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Les transformations microbiennes de l'azote dans les grandes rivières

par
Laure TALL

Département de Sciences biologiques
Faculté des Arts et Sciences

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Cette thèse intitulée:

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Présentée par :
Laure TALL

a été évaluée par un jury composé des personnes suivantes :

Marc Amyot, président-rapporteur
Roxane Maranger, directrice de recherche
Yves Prairie, membre du jury
Andrew Laursen, examinateur externe
Michel A. Bouchard, Représentant du doyen de la FES

Résumé

Les rivières reçoivent de l'azote de leurs bassins versants et elles constituent les derniers sites de transformations des nutriments avant leur livraison aux zones côtières. Les transformations de l'azote inorganique dissous en azote gazeux sont très variables et peuvent avoir un impact à la fois sur l'eutrophisation des côtes et les émissions de gaz à effet de serre à l'échelle globale.

Avec l'augmentation de la charge en azote d'origine anthropique vers les écosystèmes aquatiques, les modèles d'émissions de gaz à effet de serre prédisent une augmentation des émissions d'oxyde nitreux (N_2O) dans les rivières. Les mesures directes de N_2O dans le Lac Saint-Pierre (LSP), un élargissement du Fleuve Saint-Laurent (SLR) indiquent que bien qu'étant une source nette de N_2O vers l'atmosphère, les flux de N_2O dans LSP sont faibles comparés à ceux des autres grandes rivières et fleuves du monde. Les émissions varient saisonnièrement et inter-annuellement à cause des changements hydrologiques. Les ratios d'émissions N_2O: N_2 sont également influencés par l'hydrologie et de faibles ratios sont observés dans des conditions de débit d'eau plus élevée et de charge en N élevé. Dans une analyse effectuée sur plusieurs grandes rivières, la charge hydraulique des systèmes semble moduler la relation entre les flux de N_2O annuels et les concentrations de nitrate dans les rivières.

Dans SLR, des tapis de cyanobactéries colonisant les zones à faible concentration de nitrate sont une source nette d'azote grâce à leur capacité de fixer l'azote atmosphérique (N_2). Étant donné que la fixation a lieu pendant le jour alors que les concentrations d'oxygène dans la colonne d'eau sont sursaturées, nous supposons que la fixation de l'azote est effectuée dans des micro-zones d'anoxie et/ou possiblement par des diazotrophes hétérotrophes. La fixation de N dans les tapis explique le remplacement de près de 33 % de la perte de N par dénitrification dans tout l'écosystème au cours de la période d'étude.

Dans la portion du fleuve Hudson soumis à la marée, la dénitrification et la production de N_2 est très variable selon le type de végétation. La dénitrification est associée à la dynamique en oxygène dissous particulière à chaque espèce durant la marée descendante. La production de N_2 est extrêmement élevée dans les zones occupées par les plantes envahissantes à feuilles flottantes (*Trapa natans*) mais elle est négligeable dans la végétation indigène submergée. Une estimation de la production de N_2 dans les lits de *Trapa* durant l'été, suggère que ces lits représentent une zone très active d'élimination de l'azote. En effet, les grands lits de *Trapa* ne représentent que 2,7% de la superficie totale de la portion de fleuve étudiée, mais ils éliminent entre 70 et 100% de l'azote total retenu dans cette section pendant les mois d'été et contribuent à près de 25% de l'élimination annuelle d'azote.

Mots-clés : Assimilation par les plantes, bilan massique, dénitrification, fleuve, hétérogeneité spatiale et temporelle, limitation en azote, ratio N_2O: N_2, service écosystémique, temps de résidence de l'eau, plante invasive, chataigne d'eau.

Abstract

Rivers receive nitrogen (N) from their watershed and are the final sites of nutrient processing before delivery to coastal waters. Transformations of dissolved inorganic N (DIN) to gaseous N are highly variable and can impact both coastal eutrophication and greenhouse gas emissions.

With anthropogenic N loading to aquatic ecosystems on the rise, nitrous oxide (N_2O) emission from rivers should increase. Direct measurements of N_2O from lake St. Pierre (LSP), an enlargement of the St. Lawrence River (SLR) indicate that although LSP is a net atmospheric source of N_2O to the atmosphere fluxes are low compared to others rivers. Emissions are seasonally and inter-annually highly variable due to changes in hydrological conditions. N_2O: N_2 is also influenced by hydrology and lower ratios are observed in conditions of higher water discharge and elevated N charge into the ecosystem. In a cross system analysis, hydraulic load mitigates the relation between annual N_2O flux and nitrate concentrations in rivers.

In SLR, cyanobacterial mats colonizing low nitrate areas are a net source of N with high negative di-nitrogen (N_2) fluxes. Given that fixation occurred during daylight and that oxygen concentrations in the water column were supersaturated, we hypothesize that N_2 fixation is performed by the dominant cyanobacteria in anoxic micro-zone of the mat and/ or possibly by heterotrophic diazotrophs. Our estimates indicate that N fixation in the mats account for the replacement of up to 33% of the N loss via denitrification in the entire ecosystem during the study period.

In the tidal Hudson River N_2 production is highly variable between vegetated shallows and was associated with species-driven differences in dissolved oxygen (DO) dynamics during the ebb tide. N_2 production was extremely high in invasive floating-leaved plants (*Trapa natans*) but was insignificant in submersed native vegetation. An estimate of summertime N_2 production in *Trapa* beds suggests that these beds are a major seasonal hotspot for N removal. Large *Trapa* beds represent only 2.7% of the total area of the tidal

Hudson but they remove between 70 and 100% of the total N retained in this section of the river during summer months and contribute to as much as 25% of the annual N removal.

Keywords : Denitrification, ecosystem services, large river, mass balance, N_2O: N_2 ratio, nitrogen limitation, plant uptake, spatial and temporal heterogeneity, water residence time, invasive plant, water chesnut.

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A : Superficie de l'écosystème

ANAMMOX: Oxydation anaérobie de l'ammonium

ANCOVA: Analyse de covariance

ANOVA: Analyse de variance

Ar: Argon

BNF: Fixation biologique d'azote

C : Carbone

CO_2: Dioxyde de carbone

D : Débit de l'eau

Df: Degré de liberté

DIN: Azote inorganique dissous

DNRA: Réduction dissimilatoire des nitrates en ammonium

DO: Oxygène dissous

DOC: Carbone organique dissous

DON: Azote organique dissous

HIP : Modèle conceptuel du « Hole In the Pipe »

HL : Charge hydraulique

***k* :** Constante d'échange gazeux à l'interface air-eau

L : Charge

LSL: Lac Saint-Louis

LSP: Lac Saint-Pierre

mT : Tonne métrique

N: Azote

N_2: Di-azote

N_2O: Oxyde nitreux

NH_3: Ammoniaque

NH_4^+: Ammonium

NO: Oxyde nitrique

NO_2^-: Nitrite

NO_3^-: Nitrate

NO_x: ions oxidés de l'azote (nitrate + nitrite)

Nr: Azote réactif

O_2: Oxygène

P: Phosphore

***p*N_2O** : Pression partielle d'oxyde nitreux dans l'air

PON: Azote organique particulaire

Q : Concentration en N

R : Rétention

***R^2*:** Coefficient de détermination

SLR: Fleuve Saint-Laurent

t : Temps en jour

T: Tonne métrique

TDN: Azote total dissous

Temp: Tempéraure

TFH: Portion non salée et soumise à la marée du Fleuve Hudson

TN: Azote total

WRT : Temps de résidence de l'eau

z : Profondeur

À mes parents

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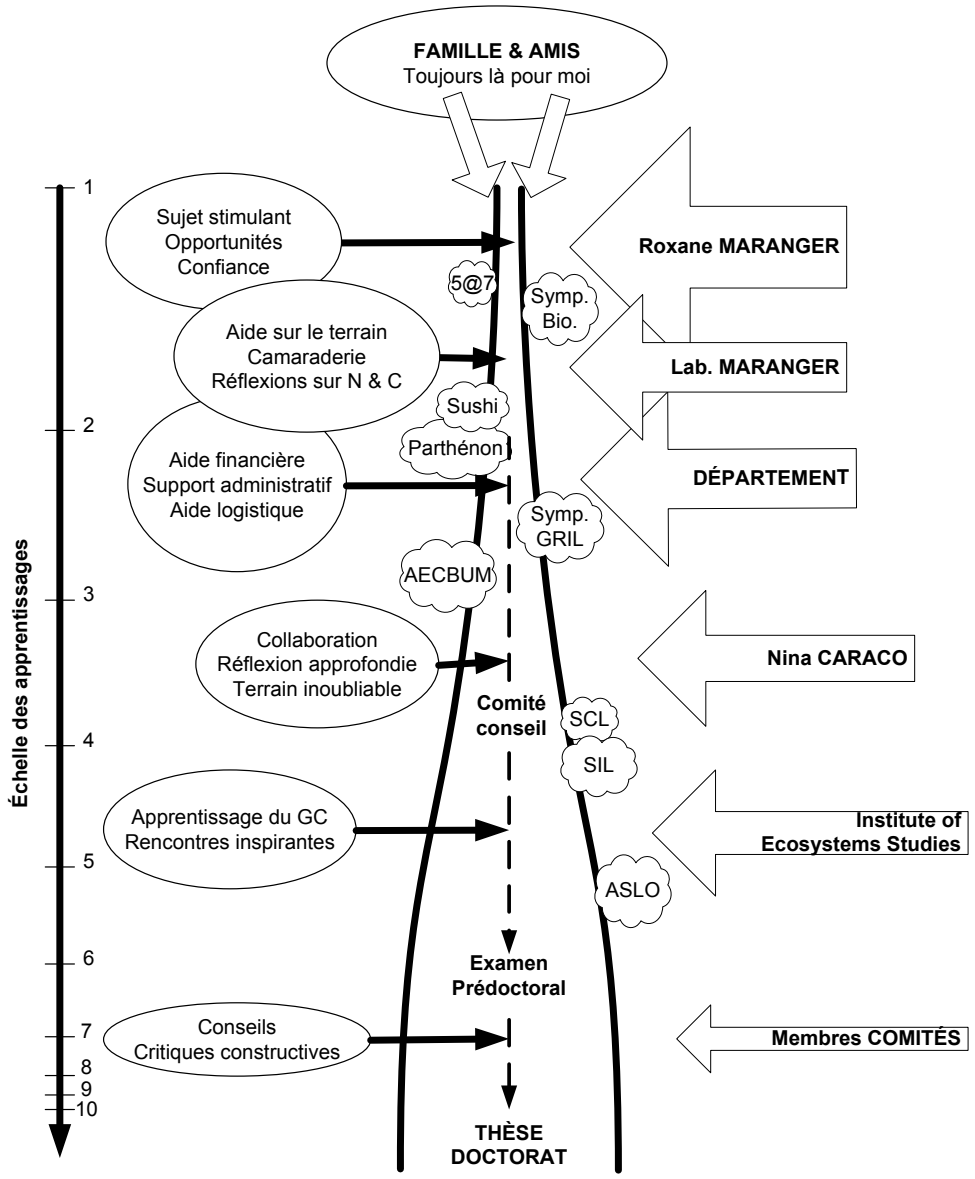
Modèle conceptuel du « Student Continuum » inspiré du « River Continuun » illustrant les apports essentiels de nombreuses personnes dans la réalisation de cette thèse. L'augmentation des apports stimule l'apprentissage et favorise le passage des étapes-clé qui permettent d'atteindre la thèse de doctorat.



1. Introduction Générale

1.1. Problématique de l'azote

L'azote, un élément complexe et essentiel

L'azote (N) est un élément essentiel à la vie des organismes. Pour cent atomes de carbone incorporés dans une cellule, il faut de deux à vingt atomes de N (Sterner et Elser 2002). C'est un des nutriments limitant, avec le phosphore, la production primaire dans plusieurs systèmes terrestres et aquatiques (Vitousek et Howarth 1991). Contrairement au phosphore, N est très actif dans le cycle d'oxydation-réduction, avec sept états d'oxydation possibles. Comme l'eau sur Terre, la majorité de N est sous une forme non utilisable par la plupart des organismes. En définitive, le cycle de N est probablement le cycle le plus complexe de tous les éléments essentiels, avec ses nombreux processus de transport et de stockage dans l'environnement.

Cependant le cycle de N et ses excès sont aussi décrits comme :

- *'le cycle le plus perturbé par l'activité humaine'* (Howarth et Gene 2009)
- *'la troisième menace la plus importante pour la planète'* (Giles 2005)

L'activité humaine a modifié le cycle de N plus que celui de n'importe quel autre élément majeur sur Terre dans tous les écosystèmes (air, eau, terre) et à des échelles multiples (locale, régionale et globale).

Un peu d'histoire ou pourquoi il ne faut pas abuser des bonnes choses

Avant la révolution industrielle, la grande majorité de l'azote réactif (Nr) sur la planète, a été créée par des bactéries fixatrices de N. Ce taux de création était équilibré au cours des temps géologiques par la dénitrification, qui transforme le Nr en N non réactif (N_2) (Howarth et Gene 2009). En 1913, l'invention du procédé Harber-Bosch qui transforme l'azote atmosphérique (N_2) en ammoniaque (NH_3) a permis pour la première fois dans l'histoire, un approvisionnement illimité en Nr utilisable pour l'agriculture et « accessoirement » pour la fabrication d'explosifs. La découverte du procédé Harber-Bosch a coïncidé avec une augmentation rapide de la population et aujourd'hui près de la moitié de la nourriture consommée est produite grâce à des

engrais azotés issus du procédé Harber-Bosch. L'augmentation des cultures de légumineuses comme le soja, capables de fixer le N_2, et la production indésirable de Nr lors de la combustion d'énergies fossiles s'ajoutent aussi au cycle de N (Smil 2001).

Au début du 21ème siècle, la production de nourriture et d'énergie a provoqué l'augmentation du taux de création de Nr anthropique par un facteur de plus de dix par rapport à la fin du 19ème siècle (Galloway et al. 2004). L'ampleur de cette production de Nr soulève des questions essentielles quant aux conséquences et au devenir de ce nouvel ajout de Nr dans l'environnement. La reconnaissance de l'ampleur et des conséquences de l'intervention humaine dans le cycle de N n'est pas récente. Il y plus de 30 ans, Delwiche (1970) déclarait que l'homme produisait la même quantité de Nr que les processus naturels, et que le destin de ce Nr était incertain. Depuis, la création de Nr anthropique a doublé par rapport au Nr produit naturellement et non seulement les incertitudes demeurent mais elles sont devenues d'autant plus cruciales à résoudre (Howarth et al. 1996, Vitousek et al. 1997).

Les conséquences de l'excès de N

Les systèmes aquatiques sont particulièrement sensibles à l'augmentation de la disponibilité et de la mobilité de N dans l'environnement (Vitousek et al. 1997, Carpenter et al. 1998, Galloway et al. 2002). Une quantité croissante de Nr d'origine anthropique entre dans les écosystèmes aquatiques par des sources telles que l'élevage, l'agriculture, les eaux de ruissellement urbain et agricole, les déchets industriels et les effluents d'eaux usées (Welsh et al. 2000, Wetzel 2001, Rabalais 2002). En conséquence, les concentrations de N dans les eaux souterraines et de surface sont en augmentation dans le monde, provoquant des problèmes médicaux, environnementaux et la dégradation de la qualité de l'eau (Rabalais 2002, Camargo et al. 2005). L'utilisation des fertilisants en milieu agricole se révèle plutôt inefficace; en effet, seulement la moitié des fertilisants utilisés sur les terres agricoles restent dans les cultures (intégrée dans la biomasse) ou dans les sols. Une petite partie est émise sous forme de gaz dans l'atmosphère et de 20 à 30% du N épandu initialement est perdu dans les eaux souterraines ou de surface par lessivage (Basso et Ritchie 2005, Dumont et al. 2005) selon la cascade de l'azote ou 'Nitrogen Cascade' décrite par Galloway et al (2003). La charge en N dans les cours d'eau (**Figure 1.1**) a ainsi augmenté de 2 à 20 fois selon les écosystèmes, depuis la période

préindustrielle (Howarth et al. 1996). Dans les systèmes aquatiques, cette augmentation des flux et des concentrations de N a pour conséquences : 1) l'acidification des cours d'eau par la déposition d'acide nitrique (Aber et al. 1995); 2) le développement de fleurs d'eau d'algues toxiques telles que les cyanobactéries (Vitousek et al. 1997); 3) l'augmentation des apports en N vers les zones côtières provoquant l'eutrophisation, l'hypoxie et la dégradation des habitats côtiers (Howarth et al. 1996, Howarth 1998). Dans plusieurs régions du globe on rapporte la contamination en N des sources d'eau potable. Des concentrations élevées de N sous forme de nitrate peuvent causer ou favoriser des maladies telles que le syndrome du bébé bleu (méthémoglobinémie) ou encore certains cancers (Cantor 1997, Knobeloch et al. 2000).

1.2. Le cycle de l'azote dans les systèmes aquatiques

Dans les systèmes aquatiques, N peut se trouver sous forme organique ou inorganique. Les formes organiques sont souvent les plus abondantes et incluent le N organique particulaire (PON : N dans les organismes vivants et les détritus) et le N organique dissous (DON : divers composés tels que les acides aminés). Le N inorganique comprend du N_2 (gaz) dissous dans l'eau, des ions oxydés (les nitrates NO_3^- et les nitrites NO_2^-) des ions réduits (ammonium NH_4^+) et de l'ammoniaque (NH_3), un gaz réduit.

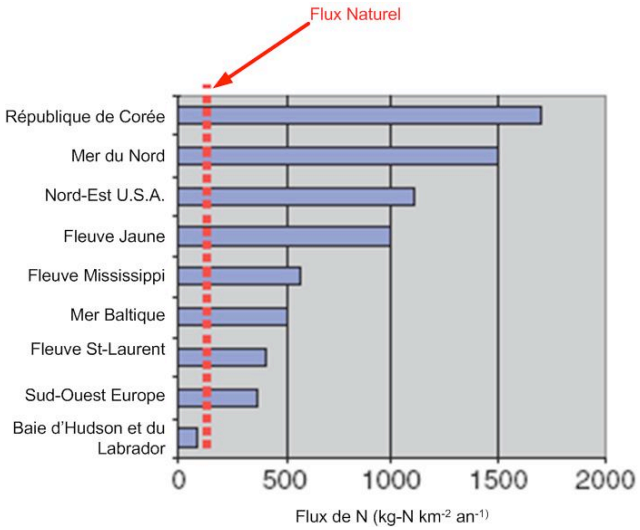


Figure 1.1: Les flux de N dans les rivières exprimés par unité de surface de bassin versant pour différentes régions situées dans la zone tempérée. Le flux naturel sans perturbation anthropique est estimé à 100 kg-N km^{-2} an^{-1}. Figure modifiée à partir de Howarth et Gene (2009).

Le cycle de N (**Figure 1.2**) est un continuum de réactions d'oxydation et de réduction dans lequel les microorganismes jouent un rôle de premier plan. Bien que certains procédés non biologiques tels que la foudre aient la capacité de transformer N d'une espèce chimique à une autre, le cycle naturel de N est reconnu pour être effectué principalement par des procaryotes.

La minéralisation et l'assimilation d'azote

La minéralisation intervient dès lors qu'un organisme – végétal ou animal – meurt dans un écosystème. Le N organique dont il est en partie constitué, est progressivement minéralisé sous la forme d'ammonium (NH_4^+). La qualité de la matière organique déterminera la rapidité avec laquelle elle sera minéralisée. Cette minéralisation est un processus réalisé par un grand nombre de micro-organismes et peut donc se faire sur une large gamme de conditions environnementales (pH, conditions redox, température).

L'assimilation de N sous forme de DIN (NO_3^-, NO_2^- ou NH_4^+) est réalisée par un groupe hétérogène de bactéries, champignons, algues et plantes. L'assimilation par l'incorporation de N dans la biomasse des organismes, permet un stockage à plus ou moins long terme de N dans un écosystème.

La fixation biologique de l'azote (BNF)

La BNF fournit un nouvel apport de Nr nécessaire aux producteurs primaires photosynthétiques à la base de la plupart des écosystèmes. Les procaryotes sont les uniques organismes, seuls ou en symbioses, à pouvoir faire la BNF et ces microbes sont donc essentiels à la Vie. La fixation biologique est conduite par des bactéries photo-autotrophes (par exemple, les cyanobactéries) ou hétérotrophes (par exemple, les bactéries symbiotiques des légumineuses en présence de l'enzyme nitrogénase dans des conditions anaérobiques) selon la réaction suivante :

$$1/2\ N_2 + 3/2\ H_2O + H^+ \rightarrow NH_4^+ + 3/4\ O_2$$

La BNF est un processus énergivore et dans le cas des cyanobactéries, cette énergie provient de la photosynthèse. Chez certains organismes fixateurs de N, l'activité de la nitrogénase

peut être inhibée par le manque de fer dans le milieu, par un apport d'ammonium dans le milieu (Fay 1992), ou par un apport d'oxygène (Burk 1930).

La nitrification

La nitrification est l'oxydation d'ammonium ou ammoniac (NH_4^+ ou NH_3) en nitrate (NO_3^-) en condition aérobie par des bactéries nitrifiantes. Les bactéries nitrifiantes ont une croissance plutôt lente, elles sont chimio-autotrophes, c'est-à-dire qu'elles créent leur propre carbone organique pour la respiration et qu'elles utilisent l'oxydation chimique comme source d'énergie. Cette transformation est réalisée en 2 étapes. Tout d'abord, les bactéries ammonium-oxydantes (AOB) oxydent NH_4^+ en NO_2^-. L'oxydation de l'ammonium elle-même se réalise aussi en 2 étapes : 1) oxydation de l'ammoniac en hydroxylamine (NH_2OH) grâce à l'enzyme catalytique, ammoniac monooxygénase; 2) oxydation de NH_2OH grâce à l'enzyme hydroxylamine oxydoréductase. Ensuite, les bactéries nitrites-oxydantes oxydent NO_2^- en NO_3^-. La nitrification conduit aussi à la production d'oxyde nitreux N_2O, un sous-produit de la réaction, qui constitue un gaz à effet de serre puissant.

La nitrification est un processus fréquent dans l'environnement lorsque les deux substrats essentiels de cette réaction sont présents (l'oxygène et l'ammonium). Plusieurs variables semblent influencer les bactéries nitrifiantes et les taux de nitrification, telles que: la disponibilité de NH_4^+ (Strauss et Dodds 1997), le pH (Sarathchandra 1978), la température, la concentration en oxygène (Stenstrom et Poduska 1980).

La dénitrification

La dénitrification est un processus anaérobie qui convertit le NO_3^- en N_2 selon la réaction suivante :

$$\text{Matière organique} + NO_3^- + H+ \rightarrow 5/4\ CO_2 + 1/2\ N_2 + 5/4\ H_2O$$

La dénitrification se fait en plusieurs étapes, chacune catalysée par une enzyme spécifique : (1) la réduction des nitrates en nitrites est catalysée par l'enzyme réductase de nitrate; (2) la réduction des nitrites en produit gazeux (NO et principalement N_2O) par l'enzyme

réductase de nitrite; (3) la réduction de N_2O en N_2 par l'enzyme réductase d'oxyde nitreux. La dénitrification est effectuée par des bactéries biochimiquement et taxonomiquement très diversifiées. Cependant la plupart des bactéries dénitrifiantes sont hétérotrophes c'est-à-dire qu'elles ont besoin d'une source de carbone organique pour produire de l'énergie. Le processus de dénitrification est inductible (activité facultative). Il est généralement contrôlé par le passage de conditions aérobies à des conditions anaérobies et la présence de NO_3^- dans le milieu (Knowles 1982). Plusieurs facteurs semblent contrôler les taux de dénitrification : la présence d'oxygène empêche la dénitrification en bloquant l'activité des enzymes, la disponibilité des électrons dans le carbone organique contrôle l'activité des bactéries hétérotrophes qui constituent la majorité des bactéries dénitrifiantes, la concentration de NO_3^- contrôle le taux de dénitrification (Knowles 1982). La dénitrification est un mécanisme très important au niveau des écosystèmes car elle permet d'éliminer le Nr des écosystèmes et de conserver ainsi un équilibre dans le cycle global de N.

D'autres processus du cycle de N ont été identifiés tels que la réduction dissimilatoire des nitrates en ammonium (DNRA), l'oxydation anaérobie de l'ammonium (anammox), la dénitrification aérobie, la production de N_2 couplée à l'utilisation du fer, du manganèse ou du sulfure (Luther et al. 1997, Thamdrup et Dalsgaard 2002, Zehr et Ward 2002, Arrigo 2005). Cependant, l'importance relative de ces nouveaux processus dans les écosystèmes d'eaux douces reste à élucider.

