0	-27.9	-27.7	-27.2	41	16	9
Stukely 2007	-31.4	-27.0	-28.1	-27.7	-27.1	23	19	4
Simoneau 2007	-32.0	-27.0	-29.3	-28.4	-28.4	28	22	21

[a]Abbreviations: nd, not determined.

[b]Algal endmember $\delta^{13}C$ derived from zooplankton isotopic signature assuming a 16% terrestrial content in biomass (*see* section 2.4 for details).

two major DOC pools. There were systematic differences in both the size of the respective labile and refractory components of these DOC pools, as well as in the degradation rate constant, *k*. The labile component of the algal DOC pool (14 ± 7 μg L^{-1}) was significantly smaller than that of the terrestrial DOC pool, which averaged 49 ± 32 μg L^{-1}, respectively (Figure 3.2a), although the proportion of algal C that was labile within the time frame of our incubations was on average two-fold greater (2.7 ± 1.7%) than that of terrestrial origin (1.2 ± 0.6%; Figure 3.2b). The algal DOC pool, on the other hand, had on average a higher *k* constant (0.245 ± 0.080 day^{-1}) as compared to the terrestrial DOC pool (0.127 ± 0.023 day^{-1}; Figure 3.2c).

3.5.2 Links between degradation dynamics and environmental gradients

Our results show not only systematic differences in the average degradation dynamics between algal and terrestrial DOC pools, but also a large range of variation in degradation kinetics within each of these pools along environmental gradients. Not surprisingly, we found that the absolute amount of labile algal DOC increased as a function of Chl *a* concentrations ($R^2 = 0.79$, $N = 6$, $P < 0.05$; Figure 3.3a), although the rate at which this DOC was degraded did not vary systematically along the same gradient (Figure 3.3b), nor with any of the other environmental variable tested. Similarly, there was a strong positive relationship between the absolute amount of labile terrestrial DOC and TP ($R^2 = 0.98$, $N = 6$, $P < 0.01$; Figure 3.4a), but in this case, there was also a strong negative relationship between its associated *k* constant and TP ($R^2 = 0.77$, $N = 6$, $P < 0.05$; Figure 3.4b).

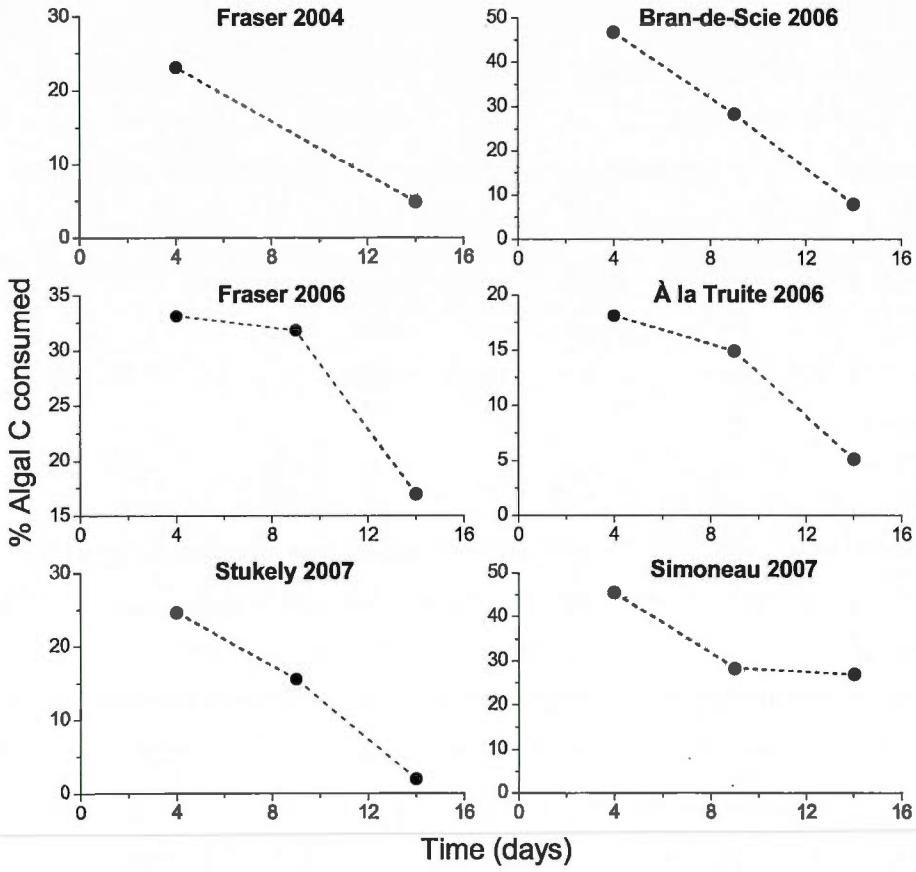

Figure 3.1 The change in the proportion of algal C consumed by lake bacteria over time in the six ReCReS experiments carried out between 2004-2007. The proportion of algal C consumed is based on the $\delta^{13}C$ of bacterial respiratory CO_2 and a coupled two-source (algal and terrestrial) mixing model (see sections 3.4.2 and 3.4.4). In the 2004 lake Fraser experiment, bacterial respiratory CO_2 was only recovered on short- (4 days) and long-term (14 days).

3.6 DISCUSSION

The biological recalcitrance of terrestrial C, as compared to its algal counterpart, is a longstanding assumption that is based on the idea that this substrate is intrinsically more structurally complex, having undergone significant decomposition in soils before reaching downstream water bodies (Breger, 1970 ; Hobbie, 1988). Surprisingly, this simple and widely assumed hypothesis has seldom, if ever, been empirically tested, likely because of the challenges associated with disentangling the C sources that are consumed within complex natural DOC mixtures. Here, we used the isotopic signature of bacterial respiratory CO_2 to trace the sources of DOC being degraded, and we were able to recreate and describe the complete short-term degradation dynamics of the algal and terrestrial DOC pools of lakes, and thus to directly test this fundamental hypothesis. Our results show that algal and terrestrial DOC differ significantly in both their overall bioavailability, expressed both in terms of amount or proportion of DOC consumed, and also in their degradation dynamics as reflected in the *k* values.

The approach we have used here, while providing new insights into the degradation dynamics of these major DOC pools, has limitations that need consideration. For example, both the total amount (57 ± 25 μg C L^{-1}) and the proportion ($1.16 \pm 0.37\%$) of total labile DOC (algal + terrestrial) that we observed in our experiments are lower than reported values for similar incubation time frames in freshwaters (del Giorgio et Davis, 2003), including for this same set of lakes (Guillemette et del Giorgio, 2011). In addition, the decay constant calculated for the bulk DOC (0.158 ± 0.030 day^{-1}, derived from the total accumulation of respiratory CO_2), is in the upper range of values reported in the same temperate lakes (Guillemette et del Giorgio, 2011) and in boreal lakes (Koehler *et al.*, 2012), although similar values have been reported in another study (Ostapenia, Parparov et Berman, 2009). These discrepancies may be mainly attributed to the difficulty in associating a specific time frame to the final amount of CO_2 (and thus to DOC consumption) harvested in the successive regrowth incubations, particularly in terms of incorporating the initial lag phase. We used bacterial production measurements to better constrain this initial lag phase (approximately two days; Appendix H), but there is clearly uncertainty is the assignment of the effective time associated to CO_2 production. Had this lag phase been assumed to be somewhat longer, it

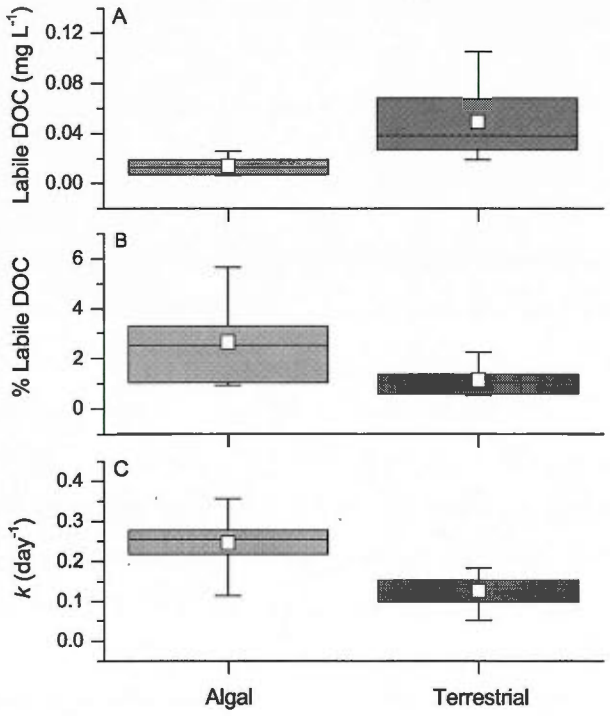

Figure 3.2 Box-and-whisker plots showing the range in algal and terrestrial (a) labile DOC concentrations, (b) % labile DOC, and (c) degradation constant, k, estimated from the first-order decay model. Whiskers and open boxes denote the min-max and mean values, respectively. Average labile DOC and k values estimated for the algal and terrestrial DOC pools were significantly different (Student's t-test; $P < 0.05$), whereas the mean % labile DOC values were only barely significantly different (Student's t-test; $P = 0.09$).

Table 3.3

Estimated parameters of the first-order decay models fitted to the algal and terrestrial DOC degradation time courses[a]

Lake	Algal DOC			Terrestrial DOC		
	k (day^{-1})	Labile ($mg\ L^{-1}$)	Refractory ($mg\ L^{-1}$)	k (day^{-1})	Labile ($mg\ L^{-1}$)	Refractory ($mg\ L^{-1}$)
Fraser 2004	0.356 ± nd	0.009 ± nd	0.965 ± nd	0.099 ± nd	0.068 ± nd	4.858 ± nd
Bran-de-Scie 2006	0.277 ± 0.020	0.026 ± 0.001	0.870 ± 0.001	0.051 ± 0.002	0.105 ± 0.002	4.531 ± 0.002
Fraser 2006	0.217 ± 0.025	0.016 ± 0.001	0.267 ± 0.001	0.153 ± 0.015	0.040 ± 0.001	4.734 ± 0.005
À la Truite 2006	0.276 ± 0.053	0.007 ± 0.001	0.207 ± 0.001	0.184 ± 0.035	0.037 ± 0.003	2.933 ± 0.003
Stukely 2007	0.231 ± 0.068	0.006 ± 0.001	0.572 ± 0.001	0.137 ± 0.060	0.027 ± 0.005	4.415 ± 0.005
Simoneau 2007	0.114 ± 0.046	0.019 ± 0.004	0.860 ± 0.004	0.137 ± 0.052	0.019 ± 0.003	3.500 ± 0.003

[a]Abbreviations: nd, not determined. Model parameters reported with ± SE.

would have resulted in higher apparent rates of DOC consumed and lower k values estimated by the model, much closer to literature values. However, we have no other means to correct our data, and bacterial production measurements clearly show that after two days, there is a steep increase in bacterial activity (Appendice D). Regardless, the objective of this study was not to quantitatively describe the degradation kinetics of bulk DOC, as this has been the focus of a previous study targeting the same lakes (Guillemette et del Giorgio, 2011). Rather, here we have focused on the potential differences in the degradation patterns of the algal and terrestrial DOC pools composing this bulk DOC, and we surmise that while the absolute values may be somewhat biased by the approach, the actual differences in the patterns of degradation between algal and terrestrial DOC within a complex mixture that we describe are likely to reflect DOC consumption processes occurring in natural lake water.

3.6.1 Degradation dynamics of algal DOC

Our results show that algal and terrestrial DOC followed very different degradation dynamics, with the algal pool being more rapidly degraded compared to its terrestrial counterpart (Figure 3.2c). These results confirm previous work suggesting that algal DOC is a preferred substrate for bacteria (Kritzberg *et al.*, 2004 ; Kritzberg *et al.*, 2005), and suggest that algal DOC is a major component of the highly reactive pool within bulk DOC. The amount of C that can be extracted from bulk DOC by bacteria has been shown to increase with lake trophy (Ostapenia, Parparov et Berman, 2009), suggesting that as the relative proportion of algal vs. terrestrial DOC increases, the overall lability of bulk DOC also increase. Our results provide evidence for the mechanistic underpinning to this pattern, by showing that the amount of algal DOC that can be utilized by bacteria actually increases as a function of lake productivity (Figure 4.3a), thus potentially driving the overall DOC lability.