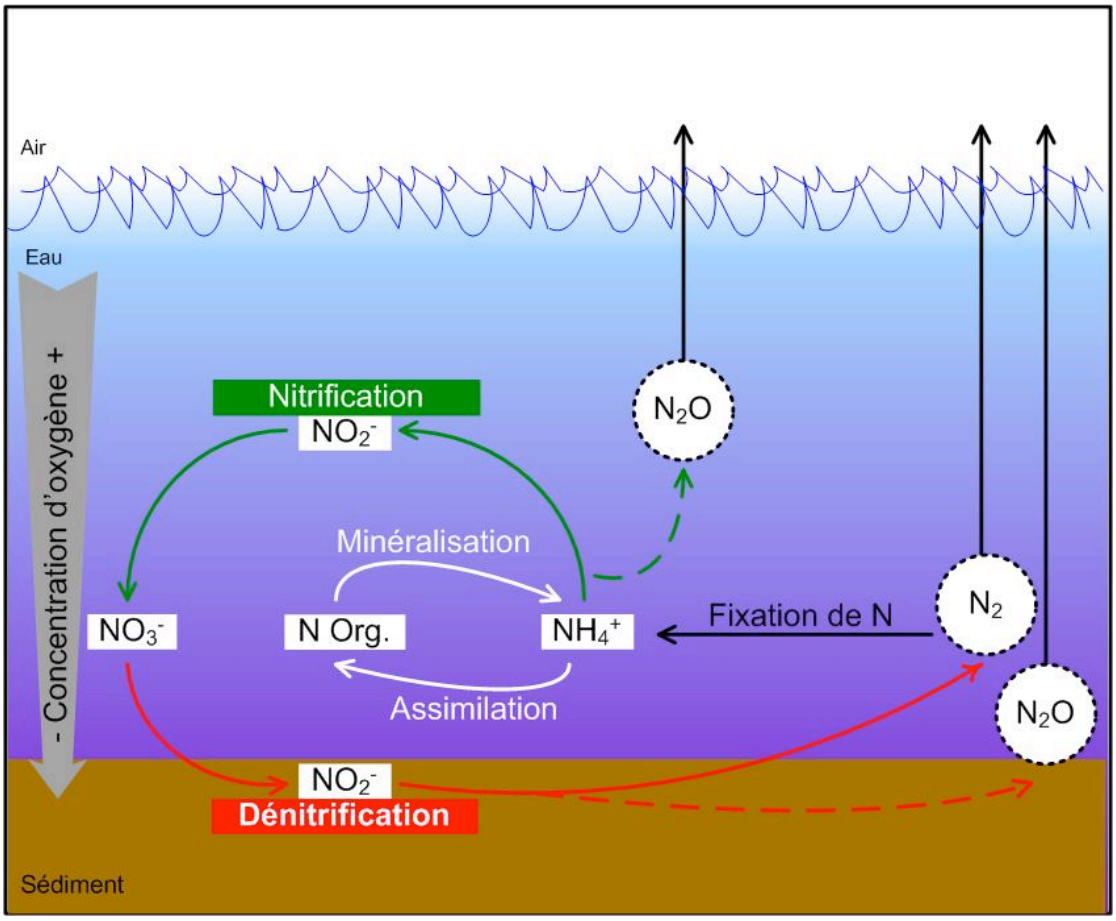


Figure 1.2 : Le cycle simplifié de l'azote dans les systèmes aquatiques.

1.3. La rétention de l'azote dans les rivières

Un service écosystémique

La mainmise de l'Homme sur l'utilisation de la planète a provoqué des changements dans la composition, la structure et la fonction des écosystèmes (Vitousek et al. 1997) ce qui par conséquent, a modifié la capacité de ces écosystèmes à produire leurs services (Palmer et al. 2004). Les services écosystémiques sont définis comme l'ensemble des fonctions d'un écosystème qui sont utiles à l'Homme. Plusieurs d'entre eux sont absolument nécessaires à sa survie (par exemple la régulation du climat, la purification de l'air ou la pollinisation des cultures) alors que d'autres services permettent de lui rendre la vie plus agréable (comme la beauté des paysages naturels ou la clarté de l'eau d'un lac). La rétention et l'élimination de N dans les écosystèmes aquatiques constituent un service majeur rendu par ces écosystèmes (Kremen 2005).

Les systèmes lotiques reçoivent leur N par des sources diffuses provenant du bassin versant (ruissellement) ou de dépôts atmosphériques, et aussi par des sources ponctuelles (tributaires). La quantité de N exportée vers les rivières est donc variable et dépendra des activités développées sur le bassin versant et de la densité de population présente sur ce même bassin versant (Caraco et Cole 1999). Ce N peut être retenu temporairement ou éliminé définitivement du système. Trois processus contribuent majoritairement à la rétention de N : la dénitrification, la sédimentation, et l'assimilation par les plantes. La dénitrification est le principal mécanisme de rétention de N dans la plupart des systèmes aquatiques (Saunders et Kalff 2001) et il permet l'élimination permanente de N. Les écosystèmes lotiques constitués d'un réseau de ruisseaux et de rivières de tailles différentes sont reconnus comme des sites très actifs de dénitrification et d'élimination de N. Globalement, les taux de dénitrification les plus élevés ont été mesurés dans des rivières, particulièrement dans les rivières riches en N et fortement affectées par l'agriculture (Piña-Ochoa et Alvarez-Cobelas 2006). Alors que les réseaux de rivières occupent moins de 1% de la surface de la Terre, la dénitrification dans ces écosystèmes peut éliminer plus de 30% de la quantité de N anthropique produite globalement (Seitzinger et al. 2006). C'est aussi dans les

rivières que la plus forte variabilité des taux de dénitrification a été observée reflétant ainsi les fluctuations dans le débit des cours d'eau.

Les facteurs contrôlant la dénitrification dans les rivières

Des études récentes portant sur l'estimation et la modélisation des taux de dénitrification dans les systèmes lotiques (Mulholland et al. 2008, Alexander et al. 2009, Bohlke et al. 2009) ont démontré l'importance des facteurs hydrologiques et biogéochimiques dans le contrôle de la dénitrification. Les facteurs hydrologiques (changement du débit de l'eau) affectent le degré d'interaction entre les nutriments contenus dans la colonne d'eau et la zone hyporhéique des sédiments. L'élimination des nutriments par la dénitrification est généralement plus élevée lorsque le débit est bas avec une baisse du niveau de l'eau et de la vitesse d'écoulement. Les facteurs biogéochimiques représentent les conditions retrouvées dans l'eau telles que la température, la disponibilité des nutriments (par exemple le NO_3^-, le carbone organique), la concentration en oxygène (cf section sur la dénitrification pour plus de détails sur les effets des facteurs biogéochimiques sur la dénitrification).

Notre compréhension empirique des facteurs qui contrôlent la dynamique et l'élimination de N dans les fleuves est basée sur des études effectuées principalement dans de petits ruisseaux (Ensign et Doyle 2006, Wollheim et al. 2006). Les conclusions étant que les ruisseaux sont des zones plus actives de transformation et d'élimination de N comparativement aux écosystèmes plus grands (Peterson et al. 2001). Malgré ces taux de dénitrification élevés, un excès de N se retrouve tout de même en aval dans les systèmes fluviaux. Les transformations de N dans les systèmes fluviaux sont donc de plus en plus étudiées car ces écosystèmes constituent la dernière protection contre l'excès de N pour les systèmes côtiers qui sont très fragiles face à la pollution en N. De plus l'estimation de la rétention de N dans les fleuves par bilans massiques (N entré moins N sorti du système) suggèrent que les grandes rivières pourraient être responsables de la plus grande quantité de N éliminée grâce à leur temps de résidence et leur distance de transport de l'eau plus long que celui des ruisseaux (Stanley et Maxted 2008, Tank et al. 2008, Alexander et al. 2009, Heffernan et al. 2010). Il manque cependant des données empiriques sur la dynamique de N dans les fleuves et les rivières de grande taille pour confirmer ces hypothèses.

L'influence des plantes aquatiques

L'assimilation de N par les plantes aquatiques peut aussi contribuer à la rétention de N (Saunders et Kalff 2001). Cette rétention de N par les plantes n'est que temporaire car lors de leur sénescence, les plantes sont décomposées et relâchent le N assimilé dans le milieu. Ainsi, les plantes aquatiques donnent une deuxième chance aux bactéries de transformer ce N par la sénescence (Hill 1986). La présence de plantes aquatiques favorise aussi la sédimentation en diminuant la vitesse du courant et en augmentant le temps de rétention de l'eau dans l'écosystème.

Les plantes aquatiques influencent la dénitrification de plusieurs façons : 1) en modifiant les conditions hydrologiques du milieu, la végétation dense diminue la vitesse du courant et augmente le temps de rétention de l'eau dans l'écosystème ou 2) en influençant les facteurs biogéochimiques tels que les apports en N, en carbone organique de leur environnement immédiat (eau et sédiment). La production de matières organiques pendant la croissance et la sénescence des plantes augmentent la dénitrification en fournissant une source de carbone nécessaire aux bactéries dénitrifiantes (Weisner et al. 1994). De plus, en altérant les concentrations en oxygène lors de la photosynthèse, les plantes aquatiques peuvent stimuler ou inhiber la dénitrification.

Avec l'augmentation des espèces invasives dans les écosystèmes en proie à des perturbations (Hobbs et Huenneke 1992), il serait intéressant d'étudier l'effet d'une plante invasive sur le cycle de N en rivière. En effet, comparativement aux systèmes terrestres les systèmes aquatiques et les rivières des zones tempérées sont particulièrement vulnérables aux invasions biologiques (Pysek et Richardson 2006). Les envahisseurs qui sont initialement favorisés par un type de perturbation donné peuvent maintenir l'écosystème dans ce même état altéré mais stable ou encore introduire un nouveau type de perturbation. Certaines espèces envahissantes interagissent très activement avec la perturbation présente dans l'écosystème. (Mack et D'Antonio 1998). Par exemple, l'invasion d'une légumineuse exotique, *Kudzu*, aux États-Unis a transformé le cycle de N dans les régions touchées (Hickman et al. 2010). La fixation de N par *Kudzu* a augmenté de façon significative les quantités de N dans des sols originairement pauvres en N et par conséquent stimulé la transformation de ce N par la

nitrification et la dénitrification. L'augmentation des transformations de N a provoqué à son tour l'accroissement massif de la production d'oxyde nitreux, un sous-produit de ces deux réactions et aussi un important gaz à effet de serre. Les scénarios de colonisation extensive prédisent que *Kudzu* pourrait affecter significativement la chimie atmosphérique et la qualité de l'air à l'échelle régionale.

1.4. La production d'oxyde nitreux

Les processus de production de l'oxyde nitreux et leurs rendements

Les effets de l'emballement observé dans le cycle régional de N peuvent aussi se faire sentir à une échelle globale par l'accélération des émissions d'oxyde nitreux (N_2O), un puissant gaz à effet de serre et un acteur influent dans la destruction de la couche d'ozone (Prather et al. 1995). Les concentrations atmosphériques de N_2O sont en hausse avec une augmentation annuelle de 0,2 – 0,3% (Forster et al. 2007). Le N_2O possède un potentiel de réchauffement par molécule 300 fois supérieur à celui du dioxyde de carbone, le gaz à effet de serre le plus célèbre. Cependant à cause de ses taux d'émissions plus bas, la contribution du N_2O au forçage radiatif du climat est de seulement 10% (Metz et al. 2007). Le N_2O est produit lors de la combustion et dans certaines réactions chimiques industrielles. Cependant, la source la plus significative de N_2O est d'origine biologique par le biais des processus de nitrification et de dénitrification qui se trouvent accélérées par la présence des engrais azotés. Avant les perturbations anthropiques, les émissions de N_2O de sources naturelles étaient en équilibre avec les puits de N_2O (destruction de N_2O dans la stratosphère et réduction microbienne de N_2O en N_2).

La production de N_2O est généralement issue de la dénitrification et de la nitrification. Lors de la nitrification, N_2O est un sous-produit de l'étape d'oxydation de NH_4^+ ainsi que de l'étape de l'oxydation de NO_2^- (Firestone et Davidson 1989). Lors de la dénitrification, N_2O est émis lorsque la dénitrification n'est pas complète et que l'étape finale de la réduction de N_2O en N_2 par l'enzyme N_2O-réductase est manquante (Zumft 1997). Cependant, le N_2O est aussi produit durant l'expression d'autres voies métaboliques associées au cycle de N telles que : le couple nitrification-dénitrification ou l'anammox. Les rendements de ces processus c'est-à-dire le ratio N_2O: N_2 pour la dénitrification ou le ratio N_2O : NO_3^- pour la nitrification, sont variables et mal

définis. Ils dépendent de facteurs environnementaux ainsi que de la composition de la communauté microbienne présente.

Le modèle du «hole in the pipe»

Le modèle du 'Hole in the pipe' (HIP) (**Figure 1.3**, Firestone et Davidson 1989) a été utilisé pour expliquer la variabilité spatiale et temporelle des émissions de N_2O (et aussi d'oxyde nitrique, NO) dans les sols (Eriksson 2001). Ce modèle est basé sur les contrôles biogéochimiques qui régissent l'émission de N_2O. Le modèle HIP considère que le flux total de N_2O ($NO + N_2O$) est proportionnel aux taux des transformations de N. La proportion relative de chaque gaz émis par le sol est contrôlée par l'espace poral du sol occupé par l'eau. Selon le modèle HIP original, la production de N_2O résulte : 1) de la haute disponibilité en N et du renouvellement rapide du pool de NO_3^- dans les sols, représenté graphiquement par la taille des tuyaux (**Figure 1.3**), 2) de la teneur en eau du sol, exprimée en pourcentage de l'espace poral du sol occupé par l'eau et représenté graphiquement par la taille des trous par lesquels s'échappe le N_2O (**Figure 1.3**).

Dans les systèmes aquatiques le modèle HIP suggère que la production de N_2O est reliée : 1) aux taux de nitrification et de dénitrification, et 2) au rendement en N_2O de chaque transformation. Ainsi, les facteurs suivants contrôlant la dénitrification ou la nitrification pourraient aussi jouer un rôle dans la production de N_2O :

- L'oxygène : L'augmentation des concentrations en oxygène inhibe l'activité des enzymes réductrices de N_2O pendant la dénitrification et favorise la production de N_2O. L'oxygène rend la nitrification plus efficace et moins de N_2O est produit.

- Le carbone organique : Lorsque le carbone organique est plus réfractaire, il devient moins disponible pour les bactéries dénitrifiantes qui produisent alors un ratio N_2O: N_2 plus élevé (Firestone et Davidson 1989, Garcia-Ruiz et al. 1999)

- Le NO_3^- et NH_4^+ : Une charge en NO_3^- ou NH_4^+ plus grande provoque l'augmentation des taux de dénitrification (NO_3^-) ou de nitrification (NH_4^+) et par conséquent un accroissement de la quantité de N_2O produite.

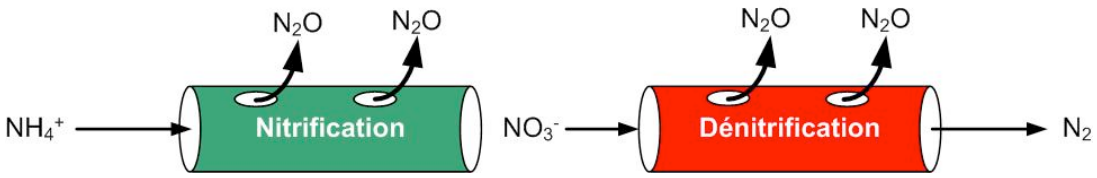


Figure 1.3 : le modèle conceptuel du 'hole in the pipe' illustre les flux d'azote inorganique et les transformations de nitrification et dénitrification dans les sols. L'oxyde nitreux fuit à travers les trous du tuyau qui dans les systèmes terrestres, représentent l'espace poral occupé par l'eau. Adapté de Firestone et Davidson 1989.

- L'hydrologie : Un débit lent et un temps de résidence long se traduisent par une plus petite charge en N mais aussi par une plus grande proportion de N transformé avec des processus complets et par conséquent un ratio N_2O: N_2 plus bas.

Les études sur les ratios N_2O: N_2 et sur les facteurs qui affectent ces ratios ont généralement été menées dans les systèmes terrestres. Malgré ces considérations générales, il existe peu de modèles pour prédire les taux de dénitrification (Alexander et al. 2002, Seitzinger et al. 2002) et, hélas, aucun modèle pour prédire les ratios N_2O: N_2 dans les rivières. Une meilleure compréhension des facteurs qui contrôlent les émissions de N_2O tout en favorisant de hauts taux de dénitrification permettra une meilleure gestion de la qualité de l'eau ainsi que la réduction d'émissions de gaz à effet de serre.

1.5. L'azote, un élément limitant dans les rivières?

Alors que les preuves scientifiques confirmant l'accroissement de Nr dans les systèmes aquatiques s'accumulent, un nouveau débat a émergé récemment pour déterminer si N est un élément limitant pour la production primaire dans les écosystèmes d'eaux douces (Schindler et al 2009, Scott et McCarthy 2010). Les théories écologiques indiquent que N est le principal élément limitant dans les écosystèmes terrestres et marins (Howarth et al. 1988b, Vitousek et Howarth 1991) alors que le phosphore est l'élément nutritif limitant dans les lacs (Schindler, 1977) et les écosystèmes d'eau douce en général. Toutefois, de récents travaux démontrant la limitation en N dans les ruisseaux (Francoeur 2001) et la colimitation en N et P dans les lacs (Elser et al. 2007, Lewis et Wurtsbaugh 2008, Scott et McCarthy 2010) remettent en question les théories précédentes. En effet, pour prédire et atténuer les effets de la modification des cycles globaux de N et du phosphore (P), il faut comprendre lequel de N ou de P est l'élément limitant la production primaire, où et comment.

Le rôle des cyanobactéries dans les rivières

Dans les rivières et les écosystèmes fluviaux la fixation de N reste peu étudiée, probablement parce que ces écosystèmes ont longtemps été considérés comme non-limités par N. Cependant dans ces systèmes, il existe des périodes, souvent durant l'été, où la charge en

nutriments diminue à cause de la baisse du débit de l'eau alors que la biomasse de producteurs primaires (algues et plantes aquatiques) est à son plus haut. Durant la saison estivale, la combinaison du métabolisme microbien dopé par la température et la forte demande en nutriment du milieu peuvent provoquer un épuisement du pool de DIN (NO_3^- et NH_4^+) et initier le début de la fixation de N dans le milieu. Dans les rivières, la fixation de N est réalisée principalement par des cyanobactéries filamenteuses qui forment d'épais tapis posés sur les sédiments et/ou par des bactéries hétérotrophes (Vitousek et al. 2002). Dans ces tapis microbiens, la fixation de N peut compenser la limitation de N pendant plusieurs jours et mêmes plusieurs semaines (Scott et al. 2005). La fixation de N par ce type de tapis peut être très élevée et atteindre les taux de fixation les plus hauts constatés dans la littérature (Howarth et al. 1988a, Bergman et al. 1997).

L'étude de tapis de cyanobactéries présents dans les estuaires et les zones côtières a démontré la coexistence et la cooccurrence des processus de dénitrification et de fixation de N au sein de ces tapis (Joye et Paerl 1993) avec la dominance de la dénitrification (production de N_2) lorsque les concentrations en NO_3^- étaient plus élevées et la dominance de la fixation (consommation de N_2) lorsque les concentrations en NO_3^- étaient diminuées. En effet, ces tapis de cyanobactéries constituent un assemblage diversifié de bactéries qui utilisent le mucus qui entoure les cyanobactéries filamenteuses, comme structure d'ancrage et qui effectuent une variété de processus métaboliques (par exemple, l'hétérotrophie, la chémolitotrophie ou l'autotrophie). À l'échelle de l'écosystème, l'importance des transformations de N dans les tapis de cyanobactéries est considéré comme faible car ces tapis bien qu'ils soient des sites très actifs, occupent des surfaces en général limitées. Dans les rivières peu profondes, les tapis de cyanobactéries pourraient coloniser de grandes surfaces relativement à la surface totale du système et ainsi jouer un rôle significatif dans le budget global de N de l'écosystème. Cependant les facteurs qui contrôlent la dominance de la fixation de N (source de Nr) ou de la dénitrification (puits de Nr) demeurent méconnus pour les rivières.

1.6. Structure et objectifs de la thèse

Cette étude a pour but général d'identifier et de caractériser les facteurs qui régulent le destin de N et en particulier la perte de N, dans les systèmes lotiques. Conséquemment, cette thèse de doctorat est organisée en cinq chapitres, le premier chapitre constitue une introduction

générale et un état des connaissances concernant les transformations de N, les trois chapitres suivants se présentent sous la forme d'articles scientifiques et développent chacun un objectif de recherche différent. Le dernier chapitre de cette thèse est consacré à la synthèse des résultats les plus importants de cette thèse et il présente l'apport de cette recherche à l'avancement des connaissances dans le domaine scientifique ainsi que les perspectives ouvertes par les résultats obtenus.

Les émissions de N_2O et la dénitrification : taux et facteurs contrôlants

Le premier objectif de recherche était de quantifier les émissions de N_2O, d'estimer la perte de N par la dénitrification et de déterminer les facteurs contrôlant la production de N_2O, la dénitrification et le ratio N_2O: N_2 dans un élargissement du Fleuve Saint-Laurent (SLR), le Lac Saint-Pierre (LSP) durant deux étés pendant lesquels les conditions hydrologiques ont été très différentes.

Bien que la charge en N dans SLR soit faible comparativement à celle des grands fleuves américains, l'accroissement des apports en N dû à l'intensification de l'agriculture et à l'urbanisation de berges, se révèle un problème grandissant. De plus, l'estuaire du Saint-Laurent connaît des conditions d'hypoxie de plus en plus préoccupantes qui pourraient être exacerbées par l'apport de nutriment venant de l'amont. Il s'avère donc nécessaire et urgent de mieux comprendre les patrons de transformation de N dans le fleuve en particuliers dans ses lacs fluviaux. En effet, LSP, le dernier lac fluvial du SLR en amont de l'estuaire pourrait se révéler un site très actif de dénitrification et de production de N_2O à cause de son hydrologie particulière et de ces riches tributaires agricoles.

Les tapis de cyanobactéries : puit ou source d'azote?

Le deuxième objectif de recherche était de mesurer les flux de N_2 dans des tapis de cyanobactéries, d'estimer les variations temporelles des taux nets de dénitrification et de fixation de N dans ces communautés et de déterminer les facteurs qui contribuent à ce que les tapis soient une source ou un puit de N.

Les tapis de cyanobactéries démontrent des taux de fixation parmi les plus élevés mesurés. Cependant leur importance au niveau de l'écosystème qu'ils occupent a toujours été faible compte tenu de la petite superficie qu'ils colonisent. Dans les lacs fluviaux du SLR, LSP et le Lac Saint-Louis (LSL), la faible profondeur du milieu favorise la présence de ces tapis sur une grande superficie. Dans ce cas, les tapis pourraient être une source significative de N qui n'a jamais été comptabilisée dans les lacs fluviaux. Ces tapis peuvent aussi accomplir la dénitrification (Joye et Paerl 1993) et donc constituer des puits de N. Dans LSP et LSL, ce phénomène se produirait-il aussi? La concentration en NO_3^- dans le milieu pourrait se révéler un des facteurs qui contrôlent la fonction de ces tapis de cyanobactéries. Ainsi lorsque le NO_3^- est abondant, la dénitrification dominerait et lorsque les NO_3^- sont entièrement consommés la fixation prendrait le relais.

Le rôle d'une plante exotique et envahissante dans le cycle de l'azote

Le dernier objectif de recherche était d'évaluer les effets de la châtaigne d'eau (*Trapa natans*), une plante aquatique exotique et envahissante, sur la dynamique de N dans la rivière Hudson (New York), une rivière riche en N.

Des études réalisées précédemment (Caraco et Cole 2002, Goodwin et al. 2008) démontrent que *Trapa natans*, une plante aquatique à feuilles flottantes peut réduire la concentration en oxygène de la colonne d'eau en rejetant l'oxygène produit durant la photosynthèse dans l'atmosphère et en favorisant la respiration bactérienne dans l'eau grâce à une production importante de matière organique. Ces études ont aussi mis en évidence une diminution de la concentration en DIN dans les milieux colonisés par cette espèce exotique. Nous avançons l'hypothèse que l'hypoxie dans la colonne d'eau stimulerait la dénitrification. Le fleuve Hudson étant soumis à des marées qui renouvellent tous les jours l'eau dans les baies colonisées par *Trapa*, l'élimination de N dans les sites occupés par Trapa pourraient s'avérer particulièrement importante à l'échelle du fleuve.

2. N_2O Emissions And Nitrogen Fate In A Fluvial Lake Of The St. Lawrence River: Comparison With Other Large Rivers

En préparation, pour soumission au “Journal of Environmental Quality”

Auteures : Laure Tall et Roxane Maranger

2.1. Abstract

Aquatic ecosystems, per unit area are considered to be hotspots on the landscape for denitrification. Nitrous oxide (N_2O), a byproduct of denitrification and nitrification, is a potent greenhouse gas. With anthropogenic N loading to aquatic ecosystems on the rise, N_2O emission from rivers and lakes should increase. In this study we measured N_2O concentrations over the summer for two consecutive years in Lake Saint-Pierre (LSP), a broadening of the St Lawrence River. LSP is a net atmospheric source of N_2O to the atmosphere with fluxes averaging 3.4 μmol-N m^{-2} d^{-1} where nitrate concentrations alone explained up to 60% of the variance in N_2O fluxes. Emissions were seasonally and inter-annually highly variable due to changes in hydrological conditions. A mass balance approach estimated retention rates of 22 to 30% of the total inorganic nitrogen load with denitrification accounting for 6 up to 82% of the amount retained within LSP. In a cross system analysis, hydraulic load seemed to mitigate the relation between annual N_2O flux and NO_3 concentrations in rivers. Systems with lower hydraulic load emitted more N_2O per unit NO_3^-. The influence of hydraulic load and relative N_2O emissions calls for further investigation to improve current N_2O emissions models in large rivers.

2.2. Introduction

Nitrous oxide (N_2O) is a potent greenhouse gas with 310 times the global warming potential of CO_2 (Firestone and Davidson 1989, Matson and Vitousek 1990). N_2O is produced in soils and aquatic ecosystems mainly via two microbially mediated processes: 1) nitrification the transformation of ammonium to nitrate and 2) denitrification the transformation of nitrate to N_2 gas, which removes permanently nitrogen (N) from the ecosystem. While the importance of soils particularly agricultural soils in global N_2O emissions has been extensively studied (litterature reviews by Mosier et al. 1998 and Stehfest et al. 2006), the role of aquatic ecosystems in the global N_2O budget particularly inland water, remains poorly evaluated. Among the aquatic systems, riverine networks are seemingly hotspots of N_2O production. While aquatic systems occupy less than 1% of the surface area of watersheds, a substantial amount of the N applied on the watershed enters rivers and streams through runoff, leaching and direct discharge (Galloway et al. 2003). The biological cycling of N through these inland networks results in high emissions of N_2O that can contribute up to 20% of the current global anthropogenic N_2O emissions (Seitzinger and Kroeze 1998, Beaulieu et al. 2011).

However uncertainties in model estimates of N_2O emissions remain considerable due to the lack of direct measurements particularly in large rivers and over temporal scale. Until recently the focus was on small and first order streams (Laursen and Seitzinger 2004, Beaulieu et al. 2008, Smith et al. 2009) which were found to transform N more actively, and therefore believed to produce more N_2O per unit area. This is due to their higher benthic to surface water ratios compared to larger rivers (Wollheim et al. 2006). In general N_2O emissions and biological N transformations concepts in large rivers are infered from models based on these smaller ecosystems (Tank et al. 2008).

Cole and Caraco (2001) reported low N2O emissions in large rivers (i.e. Hudson, Humber, Colne and Tamar rivers). These potentially lower rates of N_2O emissions in rivers combined with high rates of N transformations particularly denitrification can result in lower N_2O: N_2 ratio of emissions compared to terrestrial environments (Schlesinger 2009). Although permanent loss of N in the form of N_2 gas is favourable in N-rich ecosystems, a relatively high

N_2O: N_2 ratio is not. Reported ratios of N_2O: N_2 emissions for aquatic systems are extremely variable ranging from 0.01% to 3% and topping at 6% in hypereutrophic environments (Seitzinger et al. 2000, Dong et al. 2002). Understanding and predicting the amount of N_2O gas emitted relative to the total amount of N processed in aquatic systems is therefore an important ecological question particularly in large rivers.

In Canada, there is an increasing amount of N loading to the St Lawrence River particularly from intensively farmed watershed of the St Lawrence Lowlands (Anderson and Cabana 2006, Hudon and Carignan 2008). Yet, there is little knowledge on processing of N in this system and nothing on the factors regulating N_2O emissions. In this study, we measured N_2O emissions in Lake Saint Pierre (LSP) which is a fluvial lake of the St Lawrence River. We measured rates of N_2O production during the summer and fall months for two consecutive years. Our objectives were to: 1) identify the controlling factors of N_2O emissions; 2) construct a N budget estimating N retention via mass balance and 3) assess the spatial variability in N_2O: N_2. We performed a cross system comparison of N_2O emission rates and considered our findings in a global context with other river ecosystems.

2.3. Materials and Methods

Study site

LSP (46°12'N 72°49'W, **Figure 2.1**) is a 30 km long, 300 km^2 broadening of the St. Lawrence River located about 70 km North East of Montreal, Quebec, Canada (**Figure 2.1**). LSP is a slow flowing (0.5 m s^{-1}) and a relatively shallow system (average depth <3 m), with the exception of the deep navigation channel (>12 m) that runs through the center of the lake. Given the shallow depth and gently sloping shores of this ecosystem, aquatic vegetation occupies up to 80% of the lake surface (Vis et al. 2007). LSP's large width/depth ratio is responsible for a very limited lateral mixing within the lake's three main water masses (Frenette et al. 2003).

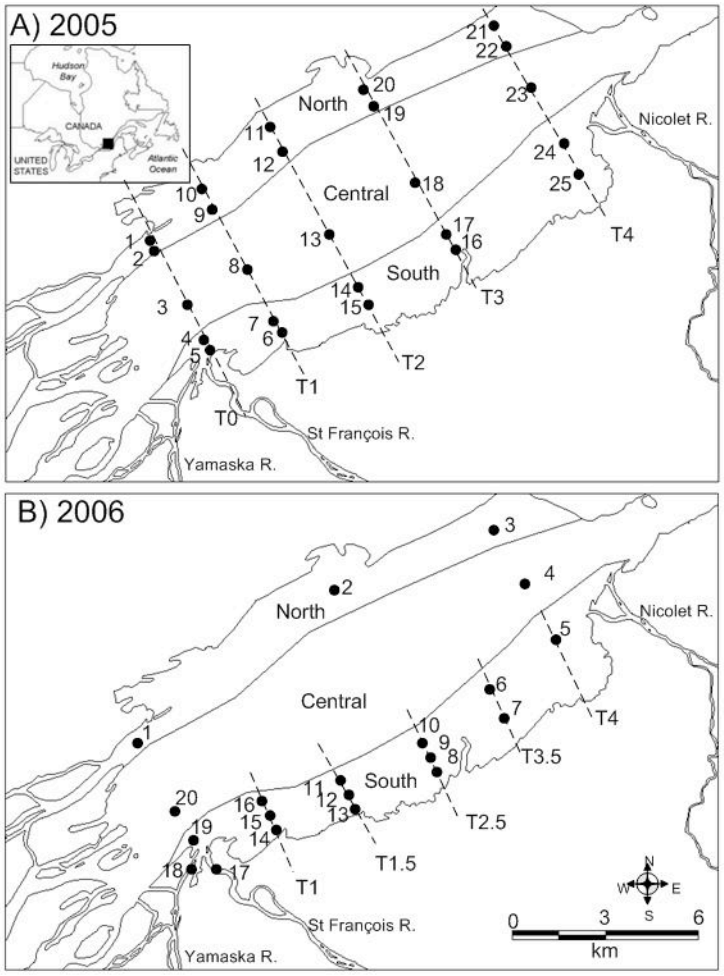


Figure 2.1: Map of Lake St. Pierre with A) 25 sites sampled in 2005 along 5 transects (T0, T1, T2, T3 and T4); and B) 20 sites sampled in 2006 located mainly in the south shore (15 sites) along 5 transects (T1, T1.5, T2.5, T3.5 and T4)

LSP is spatially very heterogeneous with three distinct water masses originating from several tributaries flowing into this section of the St. Lawrence River. Waters from the Ottawa River and other tributaries draining the Precambrian Shield flowed along the north shore of LSP (north water mass) and represented 13 to 17% of the mean summer discharge respectively in 2005 and 2006 (data from gauging stations). Waters originating from Lake Ontario dominated in terms of flow (82% in 2005 and 78% in 2006 of the total LSP discharge, data from gauging stations), but were funnelled into the navigation channel and were restricted to the central water mass. The south mass was fed primarily by three tributaries with agriculturally intensive watersheds: the Richelieu, the Yamaska and the Saint François rivers. Nutrient loading is on the rise in the St. Lawrence basin primarily as a function land-use change. Over the last 15 years, agricultural practices have shifted from pasture and dairy production to intensive corn (14-fold rise), chicken (3-fold rise) and hog (4-fold rise) production (Statistics Canada 2001), resulting in increased fertilizer use.

Sample collection and chemical analysis

The study was conducted for two consecutive years (2005 and 2006) from spring to fall. In 2005, we sampled 25 sites in LSP along 5 transects (**Figure 2.1**A) on 5 dates (June 28, July 12, July 26, August 18, September 13). In 2006, we focused our study on the south water mass and sampled 20 sites on 6 dates (May 9, June 6, July 4, August 15, October 15, November 23). On each date and site, partial pressure of N_2O (pN_2O) was measured by headspace equilibration at ambient temperature (Cole and Caraco 2001). A volume of 1.1 L of water, taken at the surface (approximately 5 cm depth) was equilibrated in a gastight bottle with ambient air (120 ml). Equilibrations were made in duplicate for each site as were ambient air samples. After equilibration, 9 mL-sample of headspace gas was injected into pre-evacuated vials with a thick butyl stopper and aluminum ring in duplicate. N_2O was measured by gas chromatography using an ECD detector on a Tracor model 540 GC with a Tekmar 7050 autosampler. We used a Poropaq Q (80/100) column to separate gases and P5 (95% argon and 5% methane) as the carrier gas. Standards consisted of vials treated exactly as described above with N_2O concentrations of 0.336, 1.5 and 5.28 ppm. Water samples were collected at each site for analysis of nitrate + nitrite (NO_2^- + NO_3^-), ammonium (NH_4^+), total dissolved N (TDN), total N (TN), dissolved organic

carbon (DOC) and total phosphorus (TP). With the exception of samples taken for TN and TP analysis, all samples were pre-filtered (Filtropur 0.45 µm pore size). NO_2^- + NO_3^- (which will be referred to as NO_3^- for the remainder of the text) and NH_4^+ were measured with a Latchat Instrument analyzer (methods number 10-107-04-1-B and 11-104-03-1-B). TN, TDN water samples were autoclaved with potassium persulfate and the resulting NO_3^- was measured as above. TP concentration was also measured after autoclaving with potassium persulfate (Stainton et al. 1977). DOC was measured using the high-temperature combustion method with a Shimadzu TOC-5000 analyser. Water temperature, pH, conductivity and dissolved oxygen were also measured at a depth of 10 to 20 cm below the surface, using a YSI probe (model 556MPS, Yellow Springs Instruments).

N_2O fluxes

Measured values of N_2O from the headspace equilibrium were corrected for the introduction of air using temperature-dependent Bunsen coefficients from Weiss and Price (1980) to obtain N_2O partial pressure in the water. The flux of N_2O from the surface water to the atmosphere depends on the differential concentrations and on physical transfer between air and water as a function of turbulence. Thus N_2O flux was calculated using Eq. 1.

$$Flux = k_{N_2O} * k_H \left(pN_2O_{water} - pN_2O_{air} \right) \quad \text{(Eq. 1)}$$

Where k_{N_2O} is the piston velocity for N_2O, k_H is the Henry's constant for N_2O, $p\mathrm{N_2O_{water}}$ is the corrected partial pressure of N_2O in water, and $p\mathrm{N_2O_{air}}$ is the partial pressure of N_2O in air. The piston velocity normalized for a Schmidt number of 600 (k_{600}) was estimated using wind speed model from Cole and Caraco (1998). Even though this model was created for smaller systems, its estimated k values were in good agreement with the mean annual transfer velocity range of 0.03–0.07 m h^{-1} proposed by Raymond and Cole (2001) for large rivers and estuaries. Hourly wind speed data were obtained from Environment Canada (Lake St. Pierre station). k_{N_2O} was then calculated using Schmidt number ratio approach (Jahne et al. 1987).

Water discharge

Discharge rates for each water mass were obtained by summing the daily discharge rates of their major tributaries: Ottawa (Carillon station, data from Hydro-Québec) and Assomption Rivers for the north water mass, Great lakes (Beauharnois station, data from Environment Canada) for the central water mass and Richelieu, Yamaska and Saint-François rivers for the south water mass (gauging stations near the mouth of each river, data from Ministère du Développement durable, de l'Environnement et des Parcs 2006a).

Summer N load in the south water mass

We focused on the south water mass for the load and retention calculations as it showed the most dynamic pattern of N concentrations and N_2O emissions compared to the central and north water masses. The magnitude of internal processes (production, retention and transformation) affecting N concentrations within the south water mass was estimated from mass balance calculations for each sampling date and then integrated through time to obtain summer N load and N retention. Calculations were made for the summer between June and September for the two sampling years.

For N loaded in the south water mass, N concentrations and discharge data were taken from stations near the mouth of each of the main tributaries (data from the Ministère du Développement durable, de l'Environnement et des Parcs 2006b). For N exported out, N concentrations were taken from our downstream sampling transect (station 24 and 25 in 2005 and an average of stations 5, 6 and 7 in 2006). Instantaneous loadings per date (Load) were calculated using the following equation:

$$Load = \sum D_i * Q_i \qquad \text{(Eq. 2)}$$

Where instantaneous load (L) is the sum in all tributaries (inflow) or in all sites downstream (outflow), of the product of N concentration (Q_i) and discharge rate (D_i).

Summer N retention in the south water mass

We have defined retention as the amount of N entering the system trough inflows and atmospheric deposition minus the amount exiting in LSP from outflows. Therefore the amount defined as "retained" has multiple fates mainly: 1) permanent removal as N gases via microbial activities; 2) temporary storage in plant uptake; 3) transient storage in sediments (Saunders and Kalff 2001) or 4) simple N form transformation (e.g. DIN to DON). We estimated the time integrated percentage of N retention (%R) using the following equations:

$$TLoad = \frac{\sum \left(t * Load_{i-1} + 0.5t(Load_i - Load_{i-1}) \right)}{t_{tot}} \quad \text{(Eq. 3)}$$

$$R = TLoad_{in} - TLoad_{out} \quad \text{(Eq. 4)}$$

$$\%R = \frac{R}{TLoad_{in}} \times 100 \quad \text{(Eq.5)}$$

Where *TLoad* is the time integrated load in or out, *t* is the time in days between retention estimates, t_{tot} is the total duration of data set (77 days in 2005 and 70 in 2006) and *R* is the time integrated amount of N retained. However, it should be noted that these retention values may be slightly underestimated as we did not consider other potential inputs of N, like N fixation and groundwater inputs which are negligible in LSP.

We assumed that denitrification and plant uptake should play the most important roles in our system as sedimentation was considered to be negligible (Carignan and Lorrain 2000). This latter assumption may not be completely true in the south water mass where particle rich tributaries entered the system and where massive macrophyte beds should promote particle settling (Rooney et al. 2003). It is not unreasonable however to assume that much of N retained via sedimentation is denitrified or taken up by plant. N uptake by phytoplankton and epiphyton communities was determined based on their productivity, ranging from 86 to 114 g C m^{-2} Yr^{-1} in the south water mass (Vis et al. 2007) and using a Redfield ratio of 106C: 16N. N content of aquatic macrophytes was estimated using estimates of plant C: N ratios and C biomass estimates by Vis et al. (2007). The C: N content of macrophyte in LSP was spatially quite variable ranging

from 12 to 45 (Blanchet et al. In press). Atmospheric deposition was estimated using an areal deposition rate (Howarth et al. 1996) multiplied by the LSP surface area.

We assumed that the N retained in the system but not bound in plants was lost via denitrification. The amount of N removed via complete denitrification was estimated by difference from the total quantity of N retained in the system from the mass balance minus the amount of N retained by primary producers and the amount lost as N_2O emissions or incomplete denitrification. The amount estimated to be lost via complete denitrification was assumed to be N_2 gas.

Errors calculation

Given that our estimate of N_2 production was subject to variation in the variables used to create the mass balance, we performed an uncertainty analysis load and retention terms using error-propagation principles from Bevington and Robinson (2002) as follows:

$$\delta Load = \sqrt{\left(\frac{\delta D}{D}\right)^2 + \left(\frac{\delta D}{D}\right)^2} \times Load \qquad \text{(Eq.6)}$$

$$\delta Load_{in} = \sqrt{\delta Load_{Richelieu}{}^2 + \delta Load_{Yamaska}{}^2 + \delta Load_{St-François}{}^2} \qquad \text{(Eq.7)}$$

$$\delta TLoad = \frac{\sqrt{\sum \left(\left(t \times \delta Load_{i-1}\right)^2 + \left(0.5 \times t \times \sqrt{\delta Load_i{}^2 + \delta Load_{i-1}{}^2}\right)^2 \right)}}{T_{tot}} \qquad \text{(Eq.8)}$$

$$\delta R = \sqrt{\delta TLoad_{in}{}^2 + \delta TLoad_{out}{}^2} - 2\,\mathrm{cov}(TLoad_{in}, TLoad_{out}) \qquad \text{(Eq.9)}$$

Where *D,* the discharge rate and *Q*, the nitrogen concentration are independent variables, $\delta Load_{in}$ represents the uncertainty (δ) in the amount of N loaded in LSP from its main tributaries: Richelieu, Yamaska and St-François rivers, cov*(*$TLoad_{in}$, $TLoad_{out}$*)* represents the covariance between TLoad in and out.

Cross systems comparison

We wanted to compare the results of the relationship between N_2O emissions and NO_3^- concentrations from this study with values reported in the literature for other rivers and estuaries. A previous analysis using fewer systems suggested that N_2O emissions tended to increase with increasing average NO_3^- concentration, however the relationship between these two factors was weak (Cole and Caraco 2001), suggesting that other factors like hydraulic load (Harrison et al. 2009) may be controlling the relative amount of N_2O emitted. To further explore the relationship of N_2O emissions and NO_3^- concentrations we found data for 16 river and estuarine systems where hydraulic load (HL) was calculated as:

$$HL = \frac{D}{A} \text{ or } HL = \frac{z}{WRT} \quad \text{(Eq.10)}$$

Where D is the annual water discharge; A is the river surface area; z is the mean depth and WRT is the mean water residence time of the system.

Statistical analysis

To determine the differences in N_2O fluxes between dates, we used a one-way analysis of variance (ANOVA). For our data set, all residuals were normally distributed. We compared pairwise means between groups using a Tukey-Kramer post hoc test (with $\alpha = 0.05$). We also performed a repeated-measure ANOVA to assess differences in N_2O fluxes between transects over time in 2005. Simple regression and forward stepwise variable selection followed by a multiple regression were used to explain N_2O fluxes variation in 2005 and 2006, in LSP In 2005, we used an analysis of co-variance (ANCOVA) to determine significantly different relationship among sampling dates. Finally, we performed simple and multiple regressions to explain annual N_2O fluxes in our cross-system analyses. ANOVAs, simple and multiple regressions were run with SPSS 16.0 for Windows and ANCOVA with R language. Data were log transformed to meet normality assumptions when needed. We only reported adjusted R^2 (referred to as R^2) to allow comparison between models.

2.4. Results

Properties of the different water masses

Overall average summer daily discharge in LSP increased by 5% between 2005 and 2006, however more striking changes were observed in the south and north water masses where discharge increased by 17% in the south and 37% in the north (**Figure 2.2A,C**). The central mass (**Figure 2.2B**), which represented the larger proportion of the total summer discharge did not experience a change in its average daily discharge between 2005 and 2006. These hydrological changes between years resulted in differences of flow related variables like water masses surface area, depth and water residence time (The three water masses of LSP showed different chemical characteristics. Waters from the north water mass generally had lower conductivity and relatively high N concentrations. The south water mass had the highest TN and TDN average concentrations (**Table 2.1**). NO_3^- concentrations in the south water mass were temporally and spatially variable particularly in 2005 where we observed severe NO_3^- depletion events downstream (**Figure 2.1A**, transects 4 and 5) later in the summer. NO_3^- concentrations at all sites also tended to decrease in the south water mass throughout the summer but complete NO_3^- depletion events were not observed in 2006 (**Table 2.1**). The larger central water mass had the highest conductivity and the lowest and more stable nitrogen concentrations throughout the summer compared to the two other water masses. In all three water masses NH_4^+ was present at low concentration averaging 2µM and ranging below detection level to 8µM during our sampling period. Phosphorus (P) concentrations when measured always exceeded 0.5µM (15µg L^{-1}) the upper P limit for oligotrophic systems.

Temporal and spatial pattern

Mean N_2O fluxes per sampling date were always positive in 2005 and 2006 (**Figure 2.3A** and **B**) and mean N_2O concentration in LSP was consistently supersaturated with respect to the atmosphere averaging 143 and 126% for 2005 and 2006 respectively. Fluxes were higher in 2005 compared to 2006 (mean: 5.19 and 1.86 µmol-N m^{-2} d^{-1} respectively). N_2O emissions ranged from 0.44 on August 16 (site 21) to 42.0 µmol-N m^{-2} d^{-1} on June 28 (site 24) for 2005 and from -6.47 on August 15 (site 6) to 11.4 µmol-N m^{-2} d^{-1} on July 4 (site 10) for 2006. In 2005, N_2O

fluxes in June were significantly higher compared to other months (**Figure 2.3A**) In 2006 we found no significant differences in fluxes among months **(Figure 2.3B**). During the study period the highest N_2O fluxes were generally observed at the end of June and beginning of July. Earlier in the season, from May to June, N_2O fluxes tended to increase gradually. During the late summer months and fall, N_2O fluxes patterns were different between our two sampling years. In 2005 we observed a slight increase in fluxes whereas in 2006, a decrease of fluxes.

In terms of spatial dynamics in 2005, the south water mass generally had the highest N_2O fluxes particularly in June, July, and September and overall the central mass experienced the lowest fluxes (**Table 2.2**).Despite of this observable pattern, a repeated-measure ANOVA did not show any significant differences in N_2O flux among the water masses for each sampling date (p= 0.31).

In the south water mass, we observed an increase of N_2O fluxes for the first two transects (T0 and T1 in 2005 and T1 and T1.5 in 2006) going downstream after tributaries inputs, followed by a decrease in fluxes in the subsequent transects (**Figure 2.4A**). This pattern was repeated in 2005 and 2006 with the exception of transect 3 in 2005 which exhibited an important peak in N_2O flux.

Predictive relationships in LSP

For the whole LSP in 2005, temporal variability observed in N_2O flux could be predicted by the spatial and temporal variability in the NO_3^- concentration in this ecosystem. A significant positive relationship between N_2O flux and NO_3^- concentration ($p < 0.0001$) could explain up to 60% of N_2O fluxes variation (**Figure 2.5A**).

An ANCOVA (**Figure 2.5B**) showed a significantly steeper slope in the relationship found in June 2005 for LSP. This steeper slope was in June when NO_3^- concentrations were the highest. Slopes of the relationship between N_2O fluxes and NO_3^- concentrations decreased during the summer following the decline of NO_3^- concentrations. In July 26 however, the slope was not significant. In August 2005, the slope increased even though NO_3^- concentrations were low. In 2006, NO_3^- concentration could only explain 9% of variation in N_2O fluxes ($p < 0.001$) (**Figure 2.5C**).

Table 2.1: Summer average values of the chemical characteristics by transects for three main water masses of LSP in summer and 2006. In parenthesis we reported minimum and maximum values, *N* indicates the number of samples used to estimate average values.