The amount of labile DOC of algal origin varied several-fold across lakes and increased with lake productivity, and whereas the rate at which this labile substrate was degraded (k) also varied substantially across lakes, there was no systematic pattern along a productivity gradient (Figure 4.3b) or with any of the environmental variables tested. This suggests that the intrinsic quality of algal DOC rather than its quantity (or other external factors) influence its degradation dynamics in lake water. Culture work has shown that algal exudates derived from different phytoplankton species may be degraded at very different rates (0.19 to 0.49 d^{-1}) for a similar proportion of labile algal DOC

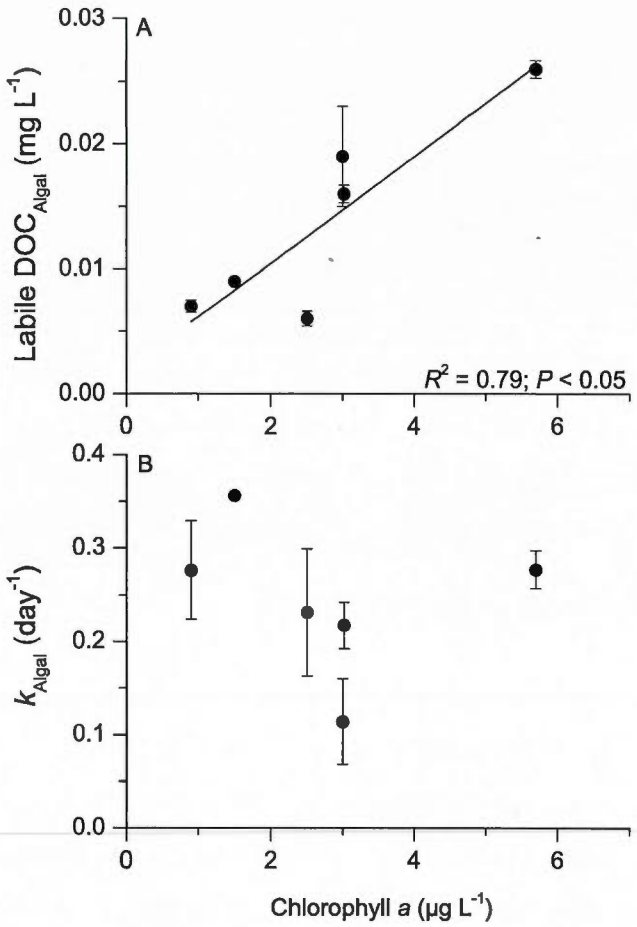

Figure 3.3 (a) The relationship between algal labile DOC and chlorophyll a concentrations (y = 0.002 + 0.004 x, R^2 = 0.79, n = 6, P < 0.05). (b) No relationship was found between the algal first-order degradation constant and chlorophyll a, nor with any of the other environmental variables tested. Data points derived from first-order decay models fitted to the different algal DOC degradation time courses and reported with ± SE.

(~10%; (Chrost et Faust, 1983)), suggesting that in lakes, the quality of algal DOC and its associated bioavailability may vary as a function of the dominant assemblages of phytoplankton. Because we sampled our lakes at different periods of the year, and over several years, we cannot discard the possibility of a temporal effect in explaining the lack of a systematic pattern of variation of the algal *k* constant along environmental gradients.

Interestingly, while algal DOC was degraded more rapidly (higher average *k* values; Figure 3.2c) and appeared to be proportionally more labile than terrestrial DOC in our incubations (Figure 3.2b), our results suggest that this algal DOC pool nevertheless has a significant refractory component. The exudation of refractory, high-molecular weight algal DOC (Chrost et Faust, 1983 ; Sundh, 1992), and the biological (Guillemette et del Giorgio, 2012 ; Romera-Castillo *et al.*, 2011) and photo-chemical (Tranvik et Bertilsson, 2001) conversion of labile algal DOC into biologically-inert compounds are probable mechanisms leading to the recalcitrance of algal C in freshwaters. Refractory algal DOC can then be transported downstream, buried in the sediments or utilized on longer time scales than those addressed here.

3.6.2 Degradation dynamics of terrestrial DOC

It has been often assumed that bacteria exhaust the algal DOC pool first before utilizing terrestrially derived material in lakes (Cole *et al.*, 2002 ; Jansson, Karlsson et Blomqvist, 2003). Recent modeling based on diverse scenarios of selective consumption of algal and terrestrial DOC suggest that this assumption is not realistic, as the amount of algal C present in lake water is generally insufficient to sustain the observed levels of bacterial C consumption (Kritzberg *et al.*, 2006 ; Kritzberg *et al.*, 2005). Our study provides the first experimental evidence to that latter contention: We observed that even at the initial stages of decomposition, the isotopic signature of respiratory CO_2 was composed of a mix of algal and terrestrial DOC (Figure 3.1), suggesting that at least a fraction of terrestrial DOC is highly reactive and also processed on short time scales. In fact, a rapid utilization of low molecular weight compounds of terrestrial origin in aquatic ecosystems has been recently reported (Berggren *et al.*, 2010a ; Covert et Moran, 2001), and because bulk DOC is dominated by terrestrial inputs in our lakes (Table 3.1), it is likely that similar terrestrial compounds may have readily fueled bacterial C consumption along with algal DOC in our incubations.

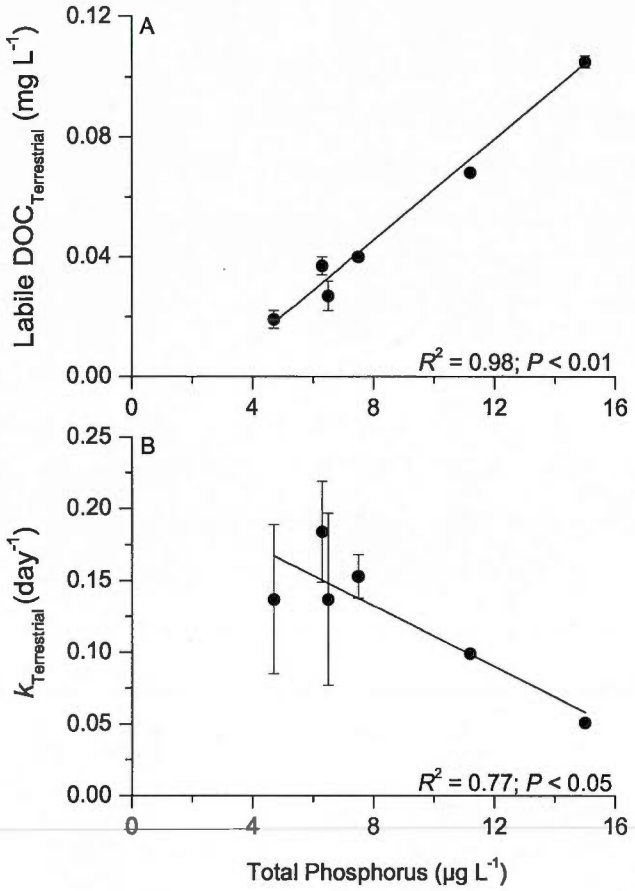

Figure 3.4 The relationship between (a) terrestrial labile DOC (y = -0.021 + 0.008 x, R^2 = 0.98, n = 6, P < 0.01), (b) terrestrial first-order degradation constant (y = 0.217 - 0.011 x, R^2 = 0.77, n = 6, P < 0.05), and total phosphorus concentrations. Data points derived from first-order decay models fitted to the different terrestrial DOC degradation time courses and reported with ± SE.

The shifts in isotopic signature of the resulting CO_2 towards the terrestrial end member suggest, however, that the labile algal pool is generally exhausted well before the labile terrestrial C pool is depleted (Figure 3.1). There was in fact a remarkable long-term convergence towards terrestrially dominated substrates in all incubations, thus pointing to a widespread terrestrial support of a residual or baseline metabolism in these lakes, independent of the composition of the initial labile pool. The concept of a baseline metabolism that is weakly connected to contemporary primary production and fueled by residual terrestrial DOC has been postulated before (del Giorgio et Williams, 2005 ; McCallister et del Giorgio, 2008), but to date poorly documented. Our results further suggest that this baseline metabolism may be more rapidly reached, in the order of days (Figure 3.1), whenever local primary production is interrupted, for example, during the ice-covered period of lakes at wintertime. Hence, the slow degradation of the major portion of the terrestrial DOC may potentially be a very important component of C cycling in lakes on an annual basis, sustaining processes such as lake net heterotrophy and buffering the overall ecosystem metabolism to environmental fluctuations.

3.6.3 Phosphorus modulation of algal and terrestrial DOC degradation dynamics

The degradation dynamics of the terrestrial C pool also varied substantially, suggesting that this pool is far from being homogenous from the point of view of its biological reactivity across lakes. While the degradation dynamics of terrestrial and algal DOC appeared to be independent from each other, since there was no relationship at all between algal and terrestrial k values ($R^2 = 0.10$, $P > 0.05$), our results suggest that they both may be influenced by phosphorus concentration in these lakes (Figure 4.5). On the one hand, phosphorus may indirectly increase the amount of labile algal DOC in lake water by stimulating primary production (Figure 4.3a). On the other hand, our results show that the k constant for terrestrial DOC tends to decline with TP, whereas the actual amount of labile terrestrial C increases with TP. This pattern suggests that phosphorus may directly influence the terrestrial DOC degradation dynamics by enhancing the amount of C that can be extracted from this pool on the short term, likely through an increase in bacterial growth and enzymatic activity (Schindler *et al.*, 1992 ; Wikner, Cuadros et Jansson, 1999 ; Zweifel, Norrman et Hagstrom, 1993). The fact that the k constant tends to decrease with TP would suggest that

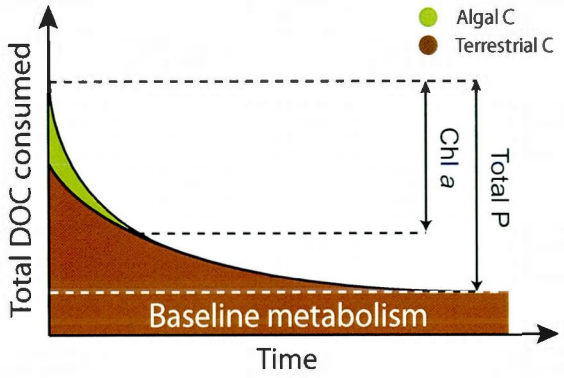

Figure 3.5 Conceptual scheme illustrating the degradation dynamics of algal and terrestrial DOC, and possible regulation pathways. According to results, a small amount of algal DOC is quickly degraded on short-time scales whereas a larger pool of terrestrial DOC is degraded on both short- and long-term following slower degradation kinetics. Phosphorus can indirectly increase the amount of labile algal DOC through a stimulation of phytoplankton productivity (Fig. 3.3a), or directly increase the amount of labile terrestrial DOC (Fig. 3.4a).

phosphorous is enhancing the use of a semilabile pool, but is not necessarily influencing the consumption of the highly labile terrestrial DOC pool. This in turn would imply that phosphorous may play a role in determining baseline metabolism in these ecosystems, by modulating the size of a terrestrial DOC pool that can be accessed by bacteria in the medium term. Thus, lakes that are rich in TP relative to DOC may have, for example, relatively higher winter and hypolimnetic metabolism (V. Ducharme-Riel et al., The contribution of winter under-ice and summer hypolimnetic CO_2 accumulation to the annual CO_2 budget of temperate boreal lakes in Québec, submitted to Ecosystems, 2012), not only because of the increase in the local production of algal DOC, but also because of a P-mediated increase of terrestrial DOC bioavailability. In addition, significant increase in nutrient delivery to lakes has been recently reported (Elser *et al.*, 2009 ; Hessen *et al.*, 2009), and our results suggest a scenario where proportionally more terrestrial DOC would be processed in freshwaters as a whole, with a resulting increase in overall aquatic CO_2 emissions to the atmosphere and a decrease in terrestrial C delivery to coastal waters and the ocean. Regardless, our results show that the degradation of the algal and terrestrial DOC pools do not appear to be solely governed by their respective chemical properties (Benner, 2003), but also by strong interactions with nutrients, with potential consequences for C cycling in freshwaters.

3.7 CONCLUSIONS

Our study presents one of the first empirical demonstrations of systematic differences in the dynamics of degradation of algal and terrestrial DOC in natural lake waters. Algal DOC tends to be proportionately more labile, and this labile C consumed more rapidly, than its terrestrial counterpart. In contrast to current assumptions that terrestrial C should be consumed once the more labile algal C is depleted, we show that the consumption of these pools is not sequential, but rather proceeds in parallel. We show that there is overall more labile terrestrial C across all lakes, and that this terrestrial C pool contributes significantly to the highly labile pool that fuels the short-term bacterial C consumption, and is also responsible for essentially the entire long-term residual (baseline) C metabolism. The degradation dynamics of terrestrial and algal DOC across lakes appeared to be independent from each other, and there was no relationship at all between algal and terrestrial k or lability. As a consequence, the resulting degradation dynamics of the bulk DOC is not simply a reflection of the relative proportions of each of these pools in the ambient waters but also of their respective patterns of degradation. In this regard, our results show that far from being uniform, the respective degradation dynamics of the algal and terrestrial DOC pools vary significantly across lakes, and that at least a portion of this variability may be related to interactions with nutrient and lake trophy. Thus, any alterations in the nutrient and allochthonous input regime to lakes due to watershed alterations may significantly alter the degradation dynamics of these pools and consequently of the bulk DOC, with possible consequences on the role of inland waters as global sinks or sources of organic C.