Year	Water Mass	Transect	N	TN (µmol L^{-1})	TDN (µmol L^{-1})	NO_3^- (µmol L^{-1})	Temp (°C)	Conductivity (mS cm^{-1})
2005	north	0	10	46.57 (32.48-77.05)	40 (25.29-69.89)	20.01 (6.63-45.73)	23.78 (20.97-25.47)	0.14 (0.09-0.21)
2005	north	1	10	44.41 (20.91-81.00)	39.02 (19.22-73.17)	18.86 (0.45-47.48)	22.89 (20.03-25.15)	0.14 (0.09-0.2)
2005	north	2	10	43.48 (20.24-79.89)	39.32 (20.01-76.44)	17.93 (0.65-48.2)	22.95 (19.51-24.86)	0.15 (0.09-0.21)
2005	north	3	10	43.60 (24.29-79.53)	39.77 (20.98-77.45)	17.4 (0-49.29)	22.74 (19.53-25.19)	0.14 (0.09-0.2)
2005	north	4	10	41.75 (27.99-77.66)	35.64 (20.66-72.89)	13.93 (0-47.42)	22.42 (15.34-26.33)	0.13 (0.08-0.2)
2005	Center	0	5	33.63 (26.42-44.65)	32.13 (26.37-42.37)	15.92 (10.11-25.26)	23.14 (21.05-24.87)	0.2 (0.14-0.25)
2005	Center	1	5	32.98 (27.03-43.17)	29.47 (23.57-39.31)	13.92 (8.33-22.69)	23.39 (21.21-24.84)	0.19 (0.14-0.25)
2005	Center	2	5	30.34 (26.91-38.76)	27.41 (24.1-36.33)	10.85 (7.01-16.53)	23.13 (20.78-24.23)	0.19 (0.14-0.24)
2005	Center	3	5	32.64 (26.45-46.15)	30.51 (23.95-44.59)	12.2 (0-26.1)	22.9 (20.81-25.1)	0.19 (0.14-0.25)
2005	Center	4	5	31.76 (24.27-47.39)	29.64 (22.09-43.03)	11.27 (0-25.56)	22.79 (20.75-24.47)	0.19 (0.14-0.25)
2005	south	0	10	45.09 (24.15-69.26)	36.44 (19.59-61.56)	11.2 (0-35.5)	24.05 (20.14-27.51)	0.16 (0.11-0.21)
2005	south	1	10	56.83 (32.94-94.51)	51.94 (29.45-86.81)	23.57 (0-55.25)	23.22 (20.17-26.04)	0.17 (0.11-0.23)
2005	south	2	10	53.75 (29.41-100.91)	48.82 (24.69-91.63)	23.25 (0-58.49)	22.73 (19.31-25.5)	0.17 (0.12-0.23)
2005	south	3	10	50.09 (26.05-88.24)	45.9 (22.68-83.86)	15.58 (0-51.55)	22.73 (15.02-27.1)	0.15 (0.11-0.21)
2005	south	4	10	41.00 (24.33-99.74)	36.79 (21.95-89.92)	9.51 (0-60.42)	22.31 (18.5-25.24)	0.15 (0.12-0.19)
2006	north	0	6	58.81 (44.14-78.90)	57.22 (44.85-71.82)	28.5 (21.1-37.66)	15.3 (4.89-21.8)	0.13 (0.08-0.18)
2006	north	3	6	54.77 (43.69-68.71)	52.53 (44.18-61.87)	28.5 (21.96-35.33)	15.17 (4.88-21.67)	0.12 (0.07-0.18)
2006	north	4	6	57.18 (52.51-64.87)	54.7 (42.42-63.82)	29.19 (20.86-34.13)	15.3 (4.62-22.09)	0.12 (0.07-0.18)
2006	center	0	6	42.13 (29.27-62.33)	42.24 (27.13-60.39)	22.91 (11.12-34.76)	15.6 (6.76-22.36)	0.21 (0.13-0.27)
2006	center	4	6	42.01 (29.20-57.16)	42.39 (27.34-55.82)	22.49 (11.09-29.85)	15.42 (6.4-21.62)	0.21 (0.13-0.27)
2006	south	Station 19	6	115.72 (38.95-278.55)	107.64 (31.58-264.25)	73.73 (8.64-205.71)	16.04 (5.62-22.41)	0.21 (0.11-0.3)
2006	south	Station 18	6	158.51 (61.57-303.45)	153.83 (48.61-288.29)	114.18 (24.69-221.43)	16.25 (5.38-22.33)	0.23 (0.11-0.36)
2006	south	Station 17	6	56.17 (38.02-79.80)	53.83 (41.21-70.17)	25.19 (17.54-33.87)	15.78 (4.39-21.88)	0.15 (0.09-0.23)
2006	south	1	18	58.33 (30.73-120.60)	55.91 (27.3-109.23)	28.59 (0.54-61.39)	16.1 (4.1-24.09)	0.17 (0.08-0.27)
2006	south	2	18	67.57 (34.58-144.95)	64.28 (29.71-137.26)	35.22 (0.23-99.76)	15.92 (4.07-24.01)	0.17 (0.08-0.28)
2006	south	3	18	63.77 (36.67-111.01)	59.38 (32.89-114.9)	30.92 (3.36-79.39)	15.81 (3.82-23.38)	0.17 (0.08-0.28)
2006	south	4	12	55.28 (34.82-85.64)	51.38 (32.33-83.92)	23.73 (0.33-41.92)	15.61 (4.05-23.36)	0.16 (0.07-0.26)
2006	south	4	6	52.94 (44.00-75.66)	50.85 (42.65-68.68)	25.69 (9.32-35.61)	15.55 (5.88-22.43)	0.17 (0.07-0.26)

Table 2.2: Summary by water mass of N_2O fluxes and flow related variables. SA is the surface area and WRT is the water residence time.

Water mass	Year	N_2O μmol m^{-2} d^{-1}	SA km^2	Depth m	WRT h	*N*
North						
	2005	5.29 (0.44−19.42)	54	1.6	24.2	50
	2006	-0.04 (-3.97 − 3.88)	66	1.8	27.8	9
Central						
	2005	3.88 (0.74−12.92)	144	4.2	24.2	25
	2006	0.38 (-3.16−4.89)	176	4.3	27.8	6
South						
	2005	5.74 (0.56−41.98)	85	1.1	46.4	50
	2006	1.66 (-6.47−11.38)	63	1.1	24.8	39

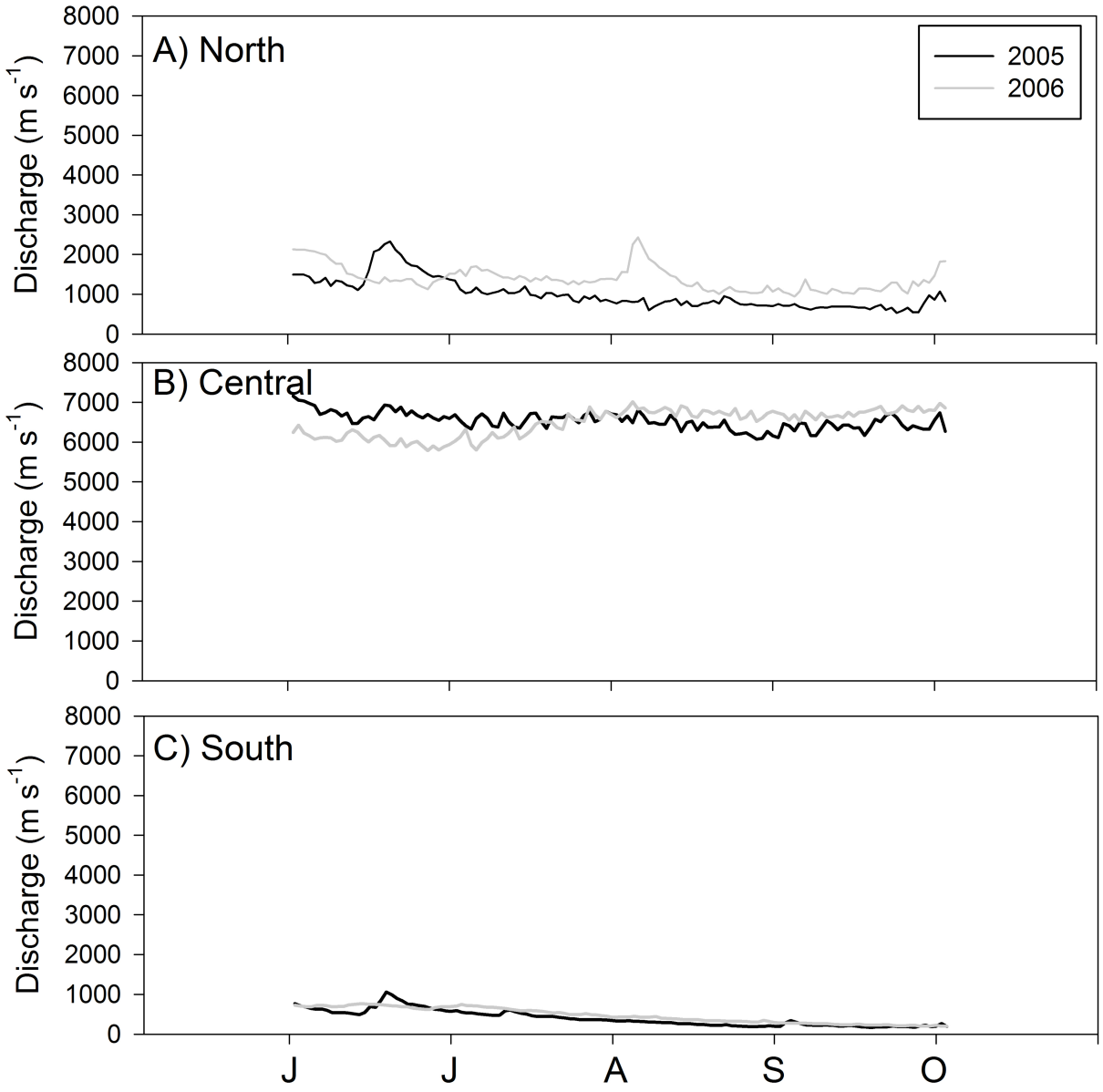


Figure 2.2: Daily discharge of A) the north; B) central and C) south water masses of LSP between June and October of 2005 and 2006.

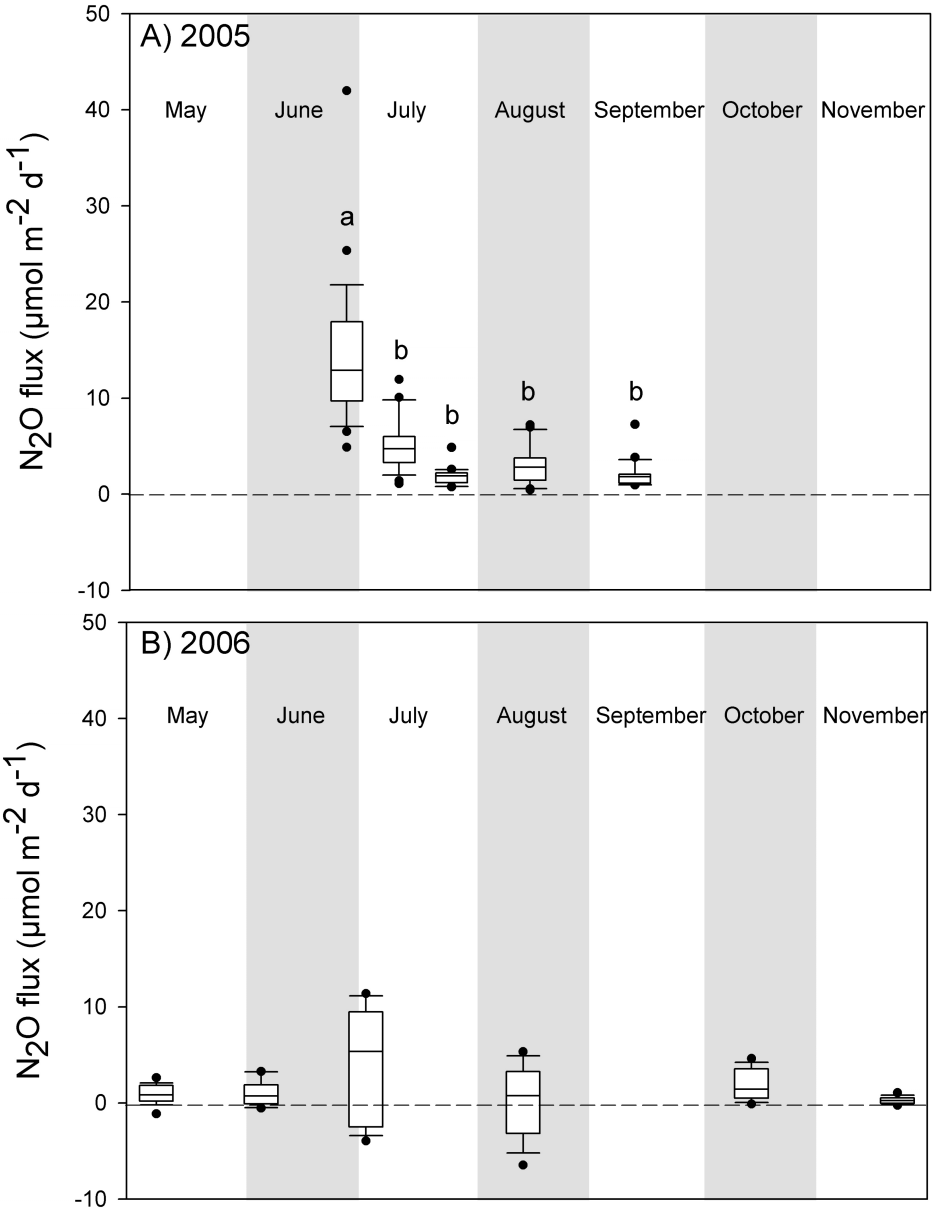


Figure 2.3: N_2O fluxes (µmol m^{-2} d^{-1}) per month in the whole LSP A) 2005 and B) 2006. The transversal line in each box represents the mean and whiskers above and below the box indicated the 90th and 10th percentiles. Letters denote a significant difference in fluxes among months (one-way ANOVA: in 2005 N= 125, p<0.001 and in 2006 N =108, p<0.061).

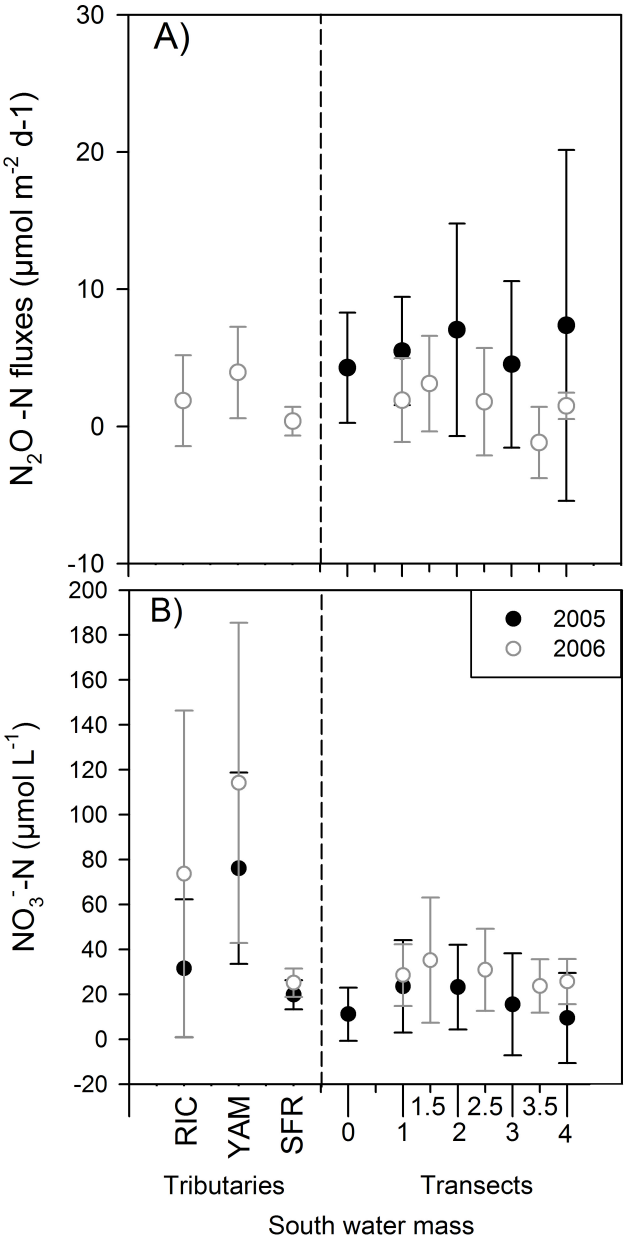


Figure 2.4: South water mass spatial pattern of A) N_2O fluxes and B) water column NO_3^- concentrations in 2005 and 2006. Mean and standard deviation were calculated for the two or three sites in the corresponding transect. Tributaries N_2O fluxes were not measured in 2005. YAM and SFR represent the sites at the mouth of Yamaska (site 18) and St. François Rivers (site 17) and RIC represents an area (site 19) influenced by the Richelieu River. In 2005, NO_3^- concentrations in tributaries were taken from the Ministry Data and in 2006 they were measured directly.

Using a forward stepwise method to select variables, we found that in 2006, the combination of DO concentrations and log (NO_3^- +1) could significantly explain 16% of N_2O flux variation. In 2005, with the same method of variables selection, we found that DO, NO_3^- concentrations and the amount of precipitation one week prior to sampling day explained 70% of N_2O fluxes variability in LSP (**Table 2.3**).

Retention processes and N_2O: N_2 in the south water mass

In the south water mass, tributaries inputs were lower in 2005 where they represented respectively 28% and 22% of TDN and NO_3^- inputs in 2006 for the same period (**Table 2.4**). However the rate of retention (% of inputs retained) in the ecosystem was higher in 2005 as compared to 2006 for TDN and NO_3^- and most of the TDN retained was under NO_3^- form. Our results showed that rates of retention were higher when expressed in NO_3^- rather than TDN. Unexpectedly, we found absolute amounts of NO_3^- retained were greater than amount of TDN (**Table 2.4**). This would indicate an internal production of DON in LSP as NH_4^+ concentration were always low averaging 2μM.

In the south water mass, tributaries were the principal sources of N representing more than 99% of the total N inputs while direct atmospheric deposition was a negligible sources of N. N assimilation by primary producers, the major N retention process beside N_2 production, was variable in the south water mass between the 2 sampling years (**Table 2.5**) representing 93% and 18% of the TDN retained in 2005 and 2006 respectively. N_2 loss through denitrification estimated by the difference between TDN retention and primary producers uptake could account for 6% of TDN retained in 2005 and 82% in 2006 (**Table 2.5**). These calculations allowed us to estimate N_2O: N_2 ratios for the south water masses in 2005 and 2006. Surprisingly, the ratio in the south water mass showed a great inter-annual variability from 1.744% in 2005 to 0.014% in 2006 (**Table 2.5**).

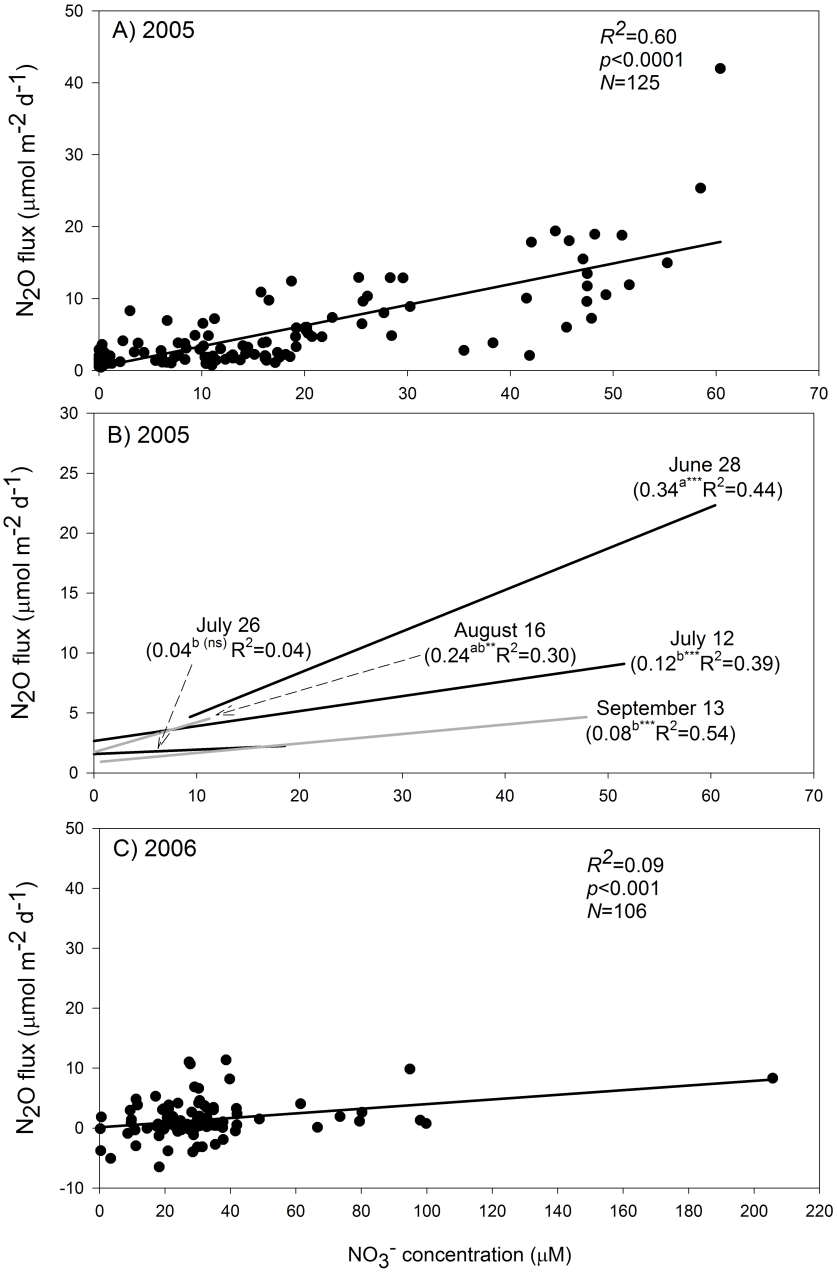


Figure 2.5: Relationships between N_2O flux (µmol m^{-2} d^{-1}) and NO_3^- concentration (µmol L^{-1}) for the entire LSP; A) in 2005 with N_2O flux = $(0.288 \times NO_3^-) + 0.515$. B) per sampling dates in 2005, slopes and adjusted R^2 are indicated in parenthesis. Letters denote ANCOVA results and *** is for P<0.0001, ** for P<0.001 and ns for 'not significant'. C) in 2006 with N_2O flux = $(0.039 \times NO_3^-) + 0.187$.

Table 2.3: Multiple regression results explaining N_2O fluxes in 2005 and 2006. The variable 'Rain' represents the amount of precipitation one week prior to sampling day (in mm) and the DO represents the dissolved oxygen concentration in the water column (in mg L^{-1}) and NO_3^- is the nitrate concentration (μM).

	Coefficient	Standard error	t-value	P	F	N	R^2
Model 2005				0.000	97.755	125	0.701
Intercept	-1.295	1.975	-0.656	0.513			
DO	0.703	0.228	3.079	0.003			
Rain	-0.196	0.031	-6.250	0.000			
NO_3^-	0.242	0.020	12.024	0.000			
Model 2006				0.000	10.954	108	0.159
Intercept	4.724	2.504	1.886	0.062			
DO	-0.752	0.222	-3.385	0.001			
log (NO_3^-+1)	2.572	0.867	2.967	0.004			

Table 2.4: Time integrated estimates of TDN and NO_3^- loaded in by tributaries, loaded out and retained for the summer months in the south water mass of LSP in 2005 (end of June to Mid September) and 2006 (beginning of June to end of August). Errors calculated using error-propagation approach.

		South water mass	
		2005	**2006**
TDN (T day^{-1})	**Tributaries Inputs**	21.6 ±1.5	76.6 ±10.0
	Load out	15.3 ±3.4	59.4 ±18.2
	Retained	6.3 ±1.9	17.2 ±18.9
	(% of inputs retained)	(29.3%)	(22.4%)
NO_3^- (T day^{-1})	**Triputaries Inputs**	12.3 ±1.3	56.9 ±5.8
	Load out	3.3 ±0.8	28.0 ±6.9
	Retained	9.0 ±0.3	28.9 ±7.1
	(% of inputs retained)	(72.9%)	(50.8%)

Table 2.5: Details of TDN inputs and retention terms in the south water mass of LSP in 2005 (end of June to Mid September) and 2006 (beginning of June to end of August). Atmospheric deposition is estimated from areal rate of deposition from Howarth et al (1996). Denitrification (N_2 production) was estimated by difference between total N retention and macrophyte and phytoplankton N uptake. Associated errors are presented and they were estimated using different methods: [a] errors derived from Vis et al. 2007; [b] errors calculated using error-propagation approach; [c] range of N_2O fluxes using the range of k promoted by Raymond and Cole (2001).

		2005	2006
Inputs (T day-1)			
	Atmospheric deposition	0.21	0.15
	Tributaries inputs	21.6 ±1.5	76.6 ±10.0
Retention (T day-1)			
	Total Retained [b]	6.3 ±1.9	17.2 ±18.9
	(% of Inputs)	(29%)	(22%)
	Phytoplankton & Epiphyton [a]	4.87±2.35	2.4±1.17
	(% of total retained)	(77%)	(14%)
	Submerged Macrophytes [a]	1.03±0.06	0.74±0.03
	(% of total retained)	(16%)	(4%)
	Loss as gaseous N_2O [c]	0.0068	0.001877
	(min – max)	(0.004−0.010)	(0.000−0.005)
	Estimated Denitrification [b]	0.39±3.01	14.06±18.94
	(% of total retained)	(6%)	(82%)
N_2O: N_2 ratio [b, c]		1.744	0.014
(min – max)		(1.026−2.564)	(0−0.036)

Cross system analysis

Average N_2O flux and NO_3^- concentrations collected for different rivers represented a wide range of NO_3^- concentrations from very low, as observed in the Amazon (Richey et al. 1988) to very high, as observed the Neuse River (Stow et al. 2005) (**Table 2.6**). The across system relationship between N_2O flux and average NO_3^- concentrations was significant and relatively modest explaining 35% of the variation in N_2O emissions (**Figure 2.6**). In fact systems with very similar NO_3^- concentrations could emit very different amount of N_2O as we observed between Hudson and Potomac Rivers or Tamar and Swale-Ouse Rivers. We hypothesized that these variations in N_2O emissions could be explained by different hydrological conditions in these rivers as it was observed between the two sampling years in the south mass. A multiple regression analysis showed that NO_3^- concentrations combined with hydrological load (HL) could explain 55% of the variability in N_2O with:

$$\log (N_2O_{flux}) = 0.558 \log(NO_3^-) - 0.251 \log(HL) - 1.238 \quad \text{(Eq. 11)}$$

With $R^2 = 0.55$, n=16, F= 10.157 and p=0.002.

The negative intercept is significant with p=0.008, suggesting that in condition of low NO_3^- concentration, the system can be net N_2O consumer.

Table 2.6: Data used for the cross-system analyses. HL : hydraulic load.

Systems	**N_2O Flux** g-N m^{-2} yr^{-1}	**Mean NO_3^-** µmol L^{-1}	**HL** m yr^{-1}	**Reference**
Adyar	0.114	20	201	Rajkumar et al. 2008
Amazon	0.060	4	34	Richey et al. 1988
Colne	0.400	400	29	Robinson et al. 1998
Hudson	0.060	60	60	Cole and Caraco 2001
Humber	0.500	300	40	Barnes and Owens 1998
Kalamazoo	1.245	89	<1	Beaulieu and et al. 2008
LSP	0.020	18	55	This study - data from 2005
Millstone	0.442	146	110	Laursen and Seitzinger 2004
Neuse	0.169	9	18	Stow et al. 2005
Potomac	0.700	70	12	McElroy et al. 1978
Seine	0.600	300	122	Garnier et al. 2006
South Platte	0.700	400	4	McMahon and Dennehy 1999
Swale-Ouse	2.649	138	18	Garcia-Ruiz et al. 1999
Tamar	0.100	150	78	Law and et al. 1992
Yangtze	0.635	103	95	Wang et al. 2007
Yaqui	3.520	220	<1	Harrison and Matson 2003

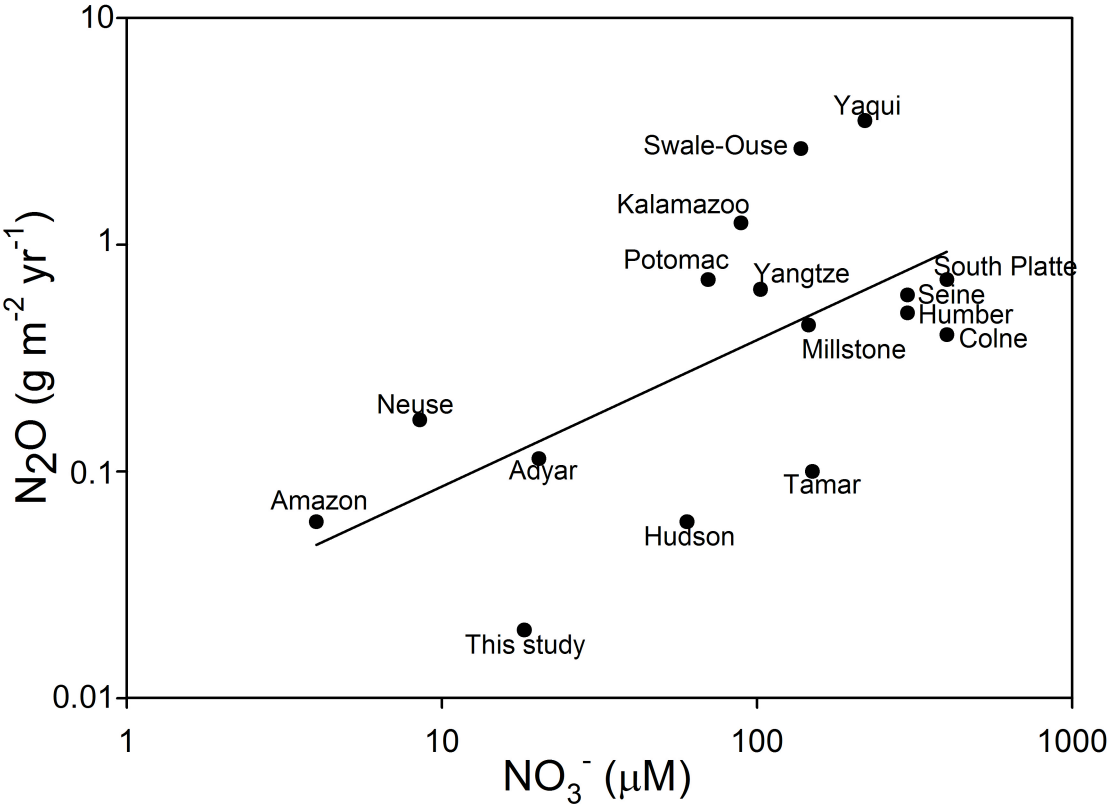


Figure 2.6: Log-log relationship between N_2O flux (μmol m^{-2} d^{-1}) and mean NO_3^- concentration (μM) for 16 rivers and estuaries around the world. Linear regression for the 16 systems: $\log N_2O = (0.64 \times \log NO_3^-) - 1.71$ ($R^2 = 0.35$, n=16, F= 9.14, p=0.009).

2.5. Discussion

N_2O source to the atmosphere

Our study showed that LSP is a net source of N_2O to the atmosphere. However summer fluxes were quite modest (mean: 5.19 and 1.86 μmol m^{-2} d^{-1} in 2005 and 2006 respectively). They were on the rather low end as compared to N_2O emissions for several nutrient rich small sized to large rivers including the Assabet River (Hemond and Duran 1989), Platte River (McMahon and Dennehy 1999) and Ohio River (Beaulieu et al. 2010) and several estuaries including Seine estuary (Garnier et al. 2006), Humber estuary (Barnes and Owens 1998) and Yangtze estuary (Wang et al. 2007). These systems exhibited N_2O emissions on average greater than 70 μmol m^{-2} d^{-1} while N_2O fluxes in this study were similar to values around 5.5 μmol m^{-2} d^{-1} reported in the Hudson River (Cole and Caraco 2001) and Amazon Rivers (Richey et al. 1988).

Spatial and seasonal patterns

LSP did not exhibit obvious seasonal pattern but our peak fluxes appeared rather early in the season, in the beginning of the summer for both years, albeit less pronounced for 2006. Even though most rivers have their maximum of N_2O emissions during the summer (Garcia-Ruiz et al. 1998, Cole and Caraco 2001, Beaulieu et al. 2010), seasonal patterns reported in the literature seem to differ significantly among ecosystems. For example the highest N_2O fluxes have typically been observed later in the summer for the Hudson River (Cole and Caraco 2001), the Humber estuary (Barnes and Owens 1998), and in subtropical agricultural streams (Harrison and Matson 2003) while several headwater streams in the Kalamazoo River Basin experienced their higher N_2O production rates during the winter (Beaulieu et al. 2009). The absence of a clear seasonal pattern was also reported in a study that determined the N_2O fluxes of 12 streams over the course of two years (Beaulieu et al. 2009). In this case, no distinct repeatable pattern was observed between the two years and N_2O production seemed to vary more as a function of N loading.

Spatially there was no significant pattern in LSP, although on average fluxes were typically higher and more variable in the south and north mass, where NO_3^- and TDN concentrations were also higher and more variable over the course of the open water season. Peaks of N_2O emissions were often located after point source inputs typically associated with sewage loading (Brion and Billen 2000, Cebron et al. 2005, Beaulieu et al. 2010). It is possible that agricultural tributaries (Richelieu, Yamaska and St. François rivers) contributed to the south mass N_2O emissions. However our low measurements of N_2O fluxes in these tributaries for 2006 did not support this hypothesis. We observed a N_2O emissions increase after the tributaries inputs but N_2O fluxes seemed to follow the increase of NO_3^- concentrations in the water column.

Controlling factors

In 2005, we found our estimated N_2O fluxes could be predicted reasonably well from NO_3^- concentrations in our large river system. This result is consistent with other relationships observed in small headwater streams (Beaulieu et al. 2009) and larger rivers (Garcia-Ruiz et al. 1999, McMahon and Dennehy 1999, Stow et al. 2005). In our case NO_3^- concentration could explain up to 60% of the variability of the estimated flux, which fall within the range of variation explained in the afore mentioned studies, varying between 29% in small streams and 89% in the Swale-Ouse River. The response of N_2O emissions to increase in NO_3^- concentrations and the significant retention in the system suggested that denitrification rather than nitrification could be responsible for most of the N_2O production in 2005. High NO_3^- concentrations are known to inhibit partially N_2O reductase in sediments and cause accumulation of N_2O and decrease of the subsequent N_2 production (Terry and Tate 1980, Knowles 1982).

DO and precipitation also influenced N_2O fluxes in 2005 with N_2O fluxes positively related to DO and negatively to precipitation. The positive relation between water column DO and N_2O emissions is counterintuitive since denitrification is performed under low oxygen concentration (Knowles 1982). There are three possible explanations for the positive effect of DO concentrations on N_2O production: 1) the higher DO concentration reflects periods or areas of intense local primary production and therefore an increase in the

availability of more labile organic substrates through autochthonous production for denitrifying bacteria (Kritzberg et al. 2005); 2) oxygen delivery to the macrophytes roots by enhanced coupled nitrification-denitrification and supplied labile C exudates to the sediments (Brix 1997); 3) higher DO concentration inhibited the N_2O reductase during denitrification causing an accumulation of N_2O (Zumft 1997) or 4) increase in DO concentration promote N_2O production by nitrification. Conditions were considerably drier and warmer in 2005, as evidenced by the reduced flow in the north and south water masses, which increased the proportion of N retained, and the average summer N_2O flux. If we look more closely at the pattern observed in 2005, there was a significant rain event in early June resulting in a pulse of N loading into the system which likely resulted in the peak flux observed in the end of June 2005. This type of pulse event was not observed at any other time during the sampling period. This higher flux observed in June really drove the overall relationship derived in 2005, and it is the combination of a large nutrient pulse followed by slower flowing conditions that resulted in this relatively hot-moment of N_2O flux.

The moderately strong relationship between N_2O fluxes and NO_3^- concentrations observed in 2005 was not repeated in 2006 where the relationship between these two variables was quite poor. The lack of a relationship between NO_3^- concentrations and N_2O emissions has been reported elsewhere (Beaulieu et al. 2010 and Beaulieu et al. 2011). Contrary to these previous studies, our poor relationship was not due to a narrow range of NO_3^- concentrations as this range was even greater in 2006. Our results indicated that N_2O fluxes in 2006 were slightly better explained (R^2= 0.17) when DO was included as a predictor variable but in this case the relation with N_2O fluxes was negative.

N_2O emissions peaks

N_2O emissions from inland waters remain on average quite modest when compared to agricultural soils where emissions span 2 orders of magnitude, varying between 2.2 (Mosier et al. 1996) and 328 μmol m^{-2} d^{-1} (Flessa et al. 1995). In agricultural soils systems, where nitrification is considered the major N_2O emission process, emission peaks were observed in relation with increase NO_3^- or urea loads after manuring operations, or after winter thaws but also with soil wetness and precipitation (Firestone et al. 1980, Franken et

al. 1992). Comparable and even higher values had been reported for peaks of N_2O emissions in freshwater systems with 10 000 μmol m^{-2} d^{-1} in Humber estuary, 457 μmol m^{-2} d^{-1} for small streams (Beaulieu et al. 2008) and 735 μmol m^{-2} d^{-1} in littoral zone of hyper-eutrophic lake (Wang et al. 2006). In LSP, our peak moment where the highest average emissions occured in early summer of 2005 when NO_3^- concentrations in the system were high, but discharge was particularly low. The discharge rate in the two weeks that preceded the observed N_2O peak was the highest observed during the entire study period, increasing N load to the south water mass. This flash flood event may have stimulated denitrification and N_2O emissions as observed in microbial mats where a pulse of inorganic N load during runoff events triggered a rapid and high denitrification response (Joye and Paerl 1993).

Hydrology and N retention

The high N_2O emissions observed in 2005 are intricately linked with the differences in overall hydrology as compared to 2006. Using a mass balance approach we were able to look at the total amount of N retained in 2005 and 2006, specifically in the south water mass. Eventhough amount of N retained in 2005 (6 T day^{-1}) and 2006 (17 T day^{-1}) were different, they were relatively, was close to the average N depletion of 10.1 T day^{-1} reported for summer 2004 in the south water mass (Hudon and Carignan 2008). We also observed a greater amount and percent of NO_3^- retained as compared with TDN. Comparison of N retention estimates in flowing systems showed that loss rate reported as NO_3^- or dissolved inorganic N were generally higher than in-stream loss rate reported for TN (Alexander et al. 2000). In LSP, part of the N consumed by plants must be exported as organic N and the discrepancy between NO_3^- and TDN retention is likely due to the conversion of dissolved inorganic N to particulate or dissolved organic forms of N within the lake that are subsequently exported out of the system. The rate of exchange among different N forms may indeed be very rapid, quickly altering different N pools during transit.

We observed an important difference in N load between the two years. In the summer of 2005, the amount of N that entered the south water mass was 3.5 times less as compared to the summer 2006. However the proportion of N load retained in both years were within the range of what is reported in published models for large rivers (Alexander et

al. 2000, Saunders and Kalff 2001) even though retention in 2005 was slitly superior (29 versus 22% in 2006). In large rivers, NO_3^- uptake and denitrification was showed to increase with stream NO_3^- concentration. However the efficiency of biotic uptake and denitrification appears to decline when concentrations increase, reducing the proportion of internal NO_3^- removal in high N load conditions (Mulholland et al. 2008). The reduction of the relative retention in 2006 could correspond to the higher N load scenario described by Mulholland et al. (2008).

The difference in rates of TDN retention between the two years was illustrated by their different uptake velocity (V_f) which was calculated as reported in Harrison et al. (2009). V_f estimated at 19 and 112 m yr^{-1} in 2005 and 2006 respectively. Conceptually, V_f is the piston velocity for N removal where N migrates from the water column to the sediment and is removed via denitrification or burial in sediments (Alexander et al. 2009). The 6-fold increase of V_f in the south water mass followed by only a 2-fold increase of its hydraulic load (HL) indicated that V_f increased rapidly contrary to the RivR-N model premises where N removal declines relatively slowly with HL, requiring increased areal biological activity at higher HL's (V_f increases from 2 to 300 m yr^{-1} over HL of 1–10000 m m yr^{-1}) (Seitzinger et al. 2002, Wollheim et al. 2006). Despite a higher N retention rate in 2005 compared to 2006, the actual amount of N retained was larger in 2006, confirming the need to better assess the role of large ecosystems receiving high loads of nutrients in N export to coastal ecosystems (Tank et al. 2008).

Interestingly in dryer years the areal extent of the southern water mass expanded by 10% while maintaining the same average depth (1.1 m) but doubling its retention time. This was due to a reduced spill over of the central water mass to the shallower areas on its southern side which were taken over by waters influenced by southern tributaries (Vis et al. 2007). This greater areal extent is the likely explanation for the estimated higher observed plant uptake in 2005 versus 2006, which resulted in proportionately less N estimated as being lost to denitrification in 2005.

Denitrification and N_2O yield

Given the increasing amount of N loaded to aquatic systems, N lost to denitrification would appear favourable, but a high N_2 yield would not. A review by Schlesinger (2009) clearly indicated that aquatic systems (mean N_2O: N_2 ratio of 8%) had lower yields than terrestrial systems (mean ratio in agricultural soils: 37%). In LSP however, N lost as gaseous N_2O emissions was small in the overall budget of the lake regardless of the year representing less than 2% of denitrification N losses. Discrepancy between ratios from Schlesinger et al. (2009) and LSP can be explained by the low-order of the streams used in the review. Measurements of potential denitrification and N_2 production in the Seine drainage network found that N_2O: N_2 ratios were smaller in large order rivers as compared to headwater streams (Garnier et al. 2010). Beaulieu et al. (2011) also found *in situ* denitrification N_2O yield was generally under 1% in aquatic systems with other N_2O sources (nitrification, groundwater) causing remaining emissions. The lower N_2O yield in the LSP could then also be explained by denitrification which we assume was the major process producing N_2O in the system rather than nitrification.

However we found N_2O: N_2 ratios varied inter-annually with a ratio more than 100-fold higher in 2005. It is currently unclear what factors might regulate variation in N_2O: N_2 production in LSP but we hypothesized that hydrological difference between the two years may have influenced N_2O: N_2 ratios. Since we were not able to differentiate between N_2O produced by nitrification from N_2O produced by denitrification, we assumed that N_2O was produced predominantly by denitrification in LSP, without ruling out a potential source of N_2O from nitrification particularly in 2005 where N_2O yield was greater than 1%. Model results indicated N_2O emissions from rivers were controlled by increased inorganic nitrogen loading accelerating nitrogen cycling processes in aquatic ecosystems (Kroeze and Seitzinger 1998, Laursen and Seitzinger 2004). Unexpectedly we found a lower N_2O: N_2 ratio in 2006 when N load was higher. The lower N_2O: N_2 ratio could be related to the high V_f and HL of the south water mass in 2006 with shorter residence time resulting in a smaller ratio of benthic surface area to water volume. The higher benthic surface to water

ratio in 2005 may have stimulated the coupled nitrification and denitrification accounting for higher N_2O: N_2 ratio estimated in 2005.

N_2O emissions across systems

Relationships between N_2O and NO_3^- have been observed within other and among different rivers and estuaries (Garcia-Ruiz et al. 1999, Cole and Caraco 2001, Stow et al. 2005), but these relationships were typically not very strong. When we added the variable HL into the model, predictive power of the relationship was enhanced by 20%. We found a negative relationship between N_2O emissions and HL and a negative intercept. In the case of stagnant conditions with a nitrate concentration near zero, we then expect net N_2O consumption. However N_2O consumption would be greater in higher HL condition that is when the system is deep or the water residence time is short. High HL reduces the contact between water and sediment consequently reducing potential N_2O production by coupled nitrification denitrification.

Different studies have shown that hydraulic load is inversely proportional to the percentage of TN retained (Saunders and Kalff 2001, Seitzinger et al. 2006, Harrison et al. 2009), which is consistent with greater relative removal via denitrification. Indeed, consistent with this observation, we found that systems with lower hydraulic loads had higher N_2O emissions per unit NO_3^- so are leakier, relative to systems with higher ones. These results supported the higher N_2O emissions relative to N_2 measured in 2005 as compared to 2006.

In the St Lawrence River, climate change are predicted to promote more frequent episodes of reduced water levels but also more variation in water discharge with an increase of flood events (Hudon 1997). Our results prove the strong influence of hydrology on N cycling and the relative amount of N_2O to N_2 emitted from aquatic systems. However how hydrology and land use may influence the relative amount of N_2O produced to N removed remains an open question. A better understanding of the factors controlling the potential variability in the N_2O: N_2 ratio of emissions would undoubtedly improve global N_2O emission models from freshwater aquatic ecosystems and provide a better management tool in order to reduce GHG emissions from rivers and aquatic systems in general.

2.6. References

Alexander, R. B., J. K. Bohlke, E. W. Boyer, M. B. David, J. W. Harvey, P. J. Mulholland, S. P. Seitzinger, C. R. Tobias, C. Tonitto, and W. M. Wollheim. 2009. Dynamic modeling of nitrogen losses in river networks unravels the coupled effects of hydrological and biogeochemical processes. Biogeochemistry 93:91-116.

Alexander, R. B., R. A. Smith, and G. E. Schwarz. 2000. Effect of stream channel size on the delivery of nitrogen to the Gulf of Mexico. Nature 403:758-761.

Anderson, C., and G. Cabana. 2006. Does delta N-15 in river food webs reflect the intensity and origin of N loads from the watershed? Science of the Total Environment 367:968-978.

Barnes, J., and N. J. P. Owens. 1998. Denitrification and nitrous oxide concentrations in the Humber estuary, UK, and adjacent coastal zones. Marine Pollution Bulletin 37:247-260.

Beaulieu, J. J., C. P. Arango, S. K. Hamilton, and J. L. Tank. 2008. The production and emission of nitrous oxide from headwater streams in the Midwestern United States. Global Change Biology 14:878-894.

Beaulieu, J. J., C. P. Arango, and J. L. Tank. 2009. The effects of season and agriculture on nitrous oxide production in headwater streams. Journal of environmental quality 38:637-646.

Beaulieu, J. J., W. D. Shuster, and J. A. Rebholz. 2010. Nitrous oxide emissions from a large, impounded river: The Ohio River. Environmental Science & Technology 44:7527-7533.

Beaulieu, J. J., J. L. Tank, S. K. Hamilton, W. M. Wollheim, R. O. Hall, P. J. Mulholland, B. J. Peterson, L. R. Ashkenas, L. W. Cooper, C. N. Dahm, W. K. Dodds, N. B. Grimm, S. L. Johnson, W. H. McDowell, G. C. Poole, H. M. Valett, C. P. Arango, M. J. Bernot, A. J. Burgin, C. L. Crenshaw, A. M. Helton, L. T. Johnson, J. M. O'Brien, J. D. Potter, R. W. Sheibley, D. J. Sobota, and S. M. Thomas. 2011. Nitrous oxide emission from denitrification in stream and river networks. Proceedings of the National Academy of Sciences 108:214-219.

Brion, N., and G. Billen. 2000. Wastewater as a source of nitrifying bacteria in river systems: the case of the River Seine downstream from Paris. Water Research 34:3213-3221.

Brix, H. 1997. Do macrophytes play a role in constructed treatment wetlands? Water Science and Technology 35:11-17.

Canada, S. 2001. Agriculture census data.

Cebron, A., J. Garnier, and G. Billen. 2005. Nitrous oxide production and nitrification kinetics by natural bacterial communities of the lower Seine River (France). Aquatic Microbial Ecology 41:25-38.

Cole, J. J., and N. F. Caraco. 1998. Atmospheric exchange of carbon dioxide in a low-wind oligotrophic lake measured by the addition of SF_6. Limnology and Oceanography 43:647-656.

Cole, J. J., and N. F. Caraco. 2001. Emissions of nitrous oxide (N_2O) from a tidal, freshwater river, the Hudson River, New York. Environmental Science & Technology 35:991-996.

Dong, L. F., D. B. Nedwell, G. J. C. Underwood, D. C. O. Thornton, and I. Rusmana. 2002. Nitrous oxide formation in the Colne estuary, England: The central role of nitrite. Applied and Environmental Microbiology 68:1240-1249.

Firestone, M. K., and E. A. Davidson. 1989. Microbial basis of NO and N_2O production and consumption in soil. Pages 7-21 in M. O. Andreae and D. S. Schimel, editors. Exchange of trace gases between terrestrial ecosystems and the atmosphere. John Wiley, New York.

Firestone, M. K., R. B. Firestone, and J. M. Tiedje. 1980. Nitrous oxide from soil denitrification - Factors controlling its biological productionI. Science 208:749-751.