CHAPITRE IV

THE SELECTIVE CONSUMPTION AND DIFFERENTIAL METABOLIC ALLOCATION OF TERRESTRIAL AND ALGAL C DEFINE ALLOCHTHONY IN LAKE BACTERIOPLANKTON

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

4.1 RÉSUMÉ

Nous avons étudié les stratégies d'utilisation et d'allocation des ressources en carbone d'origine algale et terrestre par le bactérioplancton lacustre. En nous basant sur la signature isotopique en $\delta^{13}C$ de la biomasse et du CO_2 respiratoire bactérien, nous avons quantifié la consommation de carbone organique dissous (DOC) dérivé du phytoplancton ou du milieu terrestre et l'allocation subséquente de ces sources de DOC pour la croissance ou la respiration cellulaire. Les résultats confirment l'utilisation préférentielle du carbone organique d'origine algale par les communautés bactériennes en lac, mais démontrent que contrairement aux suppositions courantes, ce substrat autochtone est principalement dirigé vers les voies respiratoires et le DOC terrigène, utilisé pour la croissance cellulaire. Nos résultats démontrent pour la première fois un effet d'amorçage, où des apports en carbone biodisponible d'origine algale stimulent la consommation et l'incorporation dans la biomasse cellulaire d'un substrat terrigène plus résistant, conduisant ainsi à un important et constant niveau d'allochtonie (~87%) dans les communautés bactériennes de lacs qui pourtant diffèrent en productivité et en apports de matière terrigène.

MOTS CLÉS: Allochtonie, Bactérioplancton, Isotope du carbone, Lac, Carbone organique dissous.

4.2 ABSTRACT

Here we explore strategies of resource utilization and allocation of algal versus terrestrially derived C by lake bacterioplankton. We quantified the consumption of terrestrial and algal dissolved organic carbon, and the subsequent allocation of these pools to bacterial growth and respiration, based on the $\delta^{13}C$ isotopic signatures of bacterial biomass and respiratory CO_2. Our results confirm that bacterial communities preferentially remove algal C from the terrestrially dominated organic C pool of lakes, but contrary to current assumptions, selectively allocate this autochthonous substrate to respiration, whereas terrestrial C was preferentially allocated to biosynthesis. These results represent the first empirical evidence of a priming mechanism whereby inputs of labile, algal derived organic C may stimulate the consumption and incorporation of a more recalcitrant, terrestrial C pool, leading to a counterintuitive pattern of high (~87%) and constant levels of allochthony in bacterial biomass across lakes that otherwise differ in productivity and external inputs.

KEY WORDS: Allochthony, Bacterioplankton, Carbon isotope, Lake, Organic C

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4.3 INTRODUCTION

Natural bacterial communities inhabiting soils and waters are typically exposed to a complex mixture of dissolved organic carbon (DOC) compounds. The bulk DOC pool of freshwater, estuarine, and coastal ecosystems is particularly diverse and complex owing to its multiple aquatic (e.g., benthic and pelagic algae, macrophytes) and terrestrial (e.g., vascular plants, soils) origins (Aitkenhead-Peterson, McDowell et Neff, 2003 ; Bertilsson et Jones, 2003), and thus represents a major challenge in terms of resource utilization for aquatic bacterial communities. Past experimental work suggests that, faced with such a highly heterogeneous substrate choice, bacteria may develop different strategies of resource utilization. For example, culture studies have shown that bacteria may selectively consume specific substrates within simple mixtures (Mateles et Chian, 1969 ; Sundh, 1992). In addition, bacteria may differentially allocate specific substrates to growth or respiration depending on the chemical properties and accessibility of the consumed substrate (Russell, 2007), and on the energy and stoichiometric requirements of cells (Vallino, Hopkinson et Hobbie, 1996). Despite extensive culture evidence, however, we do not know the extent to which these strategies of bacterial resource utilization actually occur at the community level in natural aquatic ecosystems.

A widespread view in aquatic ecology and C biogeochemistry is that bacterial communities exposed to a mix of algal and terrestrial organic compounds should develop a strategy of resource utilization whereby algal C is preferentially consumed and incorporated into biomass over terrestrially-derived C, due to its greater accessibility and nutritional quality (Bianchi, 2011 ; Hobbie, 1988). However, the preferential consumption of algal C and its subsequent allocation to growth rather than to respiration remain to be empirically tested, as past studies have never simultaneously quantified the sources of DOC (i.e., algal and terrestrial) that support these different metabolic pathways. Further, the lack of concurrent measurements of the sources of C supporting bacterial biomass production and respiration has hampered the exploration of potential interactions between the utilization of the terrestrial and algal pools. For example, it was recently hypothesized that the utilization of labile, algal-derived C by bacteria may increase the overall utilization of terrestrial C by bacterial communities through a priming effect (Guenet *et al.*, 2010). However, we do not know if the

preferential consumption of algal C may also enhance its incorporation into bacterial biomass, thus increasing the overall importance of terrestrial subsidies (or allochthony) for aquatic bacterial communities.

Assessing these resource utilization strategies is thus important not only from a bacterial bioenergetics perspective, but also critical to our understanding of the functioning of aquatic ecosystems as a whole. There is an ongoing debate concerning the importance of terrestrial subsidies to aquatic food webs (Brett *et al.*, 2009 ; Cole *et al.*, 2011), partly originating from the fact that the pathways of delivery and transfer of these subsidies are still not well understood (Berggren *et al.*, 2010b ; Cole *et al.*, 2006). Because they repackage a portion of the terrestrial DOC entering aquatic ecosystems in the form of bacterial biomass (Kritzberg *et al.*, 2004), bacteria are likely to be central for the transfer of this subsidy to lake food webs (Berggren *et al.*, 2010b ; Tranvik, 1992). In addition, there is also direct evidence for the respiration of terrestrial C by bacteria (Karlsson, Jansson et Jonsson, 2007 ; McCallister et del Giorgio, 2008), and that this process may contribute to the widespread net heterotrophy (gross primary production: ecosystem respiration, P:R < 1) that characterizes the vast majority of inland waters (Duarte et Prairie, 2005). The lack of quantitative assessments of bacterial resource utilization strategies across different types of freshwaters does not allow an accurate assessment of the true level of allochthony both at the base of aquatic food webs, and in terms of whole ecosystem metabolism, because the metabolic fate of the terrestrial vs. algal C is still poorly understood.

In this study we have explicitly tested two key hypotheses related to the strategies of resource utilization of authochthonous (algal) and allochthonous (terrestrial) sources of organic carbon by lake bacterioplankton: 1) That freshwater bacterioplankton preferentially consume organic C of algal origin from within a complex ambient DOC pool, and 2) that this algal C is preferentially allocated to growth rather than to respiration. We directly and simultaneously measured the $\delta^{13}C$ isotopic signature of bacterial biomass and respiratory CO2 in lake water incubations, and used these to quantify the proportion of terrestrial DOC fueling bacterial biomass production and respiration. We further combined these latter estimates with the actual rates of bacterial respiration (BR) and biomass production (BBP) to 1) determine the actual flow of terrestrial and algal C into biomass and respiration, 2) reconstruct the proportion of terrestrial C consumed by bacteria, and 3) assess possible

interactions between these processes. The results presented herein suggest a strong selective consumption and allocation of specific DOC sources by bacteria, and a fundamentally different perspective of the overall importance of terrestrial subsidies to the functioning of these bacterial communities relative to past studies that have assessed bacterial allochthony on the basis of fragmented and disconnected metabolic measurements.

4.4 MATERIALS AND METHODS

4.4.1 Study sites and sample collection

We sampled eight lakes in the Eastern Townships region of Southern Québec, Canada (45.50°N, 73.58°W) during the summer period of 2004 and revisited four a month later. These lakes are located within the same drainage basin, which is dominated by temperate mixed wood forest, and cover a wide range in trophic status, DOC concentrations, and morphometry (Table 4.1). Lake water samples (60 L) were collected at a depth of 1.0 m using a diaphragm pump, stored in acid-washed polycarbonate bottles, and kept cool in the dark until return to the laboratory (<3 hours). Further pumping of lake water (200 L) through a 50-µm mesh size net allowed the collection of zooplankton samples. In the laboratory, zooplankton were placed at 4°C overnight to empty their gut contents. Over 100 individual cladocerans or copepods, used to estimate the $\delta^{13}C$ isotopic signature of the algal endmember (see below), were then collected in smooth-walled tin capsules, acidified with 10% HCl, and dried (45°C) before isotopic analysis. A portion of unprocessed water (8 L) was taken for the spectrophotometric determination of chlorophyll a, and the colorimetric analysis of total phosphorus and nitrogen (Cattaneo et Prairie, 1995). The remaining water sample was filtered through a combusted (525°C for 4h) AE glass fiber filter (1.0 µm; Millipore, Billerica, MA, USA) to remove bacterial grazers (>90%), and to conduct bacterial metabolism experiments (see below). The 1.0-µm filtered water was subsequently passed through a Gelman filter capsule (0.2 µm; Pall, Port Washington, NY, USA) to collect DOC samples (poisoned with 5N sulphuric acid), and to carry out Respiratory C Recovery System (ReCReS) experiments.

4.4.2 Bacterial metabolism

Short-term bacterial respiration incubations detailed in del Giorgio, Pace et Fischer (2006b) were carried out in parallel with the ReCReS experiments described below. Briefly, bacterial respiration was determined over 6 hours as change in O_2 in 1.0-µm filtered water incubations kept in the dark at room temperature (20°C). A membrane-inlet mass spectrometer was used to determine O_2 concentrations at several time points (Kana *et al.*, 1994), and rates of O_2 consumption were converted into C units using a respiratory quotient

Table 4.1

Limnological and metabolic conditions of the sampled Northern temperate lakes*

Lake	Sampling Date	Total P ($\mu g\ L^{-1}$)	DOC ($mg\ L^{-1}$)	Chl *a* ($\mu g\ L^{-1}$)	BR ($\mu g\ C\ L^{-1}\ d^{-1}$)	BBP ($\mu g\ C\ L^{-1}\ d^{-1}$)	BGE (%)	Water retention (years)	Lake area (km^2)
Brome	20-may-04	18.5	3.41	5.7	0.52	0.43	45.4	1.0	12.4
Memphremagog	06-june-04	11.3	3.47	3.6	1.68	0.52	23.6	1.7	102
Des Monts 1	13-june-04	10.2	5.74	4.2	1.64	0.69	29.6	<0.01	0.3
Simoneau	04-july-04	8.3	4.51	2.0	1.73	0.42	19.7	0.4	0.5
Stukely 1	20-july-04	5.7	4.15	1.9	2.05	0.29	12.3	4.0	4.0
Bran-de-scie 1	03-aug-04	17.9	6.14	6.5	1.24	1.13	47.6	<0.01	0.1
Fraser 1	10-aug-04	11.2	5.93	6.9	0.19	0.26	57.4	0.4	1.6
Bran-de-scie 2	31-augt-04	16.7	7.51	5.1	1.71	1.13	39.8	<0.01	0.1
Des Monts 2	07-sept-04	12.3	7.45	4.2	0.96	0.60	38.5	<0.01	0.3
Stukely 2	14-sept-04	6.5	5.03	2.5	0.84	0.18	18.0	4.0	4.0
Bowker	14-sept-04	5.8	2.76	2.0	0.58	0.13	18.1	9.0	2.5
Fraser 2	27-sept-04	11.2	6.93	1.5	0.59	0.14	19.2	0.4	1.6

*Abbreviations: DOC is dissolved organic carbon, Chl *a* is chlorophyll *a*, BR and BBP are bacterial respiration and biomass production, respectively, BGE is bacterial growth efficiency.

of 1. In parallel, bacterial biomass production was determined using the ^{3}H-leucine incorporation technique (Kirchman, 1993). Bacterial growth efficiency, defined as the share of the C consumed used for growth, was calculated as BBP/(BBP+BR), and total bacterial C consumption (BCC) as the sum of BBP and BR.

4.4.3 Bacterial respiratory CO_2 and biomass collection

The quantitative recovery of bacterial respiratory CO_2 and biomass was achieved with the ReCReS system (McCallister, Guillemette et del Giorgio, 2006). The system allows the collection of the CO_2 derived from bacterial respiration in freshwater samples while keeping background DIC values to a minimum (< 2%). An airtight incubation system (20L), in which a 0.2-μm filtered water sample is inoculated with lake bacteria for 4 days, is coupled to a harvest system to capture the respiratory CO_2 produced during these incubations. Following harvesting, the CO_2-containing traps were mounted to a vacuum extraction line to strip the respiratory CO_2 from residual moisture prior to transfer to break seals pending isotopic analysis. Potential methodological contaminations of the ReCReS system and fractionation artifacts are assumed to be marginal after prior testing (McCallister, Guillemette et del Giorgio, 2006).

At the end of the incubation, bacterial cells present in the 20 L water sample were concentrated using tangential flow ultra-filtration (1000 kDa membrane; Millipore), and the bacterial biomass was recovered on a 0.2 μm Anodisc 47 filter (Whatman, Springfield Mill, UK). All filters containing bacterial biomass were acid fumed overnight with HCl and dried at 45°C prior to transfer into tin capsules and isotopic analysis.

4.4.4 Isotopic analysis

Within 3 days of collection, the respiratory CO_2 breakseals were transferred into exetainers, and analyzed for $\delta^{13}C$ with a continuous flow GasBench peripheral (Thermo Finnigan, Bremen, Germany) interfaced to an Isotope Ratio Mass Spectrometer Delta XP (Thermo Finnigan) with an analytical precision of 0.10 ‰ (G.G. Hatch Lab, University of Ottawa, Canada). Stable carbon isotope ratios for bacterial biomass and zooplankton were measured using a FinniganMAT Delta^{Plus} dual-inlet continuous flow isotope ratio mass spectrometer (Thermo Finnigan) with on-line sample combustion. A few zooplankton samples were assessed for analytical precision and run in duplicate (relative standard

deviation <0.3‰). Finally, DOC concentration and $\delta^{13}C$ determination were performed on a modified 1010 TIC TOC analyzer (O.I. Analytical, College Station, TX, USA) connected to a Finnigan MAT DeltaPlus IRMS with a Conflo III continuous flow interface (Thermo Finnigan) as described in St-Jean (2003). Stable isotopes values are reported in standard δ notation as:

$$\delta^{13}C = [(R_{sample} / R_{standard}) - 1] \times 103 \quad (1)$$

where R is $^{13}C:^{12}C$.

4.4.5 Apportioning terrestrial and algal C contribution

We determined the relative contribution of terrigenous and algal sources to bulk DOC, bacterial respiratory CO2 and biomass based on a two-source mixing model described by the following equations:

$$\delta^{13}C \text{ C-Comp.} = f_1\delta^{13}C_{Terrestrial} + f_2\delta^{13}C_{Algal} \quad (2)$$

$$f_1 + f_2 = 1 \quad (3)$$

where $\delta^{13}C$ C-Comp. corresponds to the isotopic signature of a given C component, f_1 and and f_2 are the relative contributions of terrestrial ($C_{Terrestrial}$) and algal (C_{Algal}) sources to this component, respectively. The terrestrial end-member was set to the commonly accepted value of -27.0‰ typically found for terrestrial C3 plants (Boschker et Middelburg, 2002 ; Lajtha et Marshall, 1994). A DOC sample collected in a forested stream entering lake Fraser corroborated this assumption with a $\delta^{13}C$ of -27.2‰ ± 0.1. The algal $\delta^{13}C$ end-point was constrained for the same set of lakes using the zooplankton isotopic signature of which ever fraction had the most depleted $\delta^{13}C$ isotopic signature. This approach was shown to yield algal $\delta^{13}C$ estimates that compares well with other approaches (Karlsson, Jansson et Jonsson, 2007 ; Marty et Planas, 2008 ; McCallister et del Giorgio, 2008). In addition, recent literature suggests that zooplankton biomass is partly supported by terrestrial C (Cole *et al.*, 2011 ; Karlsson *et al.*, 2012), hence we further assume a 16% of terrestrial C contribution to zooplankton biomass based on the average terrestrial C content reported by Mohamed et Taylor (2009) for Canadian temperate lakes (N = 25) and in accordance with the low level of allochthony previously observed in several of the lakes sampled in this study (del Giorgio et France, 1996a) (see Appendice E for details).

4.4.6 Proportion of terrestrial DOC consumed by bacteria

We estimated the relative proportion of terrestrial DOC sustaining total bacterial carbon consumption by combining our estimates of terrestrial C in bacterial biomass and respiratory CO_2 with the short-term rates of bacterial metabolism measured in the parallel metabolic incubations. This approach has previously been successfully employed to estimate the rates of bacterial respiration supported by terrestrial DOC (McCallister et del Giorgio, 2008). Here, we used the following mass-balance equation to ascertain the proportion of terrestrial DOC consumed by bacteria:

$$\text{Consumed } DOC_{Terr} = BBP/BCC \times Biomass_{Terr} + BR/BCC \times \text{Resp. } CO_{2Terr} \quad (4)$$

where Consumed DOC_{Terr}, $Biomass_{Terr}$ and Resp. CO_{2Terr} correspond to the proportion of terrestrial DOC consumed, used for growth and respired by bacteria, respectively.

4.5 RESULTS

4.5.1 Bacterial consumption of algal and terrestrial DOC

We first determined the relative proportions of algal and terrestrial C in the bulk DOC pool of lakes, and compared these to the composition of the DOC consumed by aquatic bacteria. The $\delta^{13}C$ signature of bulk DOC averaged -27.7‰ ± 0.5 across lakes (Table 4.2), close to the isotopic signature of terrestrial sources (-27.0‰). Hence, the contribution of terrestrial C to the bulk DOC pool estimated by the mixing model was high in all lakes, averaging 87% ±10 (Fig. 4.1). Bacteria consumed terrestrial C in much lower proportions than that present in bulk DOC: our mass-balance calculation, which combines the proportion of terrestrial C found in bacterial biomass and respiratory CO_2 with the actual rates of bacterial production and respiration measured in parallel experiments, indicates that the consumed DOC pool was composed on average of 64% ± 10 of terrestrial C. It appears that bacteria selectively consumed and removed algal C from the bulk DOC pool of lakes (Fig. 4.1, Selection #1), and consequently, the composition of consumed DOC cannot be predicted from the composition of bulk DOC (Fig. 4.2a). Rather, we found that the fraction of algal DOC consumed increased with the total amount of DOC consumed (i.e., total bioavailable DOC; Figure 4.3a), which itself increased as a function of chlorophyll *a* (Chl *a*) concentrations (Figure 4.3b).

4.5.2 Bacterial allocation of algal and terrestrial DOC

Bacteria did not only differentially consume algal and terrestrial DOC, but also differentially allocated these sources to biomass production or respiration (Fig. 4.1, Selection #2). The isotopic signature of respiratory CO_2 was systematically depleted compared to that of DOC consumed, with $\delta^{13}C$ values ranging from -32.5 to -28.4‰ (Table 4.2). Thus, although terrestrial C dominated both the bulk DOC pool (87%) of lakes, and the pool of DOC consumed (64%), bacteria respired a lower proportion of terrestrial C, averaging 53% ± 18 across lakes (Fig. 4.1). In contrast, $\delta^{13}C$ values of biomass only minimally departed from the terrestrial end member (-28.6 to -27.1‰; Table 3.2), resulting in a greater terrestrial contribution to biomass (86% ± 6; Fig. 4.1). In addition, the contribution of terrestrial C to biomass was relatively constant across lakes, in spite of large variations in the composition of

Table 4.2

Estimated contribution of terrestrial C to bulk DOC, bacterial biomass and respiratory CO_2.

	Algal	Terrestrial	DOC		Biomass		Respiratory CO_2	
Lake	endmember $\delta^{13}C$ (‰)	endmember $\delta^{13}C$ (‰)	$\delta^{13}C$ (‰)	% Terrestrial	$\delta^{13}C$ (‰)	% Terrestrial	$\delta^{13}C$ (‰)	% Terrestrial
Brome	-30.0*	-27.0†	—	—	-27.4	87	-28.9	37
Memphremagog	-34.5	-27.0	—	—	-27.9	88	-29.1	72
Des Monts 1	-32.7	-27.0	-27.1	99‡	-27.6	90	-30.0	48
Simoneau	-32.0	-27.0	-27.5	80	-28.1	78	-29.2	57
Stukely 1	-31.3	-27.0	-27.5	89	-28.7	89	-28.9	56
Bran-de-scie 1	-35.3	-27.0	-27.8	91	-28.5	82	-32.2	38
Fraser 1	-33.0	-27.0	-27.0	100	-28.3	79	-31.9	18
Bran-de-scie 2	-35.3	-27.0	-27.8	91	-28.5	82	-32.5	34
Des Monts 2	-34.2	-27.0	-27.8	88	-27.1	99	-28.9	73
Stukely 2	-31.3	-27.0	-27.5	89	-28.6	93	-28.5	66
Bowker	-32.0	-27.0	-28.8	64	-28.0	81	-29.2	56
Fraser 2	-33.0	-27.0	-28.0	84	-27.9	85	-28.4	78

*The algal endmember isotopic values are based on zooplankton $\delta^{13}C$ signature (Karlsson, Jansson et Jonsson, 2007 ; McCallister et del Giorgio, 2008) and assuming a 16% terrestrial C content (*see* Methods for details).

†The terrestrial endmember was set to -27.0‰ based on ref. (Boschker et Middelburg, 2002 ; Lajtha et Marshall, 1994), and on the $\delta^{13}C$ (-27.1 ‰ ±0.1) of a small forested stream sampled in this study.

‡A two-source (algal and terrestrial) mixing model was resolved to calculate the proportion of terrestrial C in ambient DOC, bacterial respiratory CO_2 and biomass using their respective $\delta^{13}C$ signature

the C consumed (Fig. 4.2b). The respiration of terrestrial C was more variable across lakes on the other hand, and decreased dramatically with declining terrestrial DOC consumed (Fig. 4.2b).

4.5.3 Incorporation efficiency of terrestrial DOC

The consistently high levels of terrestrial C in bacterial biomass, relative to the variable proportions of terrestrial C consumed, implies that the incorporation efficiency of terrestrial C into biomass must vary as an inverse function of the proportion of terrestrial C consumed, which itself is partly a function of lake productivity (Fig. 4.3). To explore this possibility, we first calculated the actual rates of bacterial respiration and biomass production based on terrestrial DOC (BR_{Terr} and BBP_{Terr}, respectively), by combining the measured BR and BBP rates with the estimated proportion of terrestrial C fueling both pathways. We then calculated the growth (or incorporation) efficiency for this terrestrial C (BGE_{Terr}) into biomass as $BGE_{Terr} = BBP_{Terr} / (BBP_{Terr} + BR_{Terr})$. Across lakes, rates of bacterial respiration based on terrestrial DOC varied from 0.03 to 1.20 $\mu g\ C\ L^{-1}\ h^{-1}$ while rates of BBP_{Terr} ranged from 0.10 to 0.92 $\mu g\ C\ L^{-1}\ h^{-1}$ (Fig. 4.4a). The resulting incorporation efficiency of terrestrial C was on average 40% higher than the overall bacterial growth efficiency (BGE), with values ranging from 18% up to 80% (Fig. 4.4b). Further, we found that the incorporation efficiency of terrestrial C into biomass was strongly and positively related to Chl *a* concentrations, and peaked in the most eutrophic lakes (Fig. 4.4c).

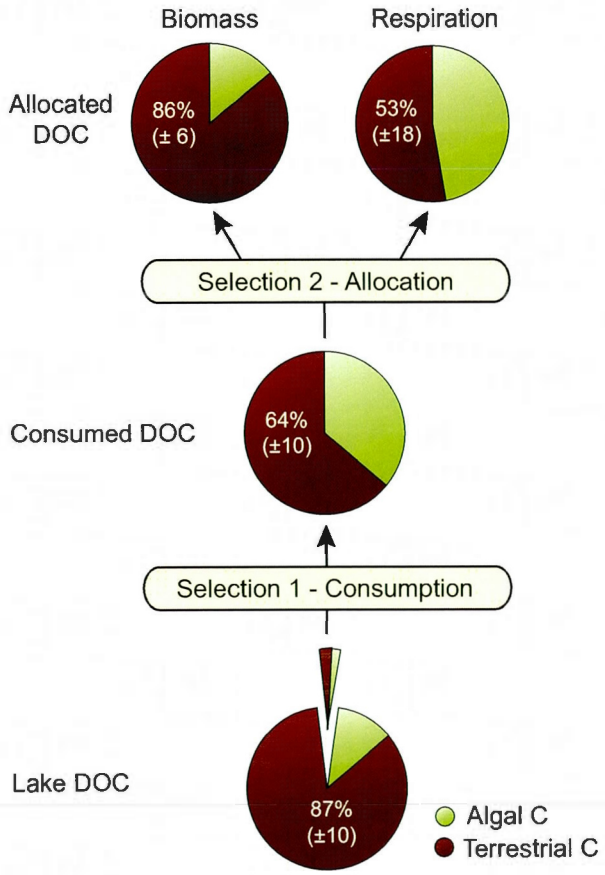


Figure 4.1 The proportion of algal (green) and terrestrial (brown) C after bacterial consumption of lake DOC and further allocation to respiration and biomass synthesis. The terrestrial contribution to each component is derived from a $\delta^{13}C$, two-source (algal and terrestrial) mixing model. The average proportion estimated in eight temperate lakes of southeastern Québec is shown along with SD in brackets.