Flessa, H., P. Dorsch, and F. Beese. 1995. Seasonal variation of N_2O and NH_4 fluxes in differently managed arable soils in southern Germany. Journal of Geophysical Research-Atmospheres 100:23115-23124.

Franken, R. O. G., W. Vanvierssen, and H. J. Lubberding. 1992. Emissions of some greenhouse gases from aquatic and semiaquatic ecosystems in the Netherlands and options to control them. Science of the Total Environment 126:277-293.

Frenette, J. J., M. T. Arts, and J. Morin. 2003. Spectral gradients of downwelling light in a fluvial lake (Lake Saint-Pierre, St-Lawrence River). Aquatic Ecology 37:77-85.

Galloway, J. N., J. D. Aber, J. W. Erisman, S. P. Seitzinger, R. W. Howarth, E. B. Cowling, and B. J. Cosby. 2003. The nitrogen cascade. Bioscience 53:341-356.

Garcia-Ruiz, R., S. N. Pattinson, and B. A. Whitton. 1998. Denitrification and nitrous oxide production in sediments of the Wiske, a lowland eutrophic river. Science of the Total Environment 210:307-320.

Garcia-Ruiz, R., S. N. Pattinson, and B. A. Whitton. 1999. Nitrous oxide production in the river Swale-Ouse, North-East England. Water Research 33:1231-1237.

Garnier, J., A. Cebron, G. Tallec, G. Billen, M. Sebilo, and A. Martinez. 2006. Nitrogen behaviour and nitrous oxide emission in the tidal Seine River estuary (France) as influenced by human activities in the upstream watershed. Biogeochemistry 77:305-326.

Garnier, J. A., E. M. Mounier, A. M. Laverman, and G. F. Billen. 2010. Potential denitrification and nitrous oxide production in the sediments of the Seine River drainage network (France). Journal of Environmental Quality 39:449-459.

Harrison, J., and P. Matson. 2003. Patterns and controls of nitrous oxide emissions from waters draining a subtropical agricultural valley. Global Biogeochemical Cycles 17.

Harrison, J. A., R. J. Maranger, R. B. Alexander, A. E. Giblin, P. A. Jacinthe, E. Mayorga, S. P. Seitzinger, D. J. Sobota, and W. M. Wollheim. 2009. The regional and global significance of nitrogen removal in lakes and reservoirs. Biogeochemistry 93:143-157.

Hemond, H. F., and A. P. Duran. 1989. Fluxes of N_2O at the Sediment-Water and Water-Atmosphere Boundaries of a Nitrogen-Rich River. Water Resources Research 25:839-846.

Howarth, R. W., G. Billen, D. Swaney, A. Townsend, N. Jaworski, K. Lajtha, J. A. Downing, R. Elmgren, N. Caraco, T. Jordan, F. Berendse, J. Freney, V. Kudeyarov, P. Murdoch, and Z. L. Zhu. 1996. Regional nitrogen budgets and riverine N and P fluxes for the drainages to the North Atlantic Ocean: Natural and human influences. Biogeochemistry 35:75-139.

Hudon, C. 1997. Impact of water level fluctuations on St. Lawrence River aquatic vegetation. Canadian Journal of Fisheries and Aquatic Sciences 54:2853-2865.

Hudon, C., and R. Carignan. 2008. Cumulative impacts of hydrology and human activities on water quality in the St. Lawrence River (Lake Saint-Pierre, Quebec, Canada). Canadian Journal of Fisheries and Aquatic Sciences 65:1165–1180

Jahne, B., K. O. Munnich, R. Bosinger, A. Dutzi, W. Huber, and P. Libner. 1987. On the parameters influencing air-water gas-exchange. Journal of Geophysical Research-Oceans 92:1937-1949.

Joye, S. B., and H. W. Paerl. 1993. Contemporaneous nitrogen fixation and denitrification in intertidat microbial mats - Rapid response to runoff events. Marine Ecology-Progress Series 94:267-274.

Knowles, R. 1982. Denitrification. Microbiological Reviews 46:43-70.

Kritzberg, E. S., J. J. Cole, M. M. Pace, and W. Graneli. 2005. Does autochthonous primary production drive variability in bacterial metabolism and growth efficiency in lakes dominated by terrestrial C inputs? Aquatic Microbial Ecology 38:103-111.

Kroeze, C., and S. P. Seitzinger. 1998. Nitrogen inputs to rivers, estuaries and continental shelves and related nitrous oxide emissions in 1990 and 2050: a global model. Nutrient Cycling in Agroecosystems 52:195-212.

Laursen, A. E., and S. P. Seitzinger. 2004. Diurnal patterns of denitrification, oxygen consumption and nitrous oxide production in rivers measured at the whole-reach scale. Freshwater Biology 49:1448-1458.

Law, C. S., A. P. Rees, and N. J. P. Owens. 1992. Nitrous oxide - Estuarine sources and atmospheric flux. Estuarine Coastal and Shelf Science 35:301-314.

Matson, P. A., and P. M. Vitousek. 1990. Ecosystem approach to a global nitrous oxide budget. Bioscience 40:667-671.

McElroy, M. B., J. W. Elkins, S. C. Wofsy, C. E. Kolb, A. P. Duran, and W. A. Kaplan. 1978. Production and release of N_2O from Potomac Estuary. Limnology and Oceanography 23:1168-1182.

McMahon, P. B., and K. F. Dennehy. 1999. N_2O emissions from a nitrogen-enriched river. Environmental Science & Technology 33:21-25.

Ministère du développement durable, de l'environnement et des parcs. 2006b. BQMA, Banque de données sur la qualité du milieu aquatique. Direction du suivi de l'environnement, Ministère du développement durable, de l'environnement et des parcs. Québec, Québec.

Ministère de l'Environnement. 2003. Synthèse des informations environnementales disponibles en matière agricole au Québec. Direction des politiques du secteur agricole, Ministère de l'environnement, Québec, Envirodoq ENV/2003/0025.

Mosier, A., C. Kroeze, C. Nevison, O. Oenema, S. Seitzinger, and O. van Cleemput. 1998. Closing the global N2O budget: nitrous oxide emissions through the agricultural nitrogen cycle - OECD/IPCC/IEA phase II development of IPCC guidelines for national greenhouse gas inventory methodology. Nutrient Cycling in Agroecosystems 52:225-248.

Mulholland, P. J., A. M. Helton, G. C. Poole, R. O. Hall, S. K. Hamilton, B. J. Peterson, J. L. Tank, L. R. Ashkenas, L. W. Cooper, C. N. Dahm, W. K. Dodds, S. E. G. Findlay, S. V. Gregory, N. B. Grimm, S. L. Johnson, W. H. McDowell, J. L. Meyer, H. M. Valett, J. R. Webster, C. P. Arango, J. J. Beaulieu, M. J. Bernot, A. J. Burgin, C. L. Crenshaw, L. T. Johnson, B. R. Niederlehner, J. M. O/'Brien, J. D. Potter, R. W. Sheibley, D. J. Sobota, and S. M. Thomas. 2008. Stream denitrification across biomes and its response to anthropogenic nitrate loading. Nature 452:202-205.

Rajkumar, A. N., J. Barnes, R. Ramesh, R. Purvaja, and R. C. Upstill-Goddard. 2008. Methane and nitrous oxide fluxes in the polluted Adyar River and estuary, SE India. Marine Pollution Bulletin 56:2043-2051.

Raymond, P. A., and J. J. Cole. 2001. Gas exchange in rivers and estuaries: Choosing a gas transfer velocity. Estuaries 24:312-317.

Richey, J. E., A. H. Devol, S. C. Wofsy, R. Victoria, and M. N. G. Riberio. 1988. Biogenic Gases and the Oxidation and Reduction of Carbon in Amazon River and Floodplain Waters. Limnology and Oceanography 33:551-561.

Robinson, A. D., D. B. Nedwell, R. M. Harrison, and B. G. Ogilvie. 1998. Hypernutrified estuaries as sources of N2O emission to the atmosphere: the estuary of the River Colne, Essex, UK. Marine Ecology-Progress Series 164:59-71.

Rooney, N., J. Kalff, and C. Habel. 2003. The role of submerged macrophyte beds in phosphorus and sediment accumulation in Lake Memphremagog, Quebec, Canada. Limnology and Oceanography 48:1927-1937.

Saunders, D. L., and J. Kalff. 2001. Nitrogen retention in wetlands, lakes and rivers. Hydrobiologia 443:205-212.

Schlesinger, W. H. 2009. On the fate of anthropogenic nitrogen. Proceedings of the National Academy of Sciences of the United States of America 106:203-208.

Seitzinger, S., J. A. Harrison, J. K. Bohlke, A. F. Bouwman, R. Lowrance, B. Peterson, C. Tobias, and G. Van Drecht. 2006. Denitrification across landscapes and waterscapes: A synthesis. Ecological Applications 16:2064-2090.

Seitzinger, S. P., and C. Kroeze. 1998. Global distribution of nitrous oxide production and N inputs in freshwater and coastal marine ecosystems. Global Biogeochemical Cycles 12:93-113.

Seitzinger, S. P., C. Kroeze, and R. V. Styles. 2000. Global distribution of N_2O emissions from aquatic systems: natural emissions and anthropogenic effects. Chemosphere - Global Change Science 2:267-279.

Seitzinger, S. P., R. V. Styles, E. W. Boyer, R. B. Alexander, G. Billen, R. W. Howarth, B. Mayer, and N. Van Breemen. 2002. Nitrogen retention in rivers: model development and application to watersheds in the northeastern USA. Biogeochemistry 57:199-237. Smith, R. L., J. K. Bohlke, D. A. Repert, and C. P. Hart. 2009. Nitrification and denitrification in a midwestern stream containing high nitrate: in situ assessment using tracers in dome-shaped incubation chambers. Biogeochemistry 96:189-208.

Stehfest, E., and L. Bouwman. 2006. N_2O and NO emission from agricultural fields and soils under natural vegetation: summarizing available measurement data and modeling of global annual emissions. Nutrient Cycling in Agroecosystems 74:207-228.

Stainton, M.P., Capel, M.J., and Armstrong, F.A.J. 1977. The chemical analysis of fresh water. 2nd ed. Canadian Fisheries and Marine Service Special Publication, no. 25. Department of Fisheries and Oceans, Winnipeg.

Stow, C. A., J. T. Walker, L. Cardoch, P. Spence, and C. Geron. 2005. N_2O emissions from streams in the Neuse River watershed, North Carolina. Environmental Science & Technology 39:6999-7004.

Tank, J. L., E. J. Rosi-Marshall, M. A. Baker, and R. O. Hall. 2008. Are rivers just big streams? A pulse method to quantify nitrogen demand in a large river Ecology 89:2935-2945.

Terry, R. E., and R. L. Tate. 1980. The effect of nitrate on nitrous oxide reduction in organic soils and sediments. Soil Science Society of America Journal 44:744-746.

Vis, C., C. Hudon, R. Carignan, and P. Gagnon. 2007. Spatial analysis of production by macrophytes, phytoplankton and epiphyton in a large river system under different water-level conditions. Ecosystems 10:293-310.

Wang, D. Q., Z. L. Chen, J. Wang, S. Y. Xu, H. X. Yang, H. Chen, L. Y. Yang, and L. Z. Hu. 2007. Summer-time denitrification and nitrous oxide exchange in the intertidal zone of the Yangtze Estuary. Estuarine Coastal and Shelf Science 73:43-53.

Wang, H. J., W. D. Wang, C. Q. Yin, Y. C. Wang, and J. W. Lu. 2006. Littoral zones as the "hotspots" of nitrous oxide (N_2O) emission in a hyper-eutrophic lake in China. Atmospheric Environment 40:5522-5527.

Weiss, R. F., and B. A. Price. 1980. Nitrous oxide solubility in water and seawater. Marine Chemistry 8:347-359.

Wollheim, W. M., C. J. Voosmarty, B. J. Peterson, S. P. Seitzinger, and C. S. Hopkinson. 2006. Relationship between river size and nutrient removal. Geophysical Research Letters 33.

Zumft, W. G. 1997. Cell biology and molecular basis of denitrification. Microbiology and Molecular Biology Reviews 61:533-616.

3. Net N_2 Fluxes in Cyanobacterial Mats – Rates and Controls in a Large River Ecosystem

En préparation pour soumission.

Auteures : Laure Tall et Roxane Maranger

3.1. Abstract

Cyanobacteria mats are found in a diverse range of nutrient enriched coastal and shallow freshwater environments where they are reported to be intense site of nitrogen transformations. In large riverine ecosystems with increasing nutrient inputs, areas experiencing nitrogen (N) depletion events can be colonized by cyanobacterial mats and little is know on their N cycling rates. Using benthic chambers installed in a large but shallow river ecosystem, the St Lawrence River (Quebec, Canada), we examined the effect of cyanobacterial mats, constituted of the filamentous non heterocystous *Lyngbya wollei*, on dinitrogen (N_2) fluxes at the water-sediment interface. *Lyngbya* mats were a net source of N with high N_2 fluxes averaging -846 μmol-N m^{-2} h^{-1} (negative N_2 fluxes equivalent to net fixation). Surprisingly oxygen flux, water column oxygen concentration and temperature, rather than nitrogen flux or concentration, were the principal factors controlling N_2 fluxes. Using these factors, we were able to explain up to 41 percent of the variation in N_2 fluxes. Given that fixation was occurring during daylight and that oxygen concentrations in the water column were supersaturated, we hypothesized that N_2 fixation could be performed by the dominant cyanobacteria *Lyngbya* in anoxic micro-zone of the mat and/ or possibly by heterotrophic diazotrophs. Our estimates indicated that N fixation by *Lyngbya* mats could account for the replacement of up to 33 percent of the N loss via denitrification in the entire ecosystem during the study period, from June to October, and represent 9% annual input to the system.

3.2. Introduction

Cyanobacterial mats are ubiquitous and can occupy disparate environments (Cohen and Rosenberg 1989). In freshwater riverine ecosystems, nitrogen dynamics and particularly N fixation in benthic mats havave not been addressed so far, probably because these systems are not believed to be limited by N availability. The current paradigme suggests that N is the primary limiting nutrient in terrestrial and marine ecosystems (Howarth et al. 1988a, Vitousek and Howarth 1991) and P is the limiting nutrient in lakes (Schindler 1977) and freshwater in general. However, more recent work demonstrates seasonal N limitation in streams (Francoeur 2001) and possible N and P co-limitation in lakes (Elser et al. 2007, Lewis and Wurtsbaugh 2008, Scott and McCarthy 2010) at least on a temporal scale.

In cyanobacteria mats, cyanobacteria filaments provide the structure to support a large range of other organisms like prokaryotes (i.e. heterotrophic, phototrophic, N_2 fixers or denitrifiers) and eukaryotes (i.e. diatoms) (Zehr et al. 1995, Severin et al. 2010). Cyanobacteria are oxygenic phototrophic bacteria and many species of cyanobacteria are capable of fixing atmospheric dinitrogen (N_2) (Stewart 1980). The array of metabolic and functional groups co-existing in the mats allow coupling of processes and exchange of substrates among organisms, which extend the physiological range from photoautotrophy to heterotrophy within small spatial scales (Paerl et al. 1989, Joye and Paerl 1993). According to a recent review by Stal et al. (2010), all cyanobacterial mats have the capacity to fix N_2 and thus can be sources of new fixed N to their environment. In fact, cyanobacterial mats in marine and coastal ecosystems experienced very high rates of N_2 fixation (Bergman et al. 1997, Fiore et al. 2010) and ecosystems showing the highest rates of N fixation were often dominated by heterocystous cyanobacteria (Howarth et al. 1988b, Stal 2003). In contrast, relatively few studies (Joye and Paerl 1994, Minjeaud et al. 2009) have estimated the losses of fixed N to N_2 gas via denitrification in mats were N fixing and denitrifing organisms co-existed.

The sub-basin of the St. Lawrence River (SLR) from Lake Ontario to Quebec City is well urbanized and receives the wastewater and municipal effluents from over 3 million inhabitants. In this fertile area, agriculture has been intensified and tributaries like the Ottawa River, or southern

Quebec rivers (i.e. Richelieu, Yamaska, St. François) draining farmland represent important sources of nutrients (Hudon and Carignan 2008). As a result, SLR is receiving an increasing amount of nutrients on a yearly basis. This combined with low flow periods; fluvial lakes of SLR are experiencing drastic nitrate reduction events during the summer (Hudon and Carignan 2008). These nitrate availability gradients are developed along the flow path of water due to plant uptake and denitrification (Tall and Maranger, Chapter 2). In two fluvial lakes of the SLR, Lake St. Pierre (LSP) and Lake St. Louis (LSL), nitrate depletion has been linked to the apparition of large benthic mats of *Lyngbya wollei* a filamentous cyanobacteria. Our goals were: 1) to estimate N_2 fluxes in the cyanobacterial mats of LSL and LSP; 2) to evaluate the importance of these fluxes at the mat scale and relative to the larger ecosystem N cycling. We hypothesized that *Lyngbya* mats because of their epiphytic bacteria community would alternate between being net fixers to net denitrifiers and the change would be controlled by nitrogen availability. *Lyngbya* mats would then be able to aleviate N limitation at their local scale.

3.3. Materials and methods

Study sites

Our study was conducted in two fluvial lakes of the St. Lawrence River: Lake St. Pierre (LSP) and Lake St. Louis (LSL). LSP is a large (>300 km^2) and shallow (3 m depth except in its navigation channel) widening of the St. Lawrence River located 65 km downstream of Montreal, Canada (**Figure 3.1**). In LSP, we sampled in the littoral zone along the south shore. This section of LSP is under the influence of the St. François and Yamaska Rivers, which both have a drainage basin heavily impacted by farmland. This area is occupied by large beds of aquatic vegetation and abundant communities of filamentous green and cyanobacterial algae (Vis et al. 2008). LSL is also a shallow ecosystem (3m depth) and a smaller (155 km^2) widening of the SLR located upstream of LSP. LSL is adjoining to the island of Montreal at the confluence of the Ottawa and SLR (**Figure 3.1**). LSL is bounded on its north and east shores by the island of Montreal. In LSL, we sampled on the north shore, which receives inputs mainly from the Ottawa River and exchanges water with SLR. The north shore is mostly built up with private houses and its drainage basin is primarily urban and industrial. Our experiments were made in 2006 and 2007

in LSP from the beginning of the summer to early fall, and in 2008 and 2009 in LSL in early fall only (**Table 3.1**) when extensive cyanobacterial mats were present.

Lyngbya wollei

Microscopic mat structure was observed with a FEI Quanta 200-3D scanning electron microscope under high vacuum at 7 KV. In situ, mat samples were immersed in a formalin - acid acetic- alcohol (FAA) fixative solution for 2 hours. After that, they were dehydrated in increasing concentrations of ethanol (30% to 100%) and dried using the critical point drying method with a Polaron E-3000. We mounted samples on stubs and coated them with gold and palladium under Technics Hummer II sputter-coater to allow observation. *Lyngbya wollei,* filamentous non herocystous cyanobacteria formed thick benthic mat on the sediment. Filaments were covered by mucous sheath that was colonized by diatoms and other bacteria (**Figure 3.2**). In LSP, incubation sites were vegetated (except in June 2006) and *Lyngbya* was often found with other green filamentous algae (mainly *Cladophora*). In LSL, we were able to sample in sites covered by dense monospecific *Lyngbya* mats.

Benthic chambers experiment

We used four benthic chambers to isolate and measure exchange across the water-sediment interface while taking mechanisms such as bioturbation and bioirrigation and possible synergistic effect between the mat and the sediments into account. The chambers were 0.45m diameter by 0.355m long acrylic tubes sealed with acrylic lids (**Figure 3.3**). A motor contained within the lid was driving stirring paddles to provide mixing within the chambers.

The stirrers exhibit stable mixing rates and uniform speeds between chambers (3 rotations per minute). A polyethylene skirt fixed around the body of the chambers and sand bags laid on the skirt helped the installation of the chambers on hard surface. One hole drilled through the acrylic tube was fitted with flexible tubing, which was used as an outlet for the sampling of water using a peristaltic pump. Another hole was made on the lid and fitted with short tubing which was left in the water and was used as an inlet to compensate for water taken out of the chamber during sampling.

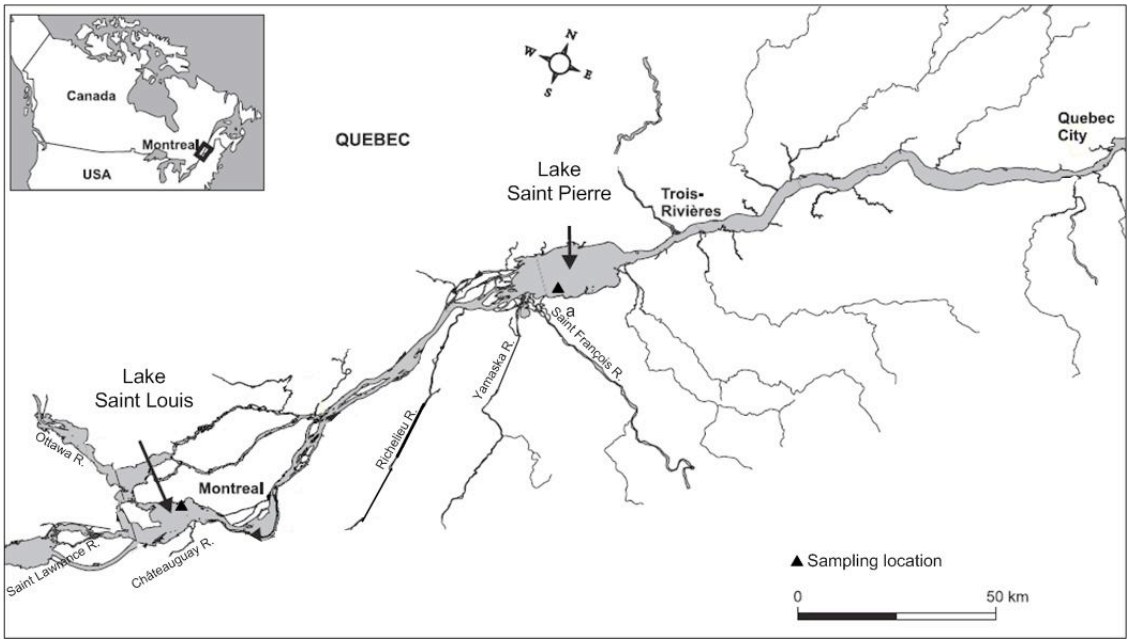


Figure 3.1: Location of Lake Saint-Louis and Lake Saint-Pierre, two fluvial lakes of the Saint-Lawrence River. Sampling sites represented by black triangles are located on the north shore of LSL and the south shore of LSP.

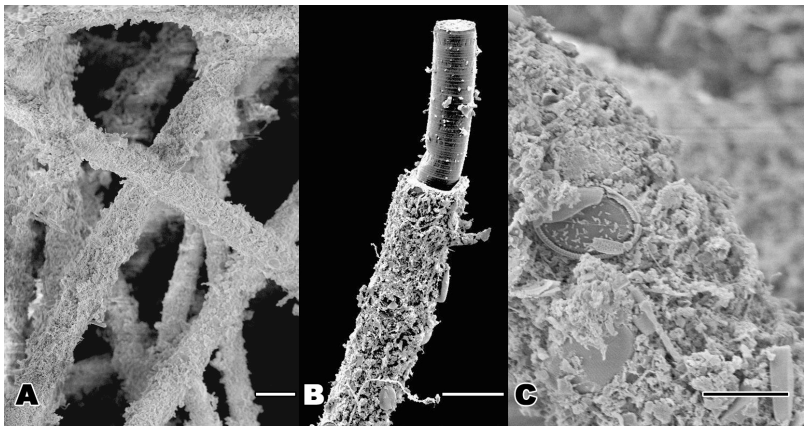


Figure 3.2: *Lyngbya* filaments under FEI Quanta 200-3D Scanning Electron Microscope showing at low magnification: A) filaments with mucous surface covered with diatoms and bacteria; B) a single filament partly freed of its mucus coating; and c) at higher magnification, a detailed view of the surface of the alga with the presence of numerous diatoms and bacteria. Scale bars: A) and B) 50µm, C) 20 µm.

Chambers deployment was done by divers when the depth was greater than 1 m otherwise it was done by foot in waders. The cylinders were first inserted 5 to 10 cm deep into the sediment and secured thanks to the skirt and sand bags. Lids were installed 6-12 hours after the cylinders to allow sediment to settle. Water was then collected for gas and nutrient analysis at regular time intervals. In LSP, water was sampled every 5 to 6 hours for 4 to 5 times totalizing a 24 hour incubation period. In LSL, samples were taken every hour for a total period of 4 to 6 hours. At each sampling dates, we considered results from our four benthic chambers as replicates.