4.6 DISCUSSION

Our findings demonstrate that the proportion of both terrestrial C consumed by bacteria, and of C allocated to growth and respiration are not simply a reflection of contribution of terrestrial C in the bulk DOC, but rather that lake bacterial communities select pools of specific origin during DOC utilization (Fig. 4.1). Had we assumed that the level of terrestrial C observed in bacterial biomass was indicative of the proportion of terrestrial C that was actually consumed, we would have largely overestimated the overall importance of terrestrial C to the functioning of the bacterial compartment in our lakes (87% terrestrial C in biomass versus 64% terrestrial C actually consumed). In addition, our results suggest a strong interaction between the preferential consumption and respiration of algal C, and the incorporation of terrestrial C in bacterial biomass. The description of these patterns of resource utilization was only possible because we simultaneously quantified the consumption, respiration and incorporation of different sources of DOC by aquatic bacterial communities. Our study relies, however, like other studies of its kind (Karlsson, Jansson et Jonsson, 2007 ; Kritzberg *et al.*, 2004 ; McCallister et del Giorgio, 2008) on different assumptions concerning the isotopic values of the algal and terrestrial end members, and the level of bacterial fractionation during biosynthesis and respiration. While we acknowledge that these potential biases may add uncertainty to the absolute values of allochthony, we argue that the patterns in resource utilization shown here are robust (see Appendce E-H).

The experiments that we carried out aimed at capturing the ambient patterns of bacterial metabolism and C consumption. The patterns observed in in vitro experiments tend to increasingly deviate from ambient conditions as incubations extend beyond the initial days (del Giorgio *et al.*, 2011), and we therefore limited the length of our incubations to the minimum time necessary to collect sufficient respiratory CO_2 and bacterial biomass for isotopic analysis. Incubations lasted between 3 and 5 days, including a systematic initial 2-day lag phase, so they were effectively equivalent to 1 to 3-day incubations, well within the range of previous metabolic experiments (del Giorgio *et al.*, 2011). The problem with this approach is that, while this length of incubation was probably effective in capturing the ambient rates of bacterial respiration and production, the accompanying changes in DOC

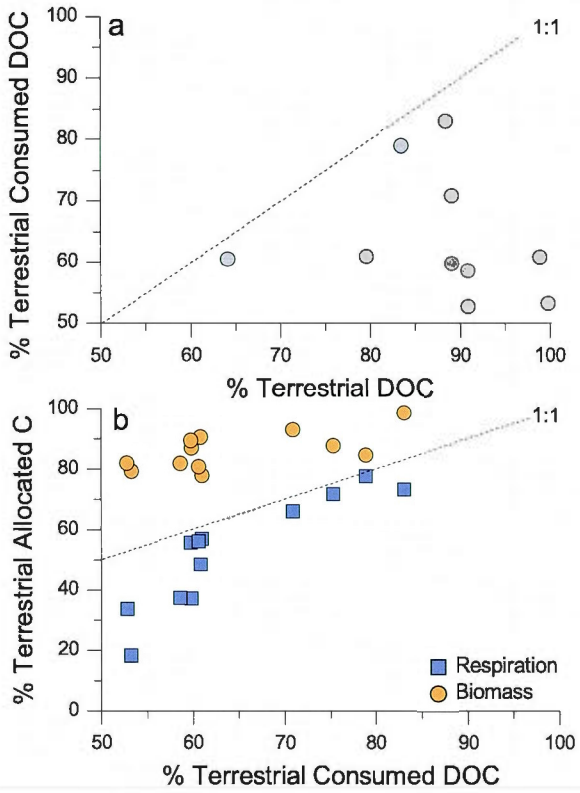


Figure 4.2 Terrestrial DOC consumption and allocation by lake bacterioplankton. (a) The proportion of terrestrial DOC consumed as a function of ambient DOC derived from terrigenous sources. (b) The relationships between the allocation of terrestrial C to bacterial respiration (blue) and biomass (orange), and the proportion of terrestrial DOC consumed ($y = -52.8 + 1.62x$; $R^2 = 0.79$, $N = 12$, $P < 0.001$ and $y = 60.4 + 0.40x$; $R^2 = 0.42$, $N = 12$, $P < 0.05$ for respiration and biomass, respectively). Dashed lines are the 1:1 lines.

(from 1 to 3% of the initial pool) were too small to be effectively resolved with current approaches, both in terms of concentration and more importantly, isotopic signature. This in turn meant that we had to back-calculate the signature and amount of DOC consumed on the basis of the measured rates and signatures of BP and BR, and that therefore, we could not actually calculate a complete isotopic mass balance that includes the consumption of DOC and its allocation to catabolic and anabolic pathways. This, however, is a limitation that is intrinsic to the type of natural sample we worked with. For example, the closest example to our work that exists in the literature is the study by Hullar *et al.* (1996), who explored the patterns of bacterial utilization of macrophyte derived DOC. These authors were indeed able to quantify both BP and BR, as well as the short-term changes in DOC, and were thus able to carry out a full mass balance, but the rates of C consumption in these highly enriched samples were orders of magnitude higher than what is commonly found in natural lakes, including our own samples. There is no simple solution to this limitation, because the alternative would have been to prolong our incubations for several weeks in order to be able to quantify the changes in DOC, but then the measurements of BP and BR would be impossible to interpret. We argue that our approach represents the best compromise possible in the circumstance, and that while it adds uncertainty to the estimates of the proportion of DOC consumed, it effectively captures the ambient pattern of bacterial C allocation, which is the core question that we address here.

4.6.1 Preferential consumption and respiration of algal DOC

The results of our mass-balance approach show that bacteria disproportionally removed algal DOC from a largely terrestrially dominated DOC pool, even in lakes of low primary productivity (Fig. 4.2a). This preferential consumption of algal DOC by lake bacterial communities has been previously postulated (Kritzberg *et al.*, 2004), but past inferences were based on the isotopic signal of bacterial biomass (Kritzberg *et al.*, 2004), or on modeling (Berggren *et al.*, 2010b), rather than the actual signature of the consumed DOC pool as we present here. Our study thus provides empirical support to the contention that algal DOC is more biologically available than its terrestrial counterpart, and therefore preferentially consumed by bacteria. This pattern of resource utilization is consistent with

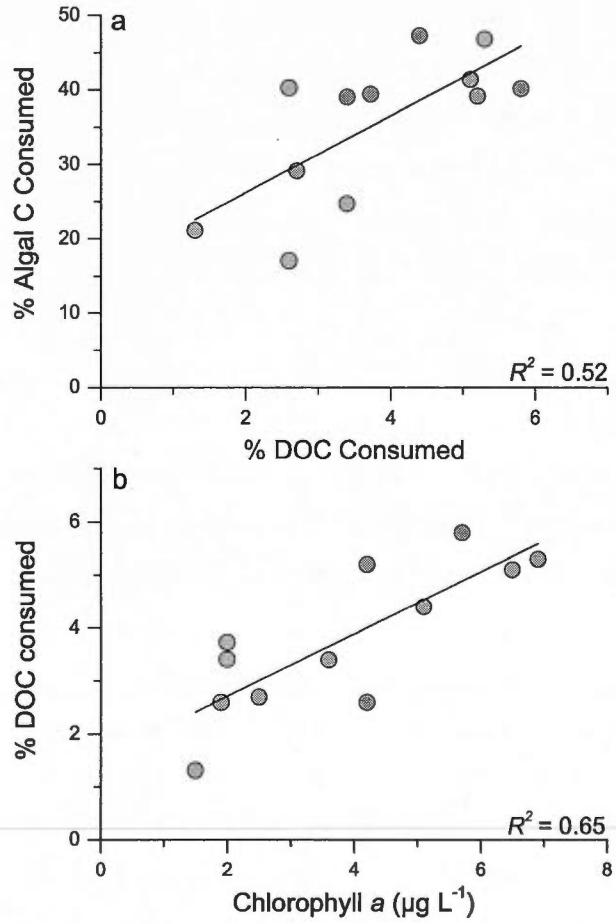


Figure 4.3 The relationships between (a) the proportion of algal C consumed and the fraction of bulk DOC consumed ($y = 16.2 + 5.1x$; $R^2 = 0.52$; $N = 12$, $P < 0.01$), and (b) the fraction of bulk DOC consumed over the incubation span and lake chlorophyll *a* concentrations ($y = 1.54 + 0.59x$; $R^2 = 0.65$; $N = 12$, $P < 0.01$).

previous work showing that simple compounds (e.g. amino acids, carbohydrates and organic acids) excreted in pure algal cultures are readily consumed by heterotrophic bacteria (Sundh, 1992), and suggests that aquatic bacteria may also be targeting these same molecules in the bulk DOC pool of lakes. Furthermore, because bacteria remove algal and terrestrial C of the bulk DOC pool in a manner that is not proportional to their absolute concentration (Fig. 4.2a), the fraction of terrestrial or algal DOC consumed by lake bacteria cannot be inferred or predicted from the bulk isotopic signature or composition of ambient DOC.

High DOC lability has been traditionally equated to nutritional quality (Thorp et Delong, 2002), and consequently to a greater potential to support growth. However, we observed that the preferentially-consumed algal pool was systematically diverted towards the respiratory pathway, and not to biomass production as previously suggested (Cole, Findlay et Pace, 1988 ; Kamjunke, Bohn et Grey, 2006). This selective respiration of algal C suggests that autochthonous DOC may be composed of molecules that are not only easily accessible but also characterized by a high energetic content (Sundh, 1992 ; Weiss et Simon, 1999). The preferential respiration of presumably energy-rich algal compounds may in fact reflect a strategy of bacteria growing under energy limitation where the cellular energy flux is maximized by allocating the high-energy substrates to respiration in order to maintain cellular functions such as membrane energetics, active transport systems, nutrient acquisition and enzymes production (del Giorgio et Cole, 1998 ; Russell, 1991).

4.6.2 Preferential incorporation of terrestrial DOC in bacterial biomass

Since the proportion of terrestrial DOC incorporated into biomass was much higher than that consumed (Fig. 4.1), we conclude that terrestrially-derived C was preferentially assimilated into bacterial biomass. It is likely that this preferential incorporation of terrestrial C may reflect the rapid consumption and efficient incorporation of low molecular weight compounds exported from soils and leaf litter, or produced via photochemical degradation (Berggren *et al.*, 2010a ; Berggren *et al.*, 2010b). For example, although these terrestrially-derived, low molecular weight organic compounds are present in low concentration in natural waters, they may account for up to 80% of total bacterial production in boreal streams and lakes of northern Sweden (Berggren *et al.*, 2010b). This latter result is in agreement with our

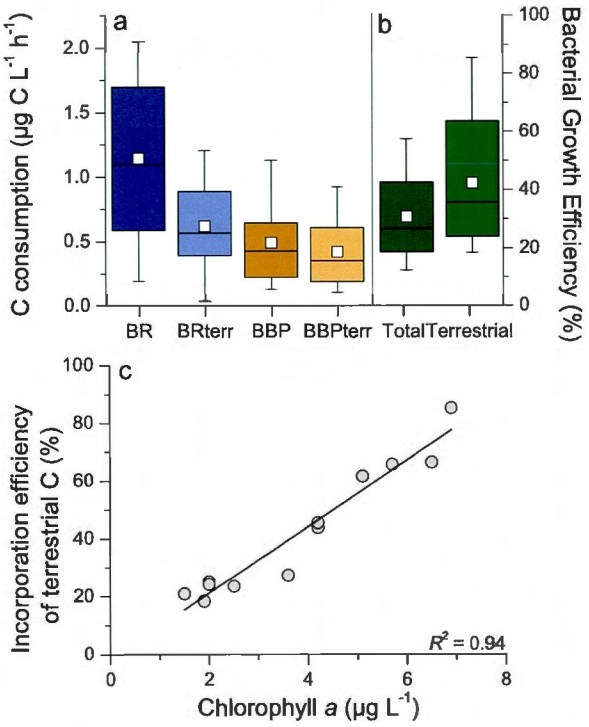


Figure 4.4 Incorporation efficiency of terrestrial C. Ranges in total and terrestrial (a) bacterial respiration and production, and (b) growth efficiency. Open squares denote mean values. (c) Incorporation efficiency of terrestrial C (BGE terrestrial) as a function of lake chlorophyll *a* concentrations ($y = -1.29 + 11.1x$; $R^2 = 0.94$; $N = 12$, $P < 0.001$).

own observations, i.e., terrestrial DOC accounted for 86% of total BBP (Fig. 4.4a). Regardless of the underlying mechanisms for this pattern, our observation is in stark contrast with the common assumption that terrestrially-derived C is of poor nutritional quality, and thus not particularly suited to support bacterial growth (Hobbie, 1988).

The constancy (87 ± 6%, Fig. 4.1) of the terrestrial signature in bacterial biomass across lakes is remarkable given the range in lake productivity (Table 4.1), and in the proportion of terrestrial C consumed along this gradient (53-83%). It appears that as lakes become more productive, bacteria consume increasing proportions of algal C (Fig. 4.3), yet the efficiency of incorporation of terrestrial C also increases along the same productivity gradient (Fig. 4.4c), such that the actual proportion of terrestrial C in bacterial biomass remains relatively constant across lakes. This interplay between the consumption and allocation of specific C sources to different metabolic pathways represents the first empirical evidence for a potential priming effect of the utilization of algal-derived C on the incorporation of terrestrial OC into bacterial biomass (Farjalla *et al.*, 2009). It has recently been proposed that an increase in the excretion of labile compounds of algal origin may stimulate the synthesis of bacterial enzymes, which may in turn enhance the degradation and subsequent utilization of recalcitrant terrestrial DOC by bacterial communities (Guenet *et al.*, 2010).

The enhancement of the degradation of the terrestrial DOC pool by algal DOC inputs may also favor the release of nutrients, such as phosphorus, which often limits bacterial growth in temperate lakes (Guillemette et del Giorgio, 2012 ; Smith et Prairie, 2004). Interestingly, we found that total phosphorus (TP) was strongly positively correlated to terrestrial DOC concentrations in our lakes (Pairwise correlation; $R = 0.69$; $N = 12$; $P < 0.05$), suggesting a common origin i.e., terrestrial sources. Since bacteria cleave organic molecules in part to access P (Jansson, Olsson et Pettersson, 1988), and that about half of the cleavage activity was recently shown to occur inside the cell (Luo *et al.*, 2009), we suggest the possibility of a co-incorporation of both phosphorus and terrestrial DOC into biomass. This co-incorporation would lead to an increased biomass production based on terrestrial C indirectly facilitated by the energy derived from the respiration of algal C (priming). It thus appears that rather than being consumed and processed independently, the consumption and differential allocation of the allochthonous or autochthonous DOC pool may influence the

fate of each other and ultimately lead to a high and relatively constant level of allochthony in the bacterial biomass compartment across a large range of lake types as we measured here (Fig. 4.1).

4.6.3 Implications for food webs and ecosystem functioning

The ecological consequence of the increase in terrestrial C incorporation efficiency into biomass with increasing system productivity is that even in eutrophic systems, where bacterial C consumption is more influenced by algal-derived C, the isotopic signature of bacterial biomass may still be overwhelmingly terrestrial. Given the large losses that occur during the transfer of C in food webs, it may be this systematically high terrestrial signature of bacterial biomass what may actually explain the widespread presence of terrestrial C measured in zooplankton even in more productive or nutrient enriched systems (Cole *et al.*, 2002). The relative importance of the bacterial pathway vs. the direct incorporation of terrestrial particles in sustaining zooplankton allochthony is still under debate (Berggren *et al.*, 2010b ; Brett *et al.*, 2009 ; Cole *et al.*, 2006), and whereas we do not assess here the ultimate fate of the bacterially-incorporated terrestrial DOC, our results do reinforce the notion that bacteria may play a key role in mediating the transfer of terrestrial subsidies to lake food webs (Berggren *et al.*, 2010b).

The respiration of much of the available algal C has significant implications not only for the incorporation of terrestrial DOC by lake bacterioplankton, but also for the overall C cycling in lakes. At the ecosystem level, bacterial respiration tends to be strongly correlated to chlorophyll and TP, and more weakly to DOC (Pace et Prairie, 2005). This is intriguing given the fact that DOC represents a major substrate for bacteria, and that most lakes appear to be net heterotrophic (Duarte et Prairie, 2005), thus strongly influenced by the metabolism of allochthonous organic C. Our results may provide some explanation to this apparent contradiction. The preferential consumption and respiration of algal C by bacteria that we describe here is consistent with the tight coupling between bacterial respiration and algal biomass (i.e., chlorophyll a concentration) that has often been observed, but because the respiration of algal C may also increase the overall mineralization of terrestrial DOC (Bianchi, 2011 ; Guenet *et al.*, 2010), significant amount of CO_2 may still be produced, supporting a negative metabolic balance in lakes (P:R < 1). In fact, the interplay between the processing of the algal and terrestrial C pools may lead to the extreme case scenario where

net heterotrophy is sustained even in more productive or nutrient enriched systems (Cole *et al.*, 2002 ; Cole *et al.*, 2000).

Human- and climate-driven alterations of catchments may result in significant shifts in both the amount and nature of the material delivered to aquatic systems (Tranvik *et al.*, 2009). For example, significant increases in DOC concentrations due to greater terrestrial C export have been observed in several temperate and boreal catchments (Roulet et Moore, 2006). In addition, increases in lake productivity, and thus in algal DOC production, are also expected due to higher nutrient concentrations resulting from atmospheric deposition and soil export (Elser *et al.*, 2009). The degree to which these changes in the delivery of resource subsidies will alter food web and gas dynamics in freshwater ecosystems remains uncertain (Tranvik *et al.*, 2009), but our results suggest that the impacts of these shifts on the allochthony of lake bacterial communities cannot be predicted simply on the basis of the absolute amounts of terrestrial C or nutrients delivered to any given system. Instead, allochthony in lake bacteria appears to be a complex interplay between resource availability and origin.

4.7 CONCLUSION

In conclusion, the results presented in this study, while reinforcing the notion that lake bacterial communities may be strongly subsidized by terrestrial DOC inputs, show that once consumed, terrestrial and algal DOC have very different metabolic fates. While algal DOC appears to be the preferred substrate, it is the terrestrial fraction that is primarily in bacterial biosynthesis. We have shown that the autochthonous DOC may in fact serve as a primer for the incorporation of terrestrial DOC into biomass, leading to the counterintuitive scenario wherein bacterial reliance on terrestrial DOC for growth may in fact increase as aquatic systems become more productive. This proposed pattern needs to be further tested, but provides a potential and coherent mechanism to explain the widespread presence of a significant terrestrial contribution to lake bacterial communities a nd higher trophic levels across a wide range of lake types. Our findings also suggest that allochthony in bacterial communities cannot be inferred from either the properties of the bulk DOC pool, or from the isotopic signature of the respired or incorporated C, because of the differential resource utilization strategies adopted by these communities. We further hypothesize that this may not just be a feature of freshwater bacterial communities, but rather a more generalized pattern within aquatic food webs, which should be considered in future studies assessing the influence of resource subsidies on organisms and ecosystem dynamics.

CONCLUSION GÉNÉRALE

L'objectif principal de cette thèse était de dresser un portrait intégratif de la consommation bactérienne en carbone organique dissous, et de la réponse métabolique subséquente, en considérant les interactions potentielles entre les pools de DOC consommés, les voies métaboliques impliquées et l'environnement. En particulier, le développement d'une nouvelle approche conceptuelle combinée à l'emploi de la modélisation et de techniques en fluorescence a permis de décrire les patrons de divers aspects de la consommation bactérienne en C c.-à-d. consommation à court terme, long terme et globale dans différent écosystèmes d'eau douce et d'en explorer les relations avec la composition du pool de DOC et l'environnement. Ces mêmes techniques en fluorescence ont par ailleurs été combinées à diverses mesures du métabolisme bactérien afin de non seulement étudier la dynamique de la consommation de certaines composantes du pool de DOC, mais aussi leurs productions, et d'en relier la dynamique à certains aspects du métabolisme. Finalement, l'utilisation d'un nouvel appareil expérimental i.e. le système ReCReS, ainsi que l'emploi de traceurs isotopiques a permis d'explorer la dynamique de consommation bactérienne des différentes sources de DOC présentes en milieu aquatique, et d'en établir la contribution relative à la consommation totale en C, à la croissance et à la respiration cellulaire.

Collectivement, les résultats de cette thèse suggèrent que la dynamique de consommation en carbone et la réponse métabolique du compartiment bactérien qui s'en suit sont régies par une forte sélectivité pour certains pools de DOC présents dans le milieu aquatique (chapitres I et III), par des interactions entre les voies métaboliques empruntées par ces pools de carbone (II et IV) et avec l'environnement (chapitres II et III). Les résultats indiquent que non seulement il existe un très faible lien entre la dynamique de consommation à court, long terme et globale, mais que ces aspects de la dynamique de consommation sont reliés à des composantes spécifiques du pool de DOC et répondent différemment à des changements dans l'environnement (chapitres I et III). Les résultats suggèrent que la consommation à court terme est davantage reliée à la présence d'un pool de carbone caractérisé par la présence de molécules potentiellement protéiniques d'origine algale, alors que la consommation à long terme est plutôt supportée par un pool de C d'origine terrigène

(chapitres I et III). Les résultats du chapitre III indiquent cependant qu'une portion du pool de C terrigène est aussi consommée à court terme, en accord direct avec les résultats du chapitre IV montrant une forte contribution de la matière terrigène au pool total de C consommé également mesuré sur une courte échelle temporelle. Les résultats démontrent par ailleurs qu'en plus d'être différemment consommées, les divers pools de DOC sont sélectivement allouées aux différentes voies métaboliques i.e. respiration, croissance et excrétion (chapitres II et IV), le carbone d'origine algale étant par exemple principalement respiré et la matière terrigène incorporée dans la biomasse bactérienne.

Une des hypothèses sous-jacente à ce travail et commune à plusieurs études récentes (Berggren, Laudon et Jansson, 2007 ; Fellman *et al.*, 2008) est que la dynamique de consommation en carbone et la réponse métabolique qui s'en suit sont directement reliées à la composition et à l'origine du pool de DOC, plutôt qu'à sa concentration. Les résultats de cette thèse supportent partiellement cette hypothèse. D'un côté, les résultats du chapitre I suggèrent que la biodisponibilité du DOC puisse être prédite par la présence de certains pools de nature protéinique, un résultat partagé par d'autres études conduites en rivières et en sols (Fellman *et al.*, 2009c ; Fellman *et al.*, 2008). Cependant, la production de ces mêmes pools par le compartiment bactérien questionne cette interprétation de simple descripteur de la composition du pool de DOC pouvant prédire la consommation en carbone. De plus, nos résultats suggèrent que la dynamique globale de consommation ne peut être prédite ou reconstruite simplement en considérant la proportion des sources de C présentes dans le pool de DOC puisque ces sources présentent elles-mêmes des degrés de réactivité variables et changeant au long de gradients environnementaux (chapitre III). Finalement, le haut degré de sélectivité observé tant au niveau de la consommation que de l'allocation métabolique des ressources consommées implique qu'il soit difficile ou presque impossible de prédire la voie métabolique empruntée par chacun des pools ou des sources de DOC consommés simplement à partir simplement de leur concentration ou contribution au pool de DOC initial.

Un des résultats inattendus de cette étude est le rôle que semble jouer les nutriments, en particulier le phosphore, comme modulateur tant au niveau de la dynamique de consommation de certains pools de DOC (chapitre III) que dans la réponse métabolique et la transformation de ces pools (chapitre II). Par exemple, il a été démontré que le phosphore peut directement accroître l'accessibilité à certains pools de DOC (ex. le pool de DOC

terrigène; chapitre III) et l'excrétion de certains types de composés carbonés (ex. les matières humiques; chapitre II) en modifiant potentiellement l'activité métabolique des communautés bactériennes (Wikner, Cuadros et Jansson, 1999 ; Zweifel, Norrman et Hagstrom, 1993). Il n'est pas non plus impossible que la réponse de divers aspects de la dynamique de consommation de certains pools de C et de leur métabolisation face à des changements dans la productivité du milieu (chlorophylle *a*). Par exemple, le taux de décomposition globale du DOC donné par la constante *k*, la taille du pool de DOC algale ou encore l'efficacité d'incorporation du DOC terrigène, résultent d'un effet indirect du phosphore via une stimulation de la production primaire algale. Collectivement, les résultats de cette thèse impliquent que la dynamique de consommation en C et le métabolisme bactérien ne sont pas uniquement régis par les qualités propres au pool de DOC et de ses différentes composantes, mais plutôt moduler par des interactions avec les nutriments. Par conséquent, des changements significatifs dans l'apport de nutriments causés par exemple par une pression anthropique accrue sur les écosystèmes aquatiques ont le potentiel d'entraîner des variations importantes autant dans la consommation de certains pools de DOC que dans le devenir de ces pools en modulant soit la quantité de C consommée et transformée en biomasse bactérienne, en CO_2 ou en une forme différente de DOC.