Analytical methods

Water samples for dissolved dinitrogen gas (N_2) analysis were collected at each sampling time in 8 ml ground-glass stopper test tubes. Four replicate samples were taken and tubes were filled to overflowing, preserved with 20 µL 1N-HgCl, capped with no head space and stored under water at a temperature slightly below *in situ* to prevent bubble formation. Samples were analyzed within 48h hours of collection. Dissolved N_2 concentrations in water were measured using a quadrupole membrane inlet mass spectrometer (MIMS, Bay Instrument, USA) and N_2 production was determined by looking at changes in N_2: Ar ratios (Kana et al. 1994). The instrument provides rapid throughput (20–30 samples per hour), small sample volume (<10 ml) and high precision measurement of concentration (CV < 0.5%) and gas ratio (CV < 0.05%).

N_2 concentration [N_2] was determined using N_2: Ar ratio and N_2 at saturation and we assumed that Ar concentration did not change in the chamber during the incubation and only N_2 did as a function of biological production or consumption:

$$[N_2] = \frac{(N_2 : Ar)_{spl}}{(N_2 : Ar)_{std}} \times (N_2)_{sat} \quad \text{(Eq. 1)}$$

Where $(N_2: Ar)_{spl}$ is the measured ratio of the water sample, $(N_2: Ar)_{std}$ is the measured ratio of the standard (both corrected for instrument drift) and $(N_2)_{sat}$ is the N_2 concentration at at equilibrium with respect to the water temperature *in situ* when first captured.

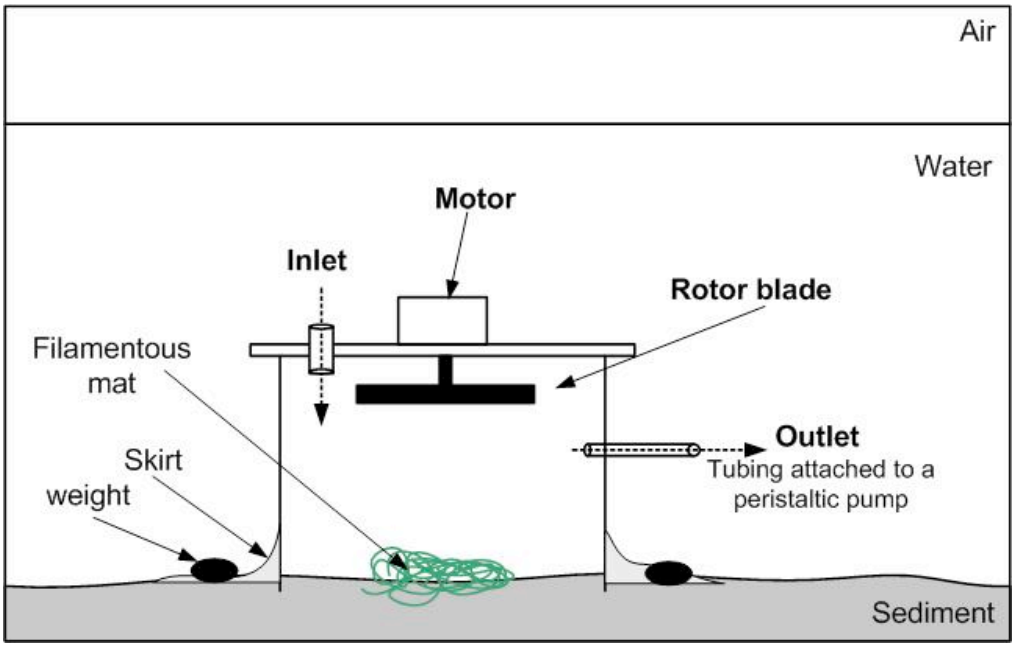


Figure 3.3: Diagram of the cylindrical benthic chamber used in this study (Diameter = 44 cm and Height =35.5 cm).

We applied the same N_2 saturation to all calculations over the course of chamber incubation. Standards consisted of air-equilibrated, continuously stirred distilled water maintained at constant temperature in a water bath for 72 h prior to analysis. Standards were measured at the beginning of the analysis and after every 12 samples to estimate and correct for instrument drift (McCutchan et al. 2003). We also estimated O_2 concentration using the same method and O_2: Ar measured with the MIMS.

At the selected time intervals, water was collected for analysis of nitrate + nitrite (NO_x), ammonium (NH_4^+), total dissolved N (TDN) and total N (TN). With the exception of samples taken for TN analysis, all samples were pre-filtered (0.45 μm pore size, Filtropur). NO_x and NH_4^+ were measured with a Latchat Instrument analyzer (methods number 10-107-04-1-B and 11-104-03-1-B). For TN and TDN analysis, samples were autoclaved with potassium persulfate and the resulting nitrate was measured as above.

Rate calculations for chamber incubations were based on linear regression of concentration changes with time. N_2 fluxes for each chamber were determined from a minimum of 3 to 5 points linear regression. These calculated N_2 fluxes represent the net benthic flux of N_2 resulting from a combination of N_2 producing and consuming processes (gross denitrification minus gross N fixation). The method used to measure N_2 did not allow us to discriminate between N_2 production pathways (canonical denitrification or anammox). Therefore positive fluxes would indicate net denitrification, whereas negative values would indicate net N fixation. Rates were also calculated for other N forms and changes in oxygen concentrations.Changes were then prorated for the volume of water and area of the chamber. For the purposes of this paper, the term ‘‘measured’’ indicates a rate calculated solely based on concentration change. Samples were not corrected for dilution as we were only tapping off aproximately 0.5 L per sampling time resulting in a maximum of 2.5 L (for 5 sampling times) out of the 59 L contained in the chamber; the difference was considered negligible.

We analysed the relationship between N_2 fluxes and other measured variables by regressions and variance partitioning using R Language.

3.4. Results

We observed that N concentrations varied seasonally in our study sites (**Table 3.1**). The higher concentrations of TDN were observed in May and June and during the summer TDN concentration tended to decrease. NO_x concentration followed the same pattern. However the two samplings made in October 2008 in LSL showed that NO_x concentration could double within a week (from 7.4 to 12.7 µmol L^{-1}) even though TDN concentration was more or less the same (28.0 to 31.7 µmol L^{-1}). NH_4^+ was present at all sampling time but in low concentration. At the beginning of the incubations the water was supersaturated in O_2 with concentrations exceeding 8 mg L^{-1}. We were always able to perform our experiments in presence of *Lyngbya* mats except for May and June in LSP. The absence of mats for these two months can be linked to NO_x concentrations. In spring, NO_x concentration was high (> 17 µmol L^{-1}) preventing *Lyngbya* growth (Vis et al. 2008). The difference between LSP and LSL was the presence of submerged vegetation in LSP (**Table 3.1**).

N_2 fluxes in LSP and LSL were negative (**Figure 3.4A-D**) ranging from -139 in September 2007 to -1732 µmol-N m^{-2} h^{-1} in October 2008 except in May 2007 (**Figure 3.4B**) when we measured a positive N_2 flux of 679 µmol-N m^{-2} h^{-1}. This positive N_2 flux indicating net denitrification was measured in chambers on sediment without *Lyngbya* mats but covered with some submerged vegetation. However in similar conditions (June 2006, **Figure 3.4A**) with bare sediment, without *Lyngbya* or vegetation, we observed a negative N_2 flux.

As observed for N_2 fluxes, O_2 fluxes were also negative for all our sampling dates ranging from near zero in September 2009 (**Figure 3.4H**: -0.036 mmol-O_2 m^{-2} h^{-1}) to -12 mmol-O_2 m^{-2} h^{-1} in July 2007 (**Figure 3.4F**) except in May 2007 when we had a positive flux of 0.6 mmol-O_2 m^{-2} h^{-1} (**Figure 3.4F**). NO_x fluxes were variable during our sampling period however we could not find any seasonal or spatial patterns. Higher fluxes of NO_x consumption were measured in July and September 2007 in LSP with respectively -77 and -64 µmol-N m^{-2} h^{-1} (**Figure 3.4J**) and in September and October 2008, NO_x fluxes were positive (**Figure 3.4K**).

Table 3.1: Descriptions of water characteristics at the beginning of chamber experiment (T0) for each sampling date.

Location	Year	Date	Depth m	Temp. °C	DO mg L^{-1}	TN	TDN µmol L^{-1}	NO_x µmol L^{-1}	NH_4^+ µmol L
Saint-Pierre									
	2006								
		June 06	1.0	23.1	8.0	62.9	58.9	24.6	2.8
		August 15	1.1	20.8	11.1	37.5	31.3	5.1	0.9
	2007								
		May 29	1.1	19.8	8.5	-	49.9	31.4	-
		July 17	0.9	25.1	16.5	-	42.4	14.8	2.1
		September 12	1.2	16.8	10.7	-	38.7	12.4	-
Saint-Louis									
	2008								
		September 25	1.2	18.9	9.5	29.6	28.9	5.3	4.3
		October 17	1.2	11.6	9.1	-	28.0	7.4	0.8
		October 23	1.1	6.5	10.5	37.7	31.7	12.7	1.1
	2009								
		September 02	2.0	19.8	8.8	32.1	29.9	11.5	0.7

We explained N_2 variance using multiple regression analysis. First we used a stepwise selection method applied to a set of nine potentially explanatory variables (O_2, TDN, NO_x, NH_4^+ fluxes and concentrations at the beginning of experiment, water temperature). The procedure selected three variables (O_2 flux, O_2 concentration and water temperature), which explained up to 36% of N_2 fluxes variance (**Table 3.2**). Our regression coefficients indicated that O_2 flux, O_2 concentration and temperature had a positive relationship with N_2 fluxes (**Table 3.2**). Positive N_2 flux would then be observed when the ecosystem produces O_2, the starting O_2 concentration and water temperature were high. Conditions promoting negative N_2 flux would be an important respiration in the ecosystem, a lower starting O_2 concentration and temperature. N_2 fluxes variance was explained principally by changes in physicochemical water variables (X1). However we hypothesized that spatio-temporal (X3) and sediment cover (X2) variables could interact with physicochemical variables to explain the variation in N_2 fluxes. We used partial regression to estimate how much of the variation of N_2 fluxes could be attributed to one set of explanatory factors, once the effect of the other set has been taken out (Legendre and Legendre 2006). The partition of the variation (**Figure 3.5**) confirmed that physicochemical variables explain N_2 fluxes better. Contrary to our hypothesis, we found that X2 and X3 and their interactions with X1 (fractions f and d) did not explain much of N_2 variation and they were not significant (**Figure 3.5**).

Table 3.2: Results of multiple regression analysis using variables selected by forward stepwise selection. Fluxes are expressed in μmol m^{-2} h^{-1} and temperature is in Celsius.

	Coefficient	Standard error	*P*	*N*	*Adj* R^2
Model			0.0008	34	0.3581
Intercept	-4973.00	1407.00	0.001		
DO Flux	0.39	0.09	0.0001		
DO Concentration	12.03	3.98	0.005		
Temperature	83.66	35.19	0.024		

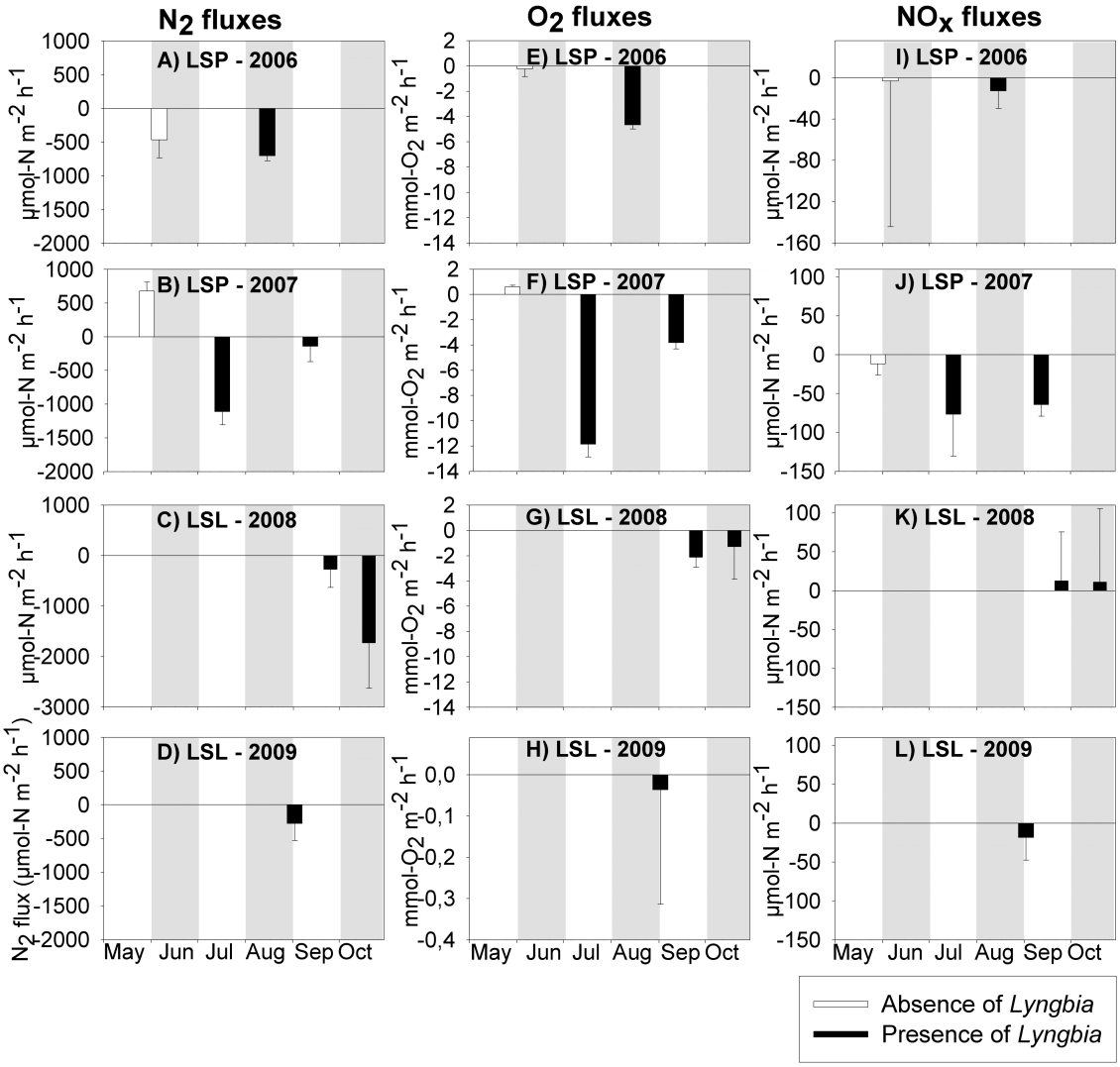
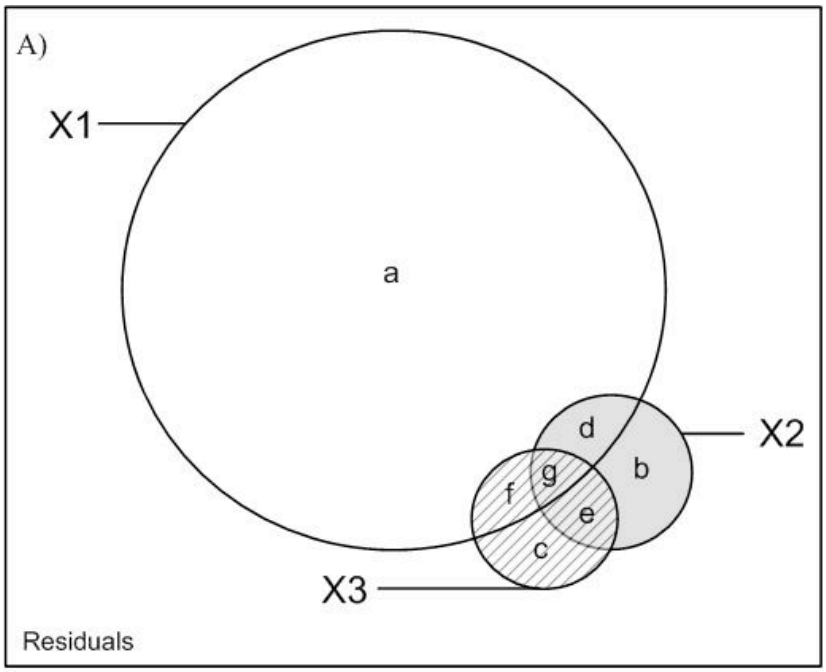


Figure 3.4: Fluxes in LSP and LSL estimated for each sampling date and grouped by year. Bars represent the average value for all chambers per date and error bars represent the standard deviation. A) –D) N_2 fluxes; E) –H) O_2 fluxes; and I) –L) NO_x fluxes. Notice different scale used in C), H) and I).



B)	R^2	Adjusted R^2	p
Water characteristic (X1)	0.45	0.36	0.00
Plant and Lyngbia presence (X2)	0.06	0.00	0.41
Spatio-temporal characteristic (X3)	0.08	-0.01	0.49
X1X2	0.55	0.43	0.00
X1X3	0.50	0.35	0.01
X2X3	0.11	-0.04	0.61
a		0.41	0.00
b		0.02	0.23
c		-0.06	0.97
d		-0.05	nt
e		0.05	nt
f		0.03	nt
g		-0.03	nt
All variables	0.55	0.37	0.01
Residuals		0.63	nt

Figure 3.5: Results of variation partitioning among water physicochemical characteristic (X1: O_2 flux and concentration, NO_x flux and concentration and Temperature), the presence of plant and *Lyngbya* (X2) and spatio-temporal variables (X3: Month, Year and location of sampling) to explain N_2 fluxes. A) Venn's diagram; B) Variance explained by each fraction and significance test ('nt' indicates that the fraction's significance was not testable).

3.5. Discussion

Our results indicated that areas occupied by *Lyngbya* in the SLR were net N fixers during summer and fall with a mean N_2 flux of -846 μmol-N m^{-2} h^{-1}. In months where *Lyngbya* were not present we observed N_2 fluxes going from net fixation (464 μmol-N m^{-2} h^{-1}) in June 2006 to net denitrification in May 2007 (679 μmol-N m^{-2} h^{-1}) in vegetated areas. Macrophytes have a well-recognised role as benthic regulators of biogeochemical cycles (Brix 1997, Pinardi et al. 2009). Their roots can provide O_2 to the otherwise anoxic zones of sediment and thus promote nitrification and supply of NO_x to the denitrification process, increasing the total rate of denitrification. In addition to potential coupled nitrification-denitrification, positive O_2 fluxes in vegetated sites may have influenced denitrification by changing NO_x to O_2 respiration among denitrifiers. Aerobic denitrification results of activation of denitrification genes occurs at a high O_2 level as it was the case in our sampling site in May 2007 (Zumft 1997).

Net fixation rates measured in *Lyngbya* mats and on bare sediments were comparable suggesting that *Lyngbya* was not the only diazotrophic organism present in the ecosystem and that N fixation could also be performed by heterotrophic bacteria. A study on the diversity of nitrogenase genes in a marine cyanobacterial mat found that there were diverse groups of non-cyanobacterial N_2-fixing microorganisms within the mat. Sulfate-reducing bacteria and anoxygenic phototrophic bacteria are two functional groups of bacteria known to play important roles in microbial mats and that have representatives capable of diazotrophic growth (Baumgartner et al. 2006, Stal et al. 2010). N_2 fixation capabilities were widely and seemingly randomly distributed among prokaryotes (Zehr et al. 1995). We assumed that N_2 fixation in cyanobacterial mats was due to the prominent cyanobacteria *L. wollei.* However mats were composed of an assemblage of prokaryotic organisms as shown in **Figure 3.2**, any of which could potentially fix (Zehr et al. 1995, Severin et al. 2010, Stal et al. 2010).

Net N_2 fixation rates measured in our experiments were comparable to those reported for heterotrophic N_2-fixation in the upper Narragansett Bay and the Providence River estuary using the same N_2: Ar method (Fulweiler et al. 2007, 2008). Estimates of

gross N fixation in cyanobacterial mat are variable and are among the highest measured (Vitousek et al. 2002) with rates ranging from 1.3 to 76 g-N m^{-2} yr^{-1} (Howarth et al. 1988b). These high rates were reported for the tropics, tidal flat in salt marshes and coastal ecosystems. LSP and LSL exhibited high net fixation rate ranging from 14 to 28 g-N m^{-2} yr^{-1}. To obtain these rates, we assumed a 12h (phototrophic fixation) to 24h (heterotrophic fixation) fixation period and a very conservative 100 days per year. Rates of N fixation in *Lyngbya* mats observed in our study are among the highest rates reported in the literature even though we compared our net N-fixing rates to gross rates.

Contrary to our hypothesis, we did not observed relationship between N_2 fluxes and N fluxes or N concentrations. We were looking for a threshold N concentration above which N_2 fluxes would be positive indicating net denitrification and below which N_2 fluxes would be negative indicating net N fixation. Joye and Paerl (1993) have reported the occurrence of simultaneous N fixation and denitrification in microbial mats of Tomales Bay. In response to a rapid increase of dissolved inorganic N induced by runoff events, mats switched from net source of N to net sink of N after runoff. NO_x, which promotes denitrification (Knowles 1982) and NH_4^+, which inhibits N fixation (Howarth et al. 1988a) did not exhibit a very high concentration during our study period. When *Lyngbya* was present, NOx and NH_4^+ concentrations were low with measured NO_x and NH_4^+ concentrations below 15 and 2 μmol L^{-1} respectively. Measured N_2 fluxes were generally negative (26 out of 35 measurements), which skewed our result toward factors controlling net fixation rather than N_2 fluxes at large.

Net fixation was positively correlated with O_2 consumption and lower concentration of O_2 in the environment. A study on *L. wollei* in a freshwater system, the Lake Okeechobee, has established that reduction of ambient O_2 concentration is required for the activation of nitrogenase, the enzyme responsible for N fixation in *L. wollei* (Phlips et al. 1992). Non-heterocystous cyanobacteria rely on diel partitioning of N fixation and photosynthesis, and to sometimes spatial partitioning of these processes, to avoid oxygen inhibition (Fay 1992). Surprisingly, *Lyngbya* mats were exhibiting N fixation even though our incubations were carried out during daytime in LSL and during day and night-time in

LSP for up to 24h. Moreover, we observed that O_2 concentrations measured in the water column at the beginning of incubations were elevated and always supersaturated. Nitrogenase activity and N fixation can exhibit peculiar daily patterns. Villbrandt et al (1990) have observed that the reduction of photosynthesis at sunset causes low levels of O_2 and the induction of nitrogenase. When the sun had set and the mat had turned anoxic, nitrogenase then became fully expressed, unhindered by the presence of O_2, and at sunrise a peak of nitrogenase activity was reported, supported by the first light, while the mat was still anoxic. Even though, this pattern did not apply to our results, we found that fixation occurred when the mats where the most heterotrophic and when O_2 concentrations where lower. There is three possible explanations for N fixation occurring in *Lyngbya* mats during daytime and in presence of O_2 in the water column: 1) *Lyngbya* filaments formed a dense and thick mat where differential O_2 diffusion could create a micro-zone with low oxygen condition and promote spatial partitioning of photosynthesis and N during daytime as it was reported in *Trichodesmium* by Paerl and Bebout (1988); 2) A close coupling of photosynthesis and respiration in the mat, where the CO_2 requirement of the photosynthetic *Lyngbya* could be covered by the respiration of associated heterotrophic bacteria, which in return would consume the produced oxygen and organic carbon (Kuhl et al. 1996). This process would also promote spatial partitioning by creating reduced oxygen condition in the mat and allow N fixation during day time; 3) N fixation could be performed not only by *Lyngbya* but also by other diazotrophic organisms like certain heterotrophic bacteria (Zehr et al. 1995, Severin et al. 2010). A study on N fixation in the littoral of a Dutch barrier island reported comparable daily rates of N fixation in two cyanobacterial mats, one consisting mainly of heterocystous (capable of phototrophic fixation) and the other of non-heterocystous filamentous species. This latter study also found that theses rates were independent from the daily incident photon flux suggesting that diazotrophic bacteria become active at different times of the day (Severin and Stal 2008) and supporting the possibility of active heterotrophic N fixers in the *Lyngbya* mats of the SLR.