Les résultats de cette thèse ont des implications pour le fonctionnement des écosystèmes aquatiques, tant au niveau écologique que biogéochimique. La rapide consommation du DOC d'origine algale (chapitres I et III) et sa contribution importante à la respiration suggèrent que le métabolisme des communautés bactériennes puisse réagir rapidement et de manière conséquente aux variations de DOC autochthones (Pace et Prairie, 2005) et impliquent qu'une partie importante de la production de CO_2 par le compartiment bactérien soit soutenue par le DOC d'origine algale et non pas uniquement supportée par le DOC terrigène comme il a été abondamment suggéré par le passé (Bianchi, 2011). En retour, la consommation et l'allocation préférentielle du DOC algal à la respiration (chapitres I, III, IV) conjuguées à la consommation rapide d'une fraction du pool de DOC terrigène (chapitres III et IV) ont probablement favorisé l'allochtonie élevée et constante observée dans la biomasse bactérienne au chapitre IV et illustrent le large potentiel du compartiment bactérien à transférer le DOC d'origine terrestre à la chaîne trophique traditionnelle, possiblement via la boucle microbienne (Berggren *et al.*, 2010b). La dégradation lente d'une partie importante

du DOC terrigène et la forte relation entre la consommation à long terme et les apports allochtones aux chapitres I et III suggèrent que le pool de DOC terrestre supporte un métabolisme résiduel ou de base dans les écosystèmes aquatiques. Cette dernière observation implique que le DOC terrigène puisse tamponner le métabolisme face à des changements rapides en ressources ou en période de rareté des ressources par exemple durant la période hivernale, où la production primaire est essentiellement inhibée par le couvert de glace. Cette possible stabilisation des écosystèmes aquatiques par le DOC terrigène est cependant très peu connue et constitue une avenue de recherche importante.

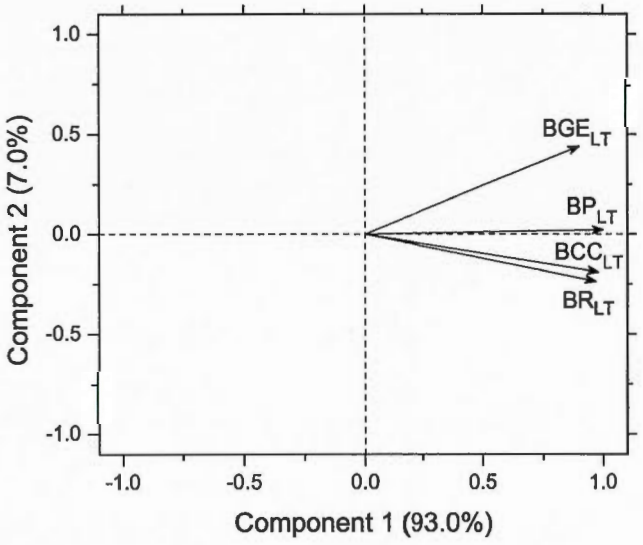
La production de certains pools de DOC comporte aussi des implications pour le fonctionnement des milieux aquatiques. En « colorant » une certaine fraction du pool de DOC, le métabolisme bactérien peut faciliter les processus de photodégradation, augmentant ainsi la minéralisation du DOC en CO_2. La conversion d'un pool de C labile en un pool réfractaire rend possible la séquestration du carbone organique dans les sédiments aquatiques (von Wachenfeldt, Bastviken et Tranvik, 2009), en plus de modifier la biodisponibilité globale du pool de DOC. La production de matière carbonée a de plus des implications pour la façon dont nous mesurons et estimons le flux de carbone dans le compartiment bactérien. Par exemple, les outils couramment utilisés pour mesurer les taux de respiration et de production bactérienne ne tiennent pas compte de l'excrétion de composés carbonés, sous-estimant ainsi la quantité totale de carbone consommée. De la même façon, les mesures d'efficacité de croissance bactérienne peuvent aussi avoir été sous-estimées par le passé (Kawasaki et Benner, 2006). Ces résultats ont une incidence directe sur les résultats du chapitre IV, où la contribution relative des sources de DOC algale et terrestre au pool de DOC consommé a été estimée à partir des taux de respiration et de production bactérienne et de la proportion des sources algales et terrestres soutenant ces processus. Les résultats de ce chapitre montrent une forte sélectivité métabolique tant au niveau de la production de biomasse que de la respiration pour une source de C donné et il n'est pas impossible que la voie excrétoire subisse une pression sélective semblable. Il est par conséquent possible que les estimations courantes de la dépendance du compartiment bactérien, et aussi des autres compartiments du réseau trophique, aux sources de C algales et terrestre soient biasées.

En conclusion, les résultats de cette thèse suggèrent que la dynamique de consommation bactérienne de DOC et la transformation de ce pool de C par le métabolisme,

que ce soit sous forme de CO_2, de nouvelle biomasse ou en une forme différente de DOC, résultent de la présence de différents pools de DOC et leurs interactions ainsi que de l'interaction avec les nutriments. L'importance des processus décrits précédemment aux estimations de flux de carbone et au fonctionnement des écosystèmes dépend directement de la transposition des mesures métaboliques effectuées en laboratoire à l'échelle de l'écosystème aquatique. Cette quantification dépasse le cadre de cette thèse qui s'est voulue plutôt mécanistique et surprenamment très peu d'études se sont adonnées à cet exercice. Cependant, cette étude suggère une contribution importante du compartiment bactérien autant à la perte de DOC transitant du milieu terrestre au milieu côtier, qu'à la transformation du pool DOC sous une forme récalcitrante menant à la séquestration du C à long terme dans les sédiments lacustres, ainsi qu'au transfert du DOC d'origine terrestre aux organismes supérieurs du réseau trophique. Ainsi, la quantification et la transposition à l'échelle de l'écosystème des patrons de consommation et d'allocation métabolique décrits dans cette étude constituent une suite logique à cette thèse et permettraient de mieux saisir l'importance du compartiment bactérien aux cycles biogéochimique et au soutient des réseaux trophiques aquatiques.

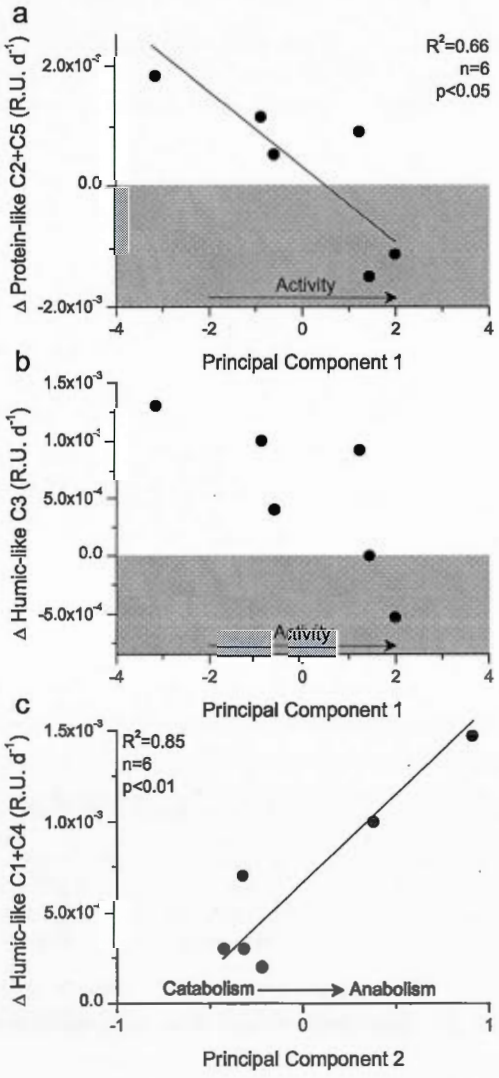
APPENDICE A

Correlations of the bacterial metabolic parameters estimated in 6 regrowth experiments over 8 days with the first two axes of a principal component analysis. The percent of explained variation is shown in brackets.



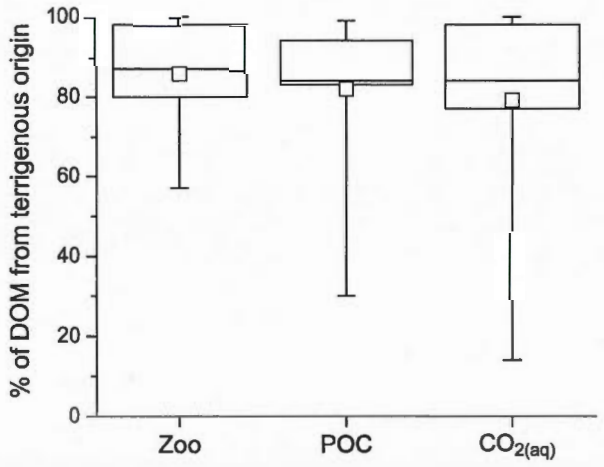
APPENDICE B

The relationships between the rates of change in protein-like components C2 and C5 (a), humic-like component C3 (b), and in humic-like components C1 and C4 (c), and the two first principal components of a PCA performed on the long-term bacterial metabolic dataset. Linear regression lines and parameter estimates were derived from ranged major axis regression models. The relationship between the rate of change in humic-like C3 and the principal component 1 was only marginally significant (p=0.07). Shaded areas denote a net disappearance of FDOM.



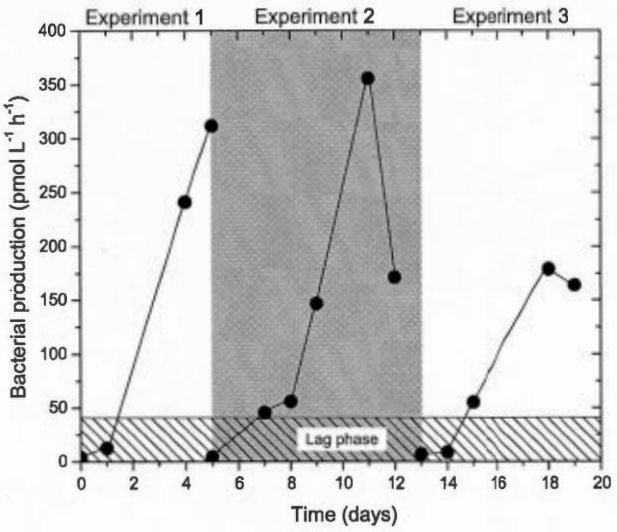
APPENDICE C

Box and wisker plot showing the comparison of the percent contribution of terrigenous organic carbon to bulk DOM estimated from mixing models that use the zooplankton, POC or $CO_{2(aq)}$ $\delta^{13}C$ as proxies for the algal-end member isotopic signature. $CO_{2(aq)}$ $\delta^{13}C$ values were derived from the DIC $\delta^{13}C$ signatures according to Mook et al. (1974) and Stumm and Morgan (1995). An algal fractionation of 14‰ was applied for the $CO_{2(aq)}$ based-estimates according to McCallister & del Giorgio (2008). The terrestrial end-member $\delta^{13}C$ signature was set to -27‰ (Boschker and Middleburg 2002) for the different models. Mean contribution values were not statistically different according to an analysis of variance (ANOVA; F_{26}=0.23, p>0.05) and are denoted as open squares. Whiskers denote the minimum and maximum values in the proportion of DOM originating from terrigenous sources.



APPENDICE D

Time course of bacterial production during the three consecutive regrowth incubations carried out using the same water sample from lake Bran-de-Scie in 2006. The lag phase was arbitrarily defined as ~10% of maximum bacterial production.



APPENDICE E

Assessment of potential errors

The recovery of bacterial biomass and respiratory CO_2 for isotopic analysis and the further comparison of the measured isotopic signatures to that of potential dissolved organic carbon sources (i.e., terrestrial vs. algal) provided valuable information about the different strategies of resource utilization adopted by bacterial communities inhabiting temperate lakes. Although we observed clear patterns in bacterial consumption and allocation of different DOC sources (Fig. 1), there are shortcomings associated to our approach that may limit our ability to accurately estimate the proportions of algal and terrestrial C present in the bulk and consumed DOC pool, and in bacterial biomass and respiratory CO_2. A first potential problem is related to the isotopic fractionation occurring during the synthesis of different metabolic products (e.g., lipids, amino acids, respiratory CO_2), which may confound comparisons of the DOC sources supporting bacterial biomass production or respiration. We could, for example, incorrectly infer a differential support of bacterial biomass production and respiration even if both cellular products originate from a similar substrate, only on the basis of this fractionation artifact. The second problem results from uncertainties in the values attributed to the source end members themselves, particularly the algal end member, in the two-source mixing models. These problems are not inherent to our study, but rather represent a common challenge shared by other studies using stable isotopes to track resource utilization by organisms. Nevertheless, our study still needs to be placed in the context of these potential uncertainties.

The first potential problem associated to our approach is related to the carbon isotopic discrimination, or fractionation, that occurs during cellular substrate uptake and processing. An effective correction of our data is difficult to perform, however, as the issue of isotopic fractionation is complex and has not been extensively assessed for natural bacterial assemblages, and because the current evidence and results are often conflicting and difficult to apply to natural settings. Isotopic fractionation has been shown to vary for individual organic compounds (Abraham, Hesse et Pelz, 1998 ; Créach, Bertru et Mariotti, 1997 ; Macko et Estep, 1984), and even for the most studied compound (i.e., glucose), a large

range of fractionation values (-1.9‰ to +0.3‰) has been reported (Abelson et Hoering, 1961 ; Blair *et al.*, 1985 ; Monson et Hayes, 1982). There is also indication that metabolic products originating from biosynthesis or respiration may differ in their isotopic signatures to the source material (Boschker et Middelburg, 2002 ; Hayes, 2001). For example, depletion of the isotopic signature of lipids and fatty acids has been well documented (Abraham, Hesse et Pelz, 1998 ; DeNiro et Epstein, 1977 ; Teece *et al.*, 1999), whereas little depletion or enrichment (~1‰), or no fractionation at all has been shown for deoxyribonucleic acid (DNA) or for bulk cell carbon (Blair *et al.*, 1985 ; Coffin *et al.*, 1990 ; Créach, Bertru et Mariotti, 1997 ; Pelz *et al.*, 1998). On the other hand, the isotopic fractionation of respiratory CO_2 generated during catabolic activities has been far less studied. In some aquatic bacterial communities, respiratory CO_2 was shown to be slightly depleted (-1.6) compared to glucose (McCallister, Guillemette et del Giorgio, 2006) or slightly enriched (+0.5‰) in comparison to macrophyte leachates (Hullar *et al.*, 1996). Extensive work on soil microbial communities has shown variable isotopic fractionation in respiratory CO_2, ranging from a small depletion (~1-2‰) in some studies (Aggarwal *et al.*, 1997 ; Bengtson et Bengtsson, 2007) to no fractionation or small enrichment (~1-2‰) in others (Boström, Comstedt et Ekblad, 2007 ; Ekblad et Högberg, 2000), and also variable fractionation (±1-3‰) depending on the stage of decomposition (Fernandez, Mahieu et Cadisch, 2003 ; Schweizer, Fear et Cadisch, 1999). Finally, variation in the isotopic fractionations may be specific to the strain or species incubated on similar simple substrates (Blair *et al.*, 1985 ; Coffin *et al.*, 1989 ; Macko et Estep, 1984), although Créach et al. (Créach, Bertru et Mariotti, 1997) did not observe variation between three different species growing on salt marsh grass leachates.

In aquatic environments, bacterial communities comprised of several hundreds or even thousands of species typically grow on a complex mixture of organic compounds. This complexity makes it difficult to apply a single fractionation correction obtained in the laboratory on the basis of results obtained with single strains of bacteria grown on individual compounds. In fact, some authors have concluded that there is an overall isotopic fidelity between bacterial community biomass and the organic pool on which they grow because species- or substrate-specific fractionations would tend to cancel each other (Coffin *et al.*, 1989 ; Coffin *et al.*, 1990 ; Macko et Estep, 1984). In addition, the apparent isotopic fractionation reported in some studies (Coffin *et al.*, 1990 ; Créach, Bertru et Mariotti, 1997 ;

Norrman *et al.*, 1995) may actually reflect selective uptake and utilization of certain compounds within a complex pool rather than true physiological fractionation. For example, it was found that estuarine bacteria growing on the halophyte *Spartina alterniflora* were enriched by 2.1‰ compared to the whole plant because they preferentially selected the more labile cellulose and hemi-cellulose components (Coffin *et al.*, 1990), which are enriched in $\delta^{13}C$ compared to the whole plant (Benner *et al.*, 1987). In this context, we argue that the difference we observed between the isotopic signature of bacterial biomass and respiratory CO_2 and the bulk DOC pool likely reflects a similar selective consumption and allocation of compounds of different origins rather than a simple physiological isotopic fractionation artifact.

We cannot, however, rule out completely the possibility of an isotopic fractionation superimposed on the effect of selective removal so we performed two independent sensitivity analyses to assess the robustness of the patterns in bacterial resource utilization that we present in this paper. We first applied a -1‰ or +1‰ isotopic fractionation to respiratory CO_2 which caused the current estimated terrestrial content to shift from 53% to 71%, and from 53% to 34% on average, respectively (Fig. S2). Applying the same fractionation to biomass resulted in variations of the terrestrial proportion in biomass ranging from 86% to 104%, and from 86% to 68% (Fig. S3). This exercise shows that it would take a >2‰ fractionation during both biosynthesis and respiration before any selectivity patterns would be blurred, which is highly unlikely given the fact that we track the isotopic signature of whole bacterial communities growing in a complex substrate as stated above. We have thus decided not to correct our data for a potential true physiological fractionation because such correction may add as much uncertainty to our allochthony estimates as not applying any correction at all.

The second potential source of error is related to our estimations of the isotopic signature of the terrestrial and algal end members. The isotopic signature of terrestrial organic carbon is fairly constrained to a $\delta^{13}C$ value of -27.0‰ (Boschker et Middelburg, 2002 ; Lajtha et Marshall, 1994), and because our own observation from a forested stream corroborated this estimate (-27.2‰ ± 0.1), we consider the error associated to the terrestrial end member to be negligible. Accurately determining the $\delta^{13}C$ signature of autochthonous producers is more difficult, however, especially because phytoplankton represents a small fraction of POC (del Giorgio et France, 1996b), and its isolation from detritus is hard to

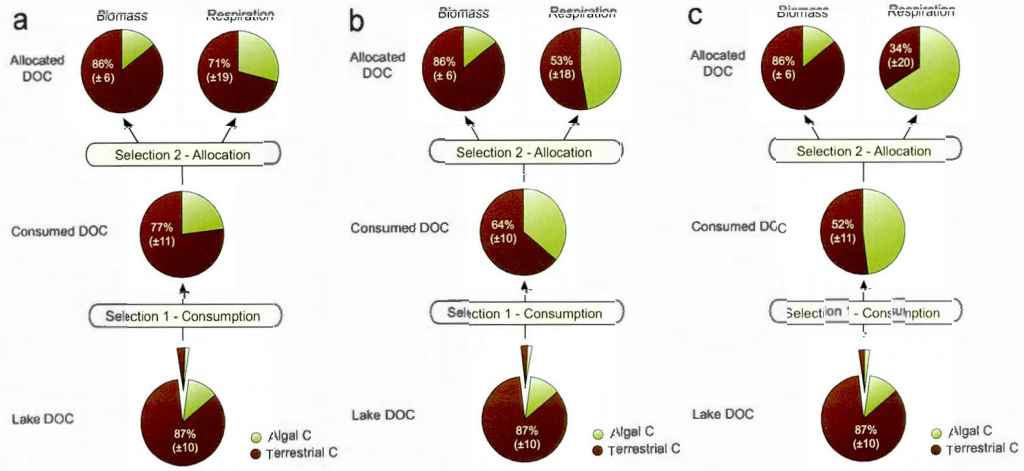
achieve. Here, we chose to use the isotopic signature of zooplankton as a proxy for the algal end member as this approach yielded realistic estimates of algal $\delta^{13}C$ in three other lake studies (Karlsson, Jansson et Jonsson, 2007 ; Marty et Planas, 2008 ; McCallister et del Giorgio, 2008). In a companion paper (McCallister et del Giorgio, 2008), the validity of these zooplankton estimates was tested against other algal proxies for the same set of temperate lakes such as the lipids extracted from particulate organic carbon, and the application of an algal fractionation to the $\delta^{13}C$ of aqueous CO_2. These different proxies yielding comparable $\delta^{13}C$ estimates, it was concluded that the use of zooplankton as a proxy of the algal end member isotopic signature is therefore appropriate.

While some studies proposed that zooplankton do not rely on terrestrial subsidies (Brett *et al.*, 2009 ; Cole *et al.*, 2002), several recent studies have shown a significant, yet variable (~10-75%) contribution of terrestrial C to zooplankton biomass (Berggren, Lapierre et del Giorgio, 2011 ; Carpenter *et al.*, 2005 ; Cole *et al.*, 2011 ; Karlsson *et al.*, 2012). Here, we use an approach similar to that of Karlsson et al. (Karlsson, Jansson et Jonsson, 2007) to derive the algal end member isotopic signature by assuming a certain fraction of terrestrial C in zooplankton biomass. We assumed a 16% contribution of terrestrial C, which corresponds to the average allochthony found for zooplankton in other Canadian temperate lakes (Mohamed et Taylor, 2009), and which agrees well with the low level of allochthony observed in our lakes (del Giorgio et France, 1996b). This approach yielded algal $\delta^{13}C$ values (-30.2 to -35.9‰) well within the range found in the literature (Cole *et al.*, 2011 ; Marty et Planas, 2008). As our estimates of bacterial allochthony depend directly on this later assumption, however, and that various levels of terrestrial C content in zooplankton biomass has been reported, we tested the robustness of our estimates of allochthony in bacteria under two different scenarios of zooplankton allochthony. We first assumed 0% terrestrial C in zooplankton, and in a second scenario we tested our patterns against a level of zooplankton allochthony of 32% (i.e., twice the amount used in this study). Assuming 0% or 32% terrestrial C in zooplankton decrease or increase the estimated terrestrial C content in bulk DOC, consumed DOC, bacterial biomass and respiration by ± 2%, ± 7%, ± 3% and ± 9%, respectively. Thus, while we acknowledge that small deviations from our zooplankton based estimates are possible and could add error to our absolute values of bacterial allochthony, we

argue that at the light of our sensitivity analysis, these should have only limited impact on the overall patterns of bacterial resource utilization we describe.

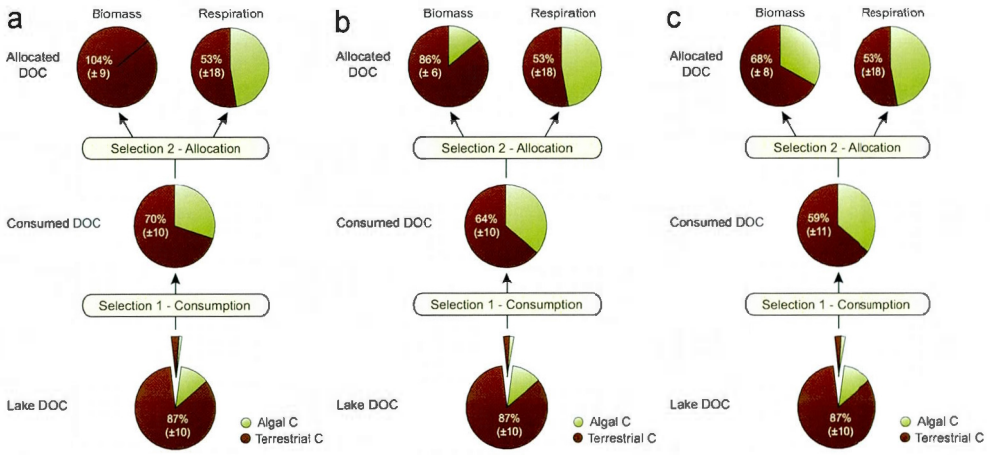
APPENDICE F

Comparison of the estimated proportion of algal (green) and terrestrial (brown) C in the bulk and consumed DOC pool, and in bacterial biomass and respiratory CO_2 assuming an isotopic fractionation following bacterial respiration of (a) -1‰, (b) 0‰ as used in this study, and (c) +1‰.



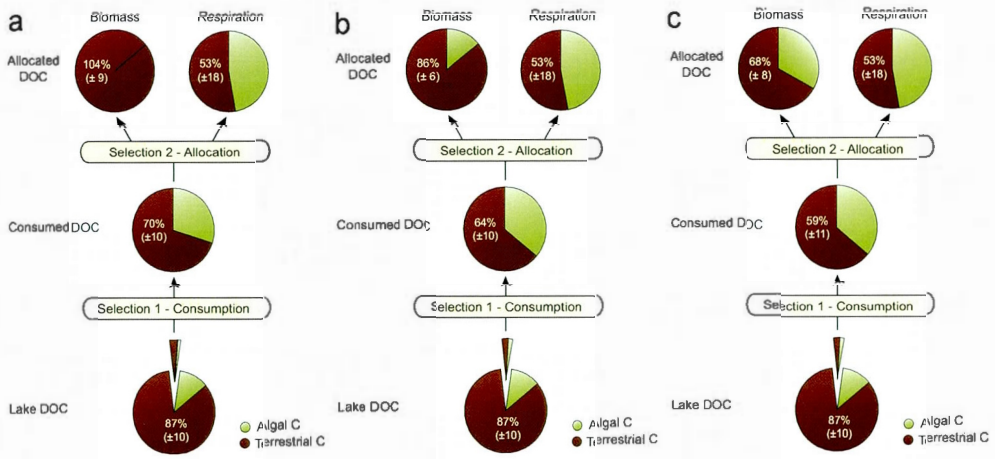
APPENDICE G

Comparison of the estimated proportion of algal (green) and terrestrial (brown) C in the bulk and consumed DOC pool, and in bacterial biomass and respiratory CO_2 assuming an isotopic fractionation following bacterial production of (a) -1‰, (b) 0‰ as used in this study, and (c) +1‰.



APPENDICE H

Comparison of the proportion of algal (green) and terrestrial (brown) C in the bulk and consumed DOC pool, and in bacterial biomass and respiratory CO_2 estimated with mixing models using the $\delta^{13}C$ isotopic signature of zooplankton as the algal end member, and assuming a terrestrial C zooplankton biomass content of (a) 0%, (b) 16% as used in this study, and (c) 32%.



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