To our knowledge there have been no detailed studies on the environmental significance of N_2 fixation by cyanobacteria mats in freshwater systems (Vitousek et al. 2002). In cyanobacteria mat, denitrification can result in loss of up to 20 percent of the

nitrogen fixed (Joye and Paerl 1994). Therefore these mats may shift from being sources to sinks for fixed N when conditions favourable to denitrification occur within mats (Joye and Paerl 1993, 1994). However zones occupied by the mats are usually limited in aquatic ecosystems. Rates of the different processes performed in these mats can also be spatially variable due to changes in standing biomass of fixers caused by hydrodynamic disturbance and export through water flow (Vitousek et al. 2002). Thus the high rates of N fixation reported in these mats can be of little importance to the larger ecosystem N budget. To test this idea , we assessed for the summer, at the whole LSP scale: 1) the net fixation rate in *Lyngbya* mats using an estimated surface area covered by *Lyngbya* in LSP of 40 km^2 (Hudon and Carignan 2008) and the average fixation rate of 846 µmol-N m^{-2} h^{-1} that we measured; 2) the net denitrification rate in the areas of LSP not covered with *Lyngbya* (240 km^2) using a combination of the average N_2 flux measured in June 2006 for the bare sediment areas (25% of the total surface area) and the net denitrification rate measured in May 2007 for the vegetated area (75% of the total surface area). These net denitrification estimates are only based on few measurements and thus could be biased. However they will give us an idea of the relative importance of the mats as a source of new N in the system. In LSP from July to October, N fixation rate in cyanobacterial mats and net denitrification amounted to 2.4 kg h^{-1} and 7.3 kg h^{-1} respectively. These results indicate that N_2 fixation in *Lyngbya* mats could be an important process in the N budget of LSP and could be responsible for the replenishment of up to 33% of the N removed by denitrification during the study period. To extrapolate these values to an annual budget, we assumed that *Lyngbya* mats were present and fixing for 100 days per year; which is very conservative considering that we measured actual N fixation in mats from July to October (120 days). For areas of LSP without *Lyngbya* mats we assumed that for the entire year, N_2 fluxes were similar to the ones measured during our study period and that plant coverage was also the same with 75% of the LSP covered with submerged macrophyte. Following these assumptions, net fixation was estimated to 6 T-N yr^{-1} in the *Lyngbya* mats and net denitrification in the rest of LSP was equivalent to 64T-N yr^{-1}. The importance of *Lyngbya* mats as a new source of N and their capacity to replace N loss via denitrification decreases when we considered N_2 fluxes at a larger temporal scale. N fixation in mats could account for the replacement of 9% of the estimated N denitrified in LSP annually.

Compared to net denitrification, net N fixation in cyanobacterial mats of the SLR was a non negligible N source during the summer season when SLR exhibited NO_x depletion events but its importance decreased when scaled up to an annual period. Because we measured net rates, it was difficult to integrate these rates in N budget and to compare them with rates found in other systems. A review on the importance of N fixation as N source to benthic communities in streams established that N fixation rarely contributed more than 5% of the annual N input in N budgets, but could contribute higher proportions when considered over daily or seasonal time scales (Marcarelli et al. 2008). Our results combined with Marcarelli et al. (2008) findings suggest that even in shallow stream and river ecosystems where cyanobacterial mats can occupy large portion of ecosystem, N fixation is not sufficient to offset the annual internal N loss by denitrification and N uptake by macrophytes as it was demonstrated in lakes (Scott and McCarthy 2010).

3.6. References

Baumgartner, L. K., R. P. Reid, C. Dupraz, A. W. Decho, D. H. Buckley, J. R. Spear, K. M. Przekop, and P. T. Visscher. 2006. Sulfate reducing bacteria in microbial mats: Changing paradigms, new discoveries. Sedimentary Geology 185:131-145.

Bergman, B., J. R. Gallon, A. N. Rai, and L. J. Stal. 1997. N_2 fixation by non-heterocystous cyanobacteria. Fems Microbiology Reviews 19:139-185.

Brix, H. 1997. Do macrophytes play a role in constructed treatment wetlands? Water Science and Technology 35:11-17.

Cohen, Y., and E. Rosenberg, editors. 1989. Microbial mats - Physiological ecology of benthic Microbial communities. American Society for Microbiology, Washington.

Elser, J. J., M. E. S. Bracken, E. E. Cleland, D. S. Gruner, W. S. Harpole, H. Hillebrand, J. T. Ngai, E. W. Seabloom, J. B. Shurin, and J. E. Smith. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecology Letters 10:1135-1142.

Fay, P. 1992. Oxygen relations of nitrogen fixation in cyanobacteria. Microbiological Reviews 56:340-373.

Fiore, C. L., J. K. Jarett, N. D. Olson, and M. P. Lesser. 2010. Nitrogen fixation and nitrogen transformations in marine symbioses. Trends in Microbiology 18:455-463.

Francoeur, S. N. 2001. Meta-analysis of lotic nutrient amendment experiments: detecting and quantifying subtle responses. Journal of the North American Benthological Society 20:358-368.

Howarth, R. W., R. Marino, and J. J. Cole. 1988a. Nitrogen fixation in freshwater, estuarine and marine ecosystems 2. Biogeochemical controls. Limnology and Oceanography 33:688-701.

Howarth, R. W., R. Marino, J. Lane, and J. J. Cole. 1988b. Nitrogen fixation in freshwater, estuarine and marine ecosystems 1. Rates and importance. Limnology and Oceanography 33:669-687.

Hudon, C., and R. Carignan. 2008. Cumulative impacts of hydrology and human activities on water quality in the St. Lawrence River (Lake Saint-Pierre, Quebec, Canada). Canadian Journal of Fisheries and Aquatic Sciences 65:1165-1180

Joye, S. B., and H. W. Paerl. 1993. Contemporaneous nitrogen fixation and denitrification in intertidal microbial mats - Rapid response to runoff events. Marine Ecology-Progress Series 94:267-274.

Joye, S. B., and H. W. Paerl. 1994. Nitrogen cycling in microbial mats -Rates and patterns of denitrification and nitrogen fixation. Marine Biology 119:285-295.

Knowles, R. 1982. Denitrification. Microbiological Reviews 46:43-70.

Kuhl, M., R. N. Glud, H. Ploug, and N. B. Ramsing. 1996. Microenvironmental control of photosynthesis and photosynthesis-coupled respiration in an epilithic cyanobacterial biofilm. Journal of Phycology 32:799-812.

Legendre, P., and L. Legendre. 2006. Numerical ecology, Second english edition. Elsevier, Amsterdam.

Lewis, W. M., and W. A. Wurtsbaugh. 2008. Control of lacustrine phytoplankton by nutrients: Erosion of the Phosphorus Paradigm. International Review of Hydrobiology 93:446-465.

Marcarelli, A. M., M. A. Baker, and W. A. Wurtsbaugh. 2008. Is in-stream N_2 fixation an important N source for benthic communities and stream ecosystems? Journal of the North American Benthological Society 27:186-211.

Minjeaud, L., V. D. Michotey, N. Garcia, and P. C. Bonin. 2009. Seasonal variation in di-nitrogen fluxes and associated processes (denitrification, anammox and nitrogen fixation) in sediment subject to shellfish farming influences. Aquatic Sciences 71:425-435.

Paerl, H. W., and B. M. Bebout. 1988. Direct measurement of O_2 depleted microzones in marine *Oscillatoria* - Relation to N_2 fixation. Science 241:442-445.

Paerl, H. W., B. M. Bebout, and L. E. Prufert. 1989. Naturally occurring patterns of oxygenic photosynthesis and N_2 fixation in a marine microbial mat: physiological and ecological ramification. Pages 326-341 in Y. Cohen and E. Rosenberg, editors. Microbial mats - Physiological ecology of benthic Microbial communities. American Society for Microbiology, Washington.

Phlips, E. J., J. Ihnat, and M. Conroy. 1992. Nitrogen fixation by benthic freshwater cyanobacterium *Lyngbya wollei*. Hydrobiologia 234:59-64.

Pinardi, M., M. Bartoli, D. Longhi, U. Marzocchi, A. Laini, C. Ribaudo, and P. Viaroli. 2009. Benthic metabolism and denitrification in a river reach: a comparison between vegetated and bare sediments. Journal of Limnology 68:133-145.

Scott, J. T., and M. J. McCarthy. 2010. Nitrogen fixation may not balance the nitrogen pool in lakes over timescales relevant to eutrophication management. Limnology and Oceanography 55:1265-1270.

Seitzinger, S., J. A. Harrison, J. K. Bohlke, A. F. Bouwman, R. Lowrance, B. Peterson, C. Tobias, and G. Van Drecht. 2006. Denitrification across landscapes and waterscapes: A synthesis. Ecological Applications 16:2064-2090.

Severin, I., S. G. Acinas, and L. J. Stal. 2010. Diversity of nitrogen-fixing bacteria in cyanobacterial mats. Fems Microbiology Ecology 73:514-525.

Severin, I., and L. J. Stal. 2008. Light dependency of nitrogen fixation in a coastal cyanobacterial mat. Isme Journal 2:1077-1088.

Stal, L. J. 2003. Nitrogen cycling in marine cyanobacterial mats. Pages 119-140 in W. E. Krumbein, D. M. Paterson, and G. A. Zavarzin, editors. Fossil and Recent Biofilms - a Natural History of Life on Earth.

Stal, L. J., I. Severin, and H. Bolhuis. 2010. The Ecology of Nitrogen Fixation in Cyanobacterial Mats. Pages 31-45 *in* P. C. Hallenbeck, editor. Recent Advances in Phototrophic Prokaryotes. Springer-Verlag Berlin, Berlin.

Stewart, W. D. P. 1980. Some aspects of structure and function in N_2 fixing cyanobacteria. Annual Review of Microbiology 34:497-536.

Villbrandt, M., L. J. Stal, and W. E. Krumbein. 1990. Interactions between nitrogen fixation and oxygenic photosynthesis in a marine cyanobacterial mat. Fems Microbiology Ecology 74:59-72.

Vis, C., A. Cattaneo, and C. Hudon. 2008. Shift from chlorophytes to cyanobacteria in benthic macroalgae along a gradient of nitrate depletion. Journal of Phycology 44:38-44.

Vitousek, P. M., K. Cassman, C. Cleveland, T. Crews, C. B. Field, N. B. Grimm, R. W. Howarth, R. Marino, L. Martinelli, E. B. Rastetter, and J. I. Sprent. 2002. Towards an ecological understanding of biological nitrogen fixation. Biogeochemistry 57:1-45.

Vitousek, P. M., and R. W. Howarth. 1991. Nitrogen Limitation on Land and in the Sea - How Can It Occur. Biogeochemistry 13:87-115.

Zehr, J. P., M. Mellon, S. Braun, W. Litaker, T. Steppe, and H. W. Paerl. 1995. Diversity of heterotrophic nitrogen fixation genes in a marine cyanobacterial mat. Applied and Environmental Microbiology 61:2527-2532.

Zumft, W. G. 1997. Cell biology and molecular basis of denitrification. Microbiology and Molecular Biology Reviews 61:533-616.

4. Denitrification Hotspots: Dominant Role of an Invasive Macrophyte (*Trapa Natans*) in Removing Nitrogen from a Tidal River

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Laure Tall, Roxane Maranger et Nina Caraco

4.1. Abstract

Rivers receive large amounts of nitrogen (N) from their watershed and are the final sites of nutrient processing before delivery to coastal waters. Transformations of dissolved inorganic N (DIN) to gaseous N within rivers can impact both coastal eutrophication and greenhouse gas emissions. Vegetated shallows of rivers are sites of active metabolism and may act as hot spots for N transformation but little is known about the variability of denitrification within shallows or the role of vegetation structure in controlling this variability. We measured in situ N loss and accumulation of N_2 and N_2O in vegetated shallows of the tidal Hudson River and used regression models to determine the role of plant species in different monospecific beds in ecosystem N loss. N_2 production was highly variable between vegetated shallows and was associated with species-driven differences in dissolved oxygen (DO) dynamics during the ebb tide. N_2 production was extremely high (67-109 mmol-N m^{-2} d^{-1}) in beds with invasive floating-leaved plants (*Trapa* natans) but was insignificant in submersed native vegetation (*Vallisneria americana*). In *Trapa* sites N_2 production was strongly related to metabolism. Change in DO concentrations in the surrounding water due to atmospheric venting by the plants during ebb tide, combined with changes in water temperatures, were the best variables to model N_2 production. Despite these high denitrification losses, beds acted as N_2O sinks where N_2O concentrations became undersaturated during ebb tide. An estimate of summertime N_2 production in Trapa beds, based on continuously measured oxygen and temperature by moored sondes, suggests that these beds are a major seasonal hot-spot for N removal. Large *Trapa* beds represent only 2.7% of the total area of the tidal Hudson but they remove between 70 and 100% of the total N retained in this river reach during summer months. Although they are active for only 3 months of the year, *Trapa* shallows contribute to as much as 27% of the annual N removal. *Trapa* activity represents an important ecosystem service, modulated by its impacts on DO as a function of their growth form trait and modulated by the physical properties of the environment.

4.2. Introduction

Humans have more than doubled new nitrogen (N) inputs to terrestrial systems over the last century (Galloway et al. 2002, Schlesinger 2009). As a result, N inputs to coastal waters have increased, but this increase has been modulated by N uptake in terrestrial, wetland and aquatic systems either by storage or permanent loss primarily via denitrification (Alexander et al. 2000, Seitzinger et al. 2006). This uptake represents an important ecosystem service (Costanza et al. 1997), without which N loads to coastal waters could be more than 5-fold greater than they are presently (Howarth 1998) leading to substantially worse episodes of algal blooms and bottom water hypoxia associated with elevated N loads (Paerl 1997).

Riverine networks can be hotspots (McClain et al. 2003) of N transformations and loss to gaseous N production (Piña-Ochoa and Alvarez-Cobelas 2006). These systems occupy less than 1% of the earth's surface, but denitrification and N_2O production within these ecosystems has been estimated to account for 30% of terrestrial values (Seitzinger et al. 2006). Until recently, small headwater streams have been the focus of studies examining N uptake and loss on the landscape. Smaller streams usually experience higher N cycling rates owing to their higher benthic to surface water ratios (Bernot and Dodds 2005). Larger rivers have been less well-studied, but are generally thought to be of lesser importance than headwater streams for N removal (Alexander et al. 2000, Peterson et al. 2001). Some recent studies suggest, however, that large rivers may play an important role in N uptake (Stanley and Maxted 2008, Tank et al. 2008, Alexander et al. 2009) as the amount of N removed per meter of reach is greater in large rivers than in small streams (Seitzinger et al. 2002). In the tidal Hudson River, almost 2 000 metric tonnes of N is retained per year. This value is estimated to be greater than N loss in freshwater wetlands of the river's watershed and equal to the sewage load to the River from a major metropolis (Lampman et al. 1999). This N uptake occurs despite a relatively short residence time of water in the tidal freshwater Hudson (TFH) (Lampman et al. 1999). The high uptake is somewhat surprising as the Hudson river does not have significant groundwater input or associated active riparian areas (Cooper et al. 1988); the sea level section of the river lacks flood plains and burial in the main stem of the Hudson is relatively low and does not seem to be a dominant fate of this N (Lampman et al. 1999).

Shallow vegetated areas could potentially play an important role in nutrient removal in part due to the physical trapping of particles, plant uptake and/or via the modification of system biogeochemistry (Wigand et al. 1997, Rooney et al. 2003). The relative importance of these various loss terms could also be a function of different plant species. Indeed, preliminary research suggests that vegetated shallows and embayments in the Hudson River may be important sites of N uptake and transformation and that this N uptake is associated with oxygen depletion within the water column of vegetated areas (Caraco and Cole 2002, Arrigoni et al. 2008). These preliminary studies did not determine if measured dissolved inorganic nitrogen (DIN) loss was a result of temporary incorporation and storage in organic end-products, or was lost from the ecosystem in a gaseous form. In this study we examined the DIN loss as well as N_2 and N_2O changes in two macrophyte beds of the Hudson with very different oxygen dynamics. Using empirical models developed in this study we related the N dynamics in these beds to total N uptake and transformations within the Hudson.

4.3. Material and Methods

Site description

The freshwater tidal Hudson River extends 140 km from Albany towards New York city, NY, USA (Figure 4.1). Dominant water inputs are from two tributaries (Mohawk and Upper Hudson) that enter near the dam at Troy, New York, while additional tributaries contribute 20% of the total water input and groundwater inputs are insignificant (Lampman et al. 1999). About 15% of the 100 km2 area of the tidal freshwater Hudson (TFH) is occupied by two macrophyte species that occur in nearly monospecific beds that can be more than 1 km2 in size (Nieder et al. 2004). *Vallisneria americana* is a submersed plant that is native to the Hudson River and is generally associated with elevated oxygen concentrations. Low oxygen conditions are extremely rare, even at night in large dense beds of this macrophyte. *Trapa natans* is an introduced exotic species to the Hudson. This plant is floating-leaved, and oxygen is vented to the atmosphere when leaves reach the water surface, resulting in oxygen-depletion events at low tide, particularly in large beds (Caraco and Cole 2002, Goodwin et al. 2008).

This study took place in a large Trapa bed (Inbocht Bay) and in a nearby large *Vallisneria* bed located in the middle of the TFH (Figure 4.1). Inbocht bay is approximately 1.5 km2 and has an average depth of 0.3 m at low tide and an average tidal amplitude of 1.2 m. During summer months (July to September), the bay is densely populated with T. natans and its floating leaves cover the water surface, blocking over 95% of incoming light. As a result photosynthesis is high in the floating vegetation but is inhibited within the water column; respiration of submersed plant tissue relies on organic matter fixed in the floating leaves. Total respiration within the water column (0.3 g O_2 m^{-2} h^{-1}) is dominated by submersed plant tissue and is high enough to deplete oxygen to below 1 mg L^{-1} during the 6.5 hours ebb tides when there is no replenishment of oxygenated water from the main channel. The nearby *Vallisneria* bed is approximately 0.6 km^2 and has an average depth near 0.5 m at low tide and an average tidal amplitude of 1.2 m. Primary production within the water column (between 0.2 and 0.7 g O_2 m^{-2} h^{-1}) is slightly greater than respiration, resulting in a slightly positive oxygen balance in these beds and a general increase in oxygen concentrations during daytime ebb tides (Caraco and Cole 2002).

Field sampling

Sampling was conducted when *Trapa* biomass was at its annual maximum and plant leaves were floating at the surface. To access our sites in the *Trapa* bed we used a channel at the eastern edge of the bay which connected the main stem of the river to the back of the bed. On July 17th and September 13th 2006, we sampled five sites: 2 sites in Inbocht Bay, 2 sites in the nearby *Vallisneria* stand and 1 site in the main channel. On July 23rd 2007, we also sampled 5 sites but only in Inbocht bay, following a transect from the main channel to the back of the bed. Sampling at a given site began at high tide and continued until low tide. Water from the main stem of the river was used to establish initial water conditions, before it had entered the bed. The *Trapa* inner site was located 700 m into the channel-side edge of the bed to ensure we were sampling water leaving the *Trapa* bed only, and not water mixing with the main-channel. For the gas and nutrient measurements, samples were collected hourly during ebbing tide just bellow the surface. There is little stratification throughout most of the tidal freshwater portion of the river (Raymond et al. 1997) so we considered surface samples as representative of the entire water column.

Oxygen measurements

Oxygen measurements were made using moored automatically recording sondes (YSI-Endico 6000 PG; YSI Inc., Yellow Springs, OH, U.S.A.) set to record at 15-min intervals. Sondes were placed simultaneously in the *Trapa* bed at Inbocht Bay at 700 m from the edge of the bed, 0.2 - 0.3 m above the sediment. Sondes were also set in a nearby open channel water site in the main stem of the river, 2 m below the surface on a permanently moored buoy in 7 m of water. For this study, we used measurements made in summer 2006. Detailed explanations on calibrations, electrode drift corrections, deployment and recovery of the sondes are provided in Goodwin et al. (2008).

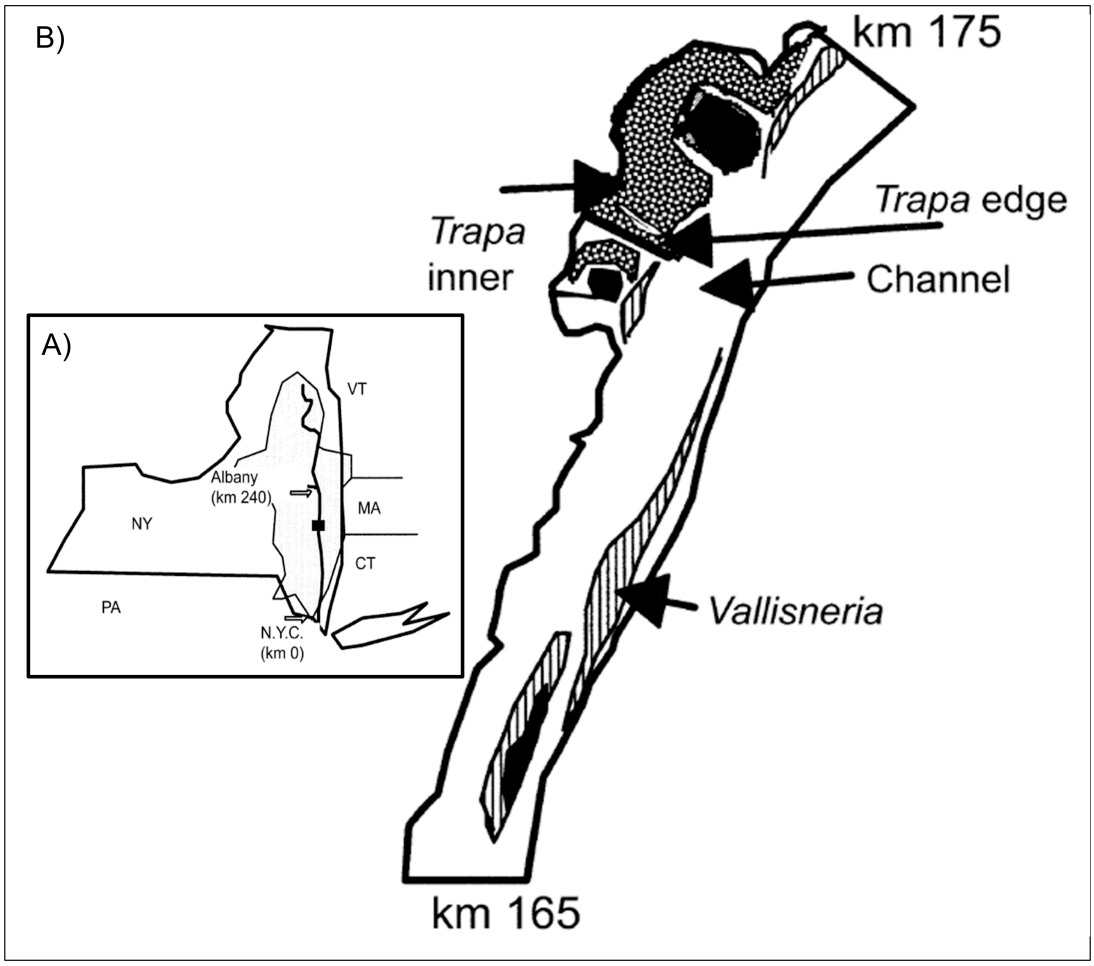


Figure 4.1: (A) Location of the tidal Hudson River from Albany to New York City (river km 0), New York. (B) River reach where the study took place. Hatched areas are *Vallisneria* beds, dark stippled areas are *Trapa* beds, black is intertidal or permanently exposed islands or jetties, and white represents open water sites. The four sites sampled in 2006 are indicated by arrows, transect sampled in 2007 runs from the *Trapa* edge site to the *Trapa* inner site. Map modified from Caraco and Cole 2002.

Analytical methods

Water samples for dissolved dinitrogen gas (N_2) analysis were collected at approximately one hour intervals from each site during ebb tide in 8 ml ground-glass stopper test tubes. Four replicate samples were taken and tubes were filled to overflowing, preserved with 20 µL 0.1 M $HgCl_2$, capped with no head space and stored under water at a temperature slightly below in situ to prevent bubble formation. Samples were analyzed within 48h hours of collection. Dissolved N_2 concentrations in water were measured using a quadrupole membrane inlet mass spectrometer (MIMS, Bay Instrument, USA) and N_2 production was determined by looking at changes in N_2: Ar ratios (Kana et al. 1994). The instrument provides rapid throughput (20–30 samples per hour), small sample volume (<10 ml) and high precision measurement of concentration (CV < 0.5%) and gas ratio (CV < 0.05%). N_2 concentration was determined from N_2: Ar ratio as:

$$[N_2] = \frac{(N_2 : Ar)_{spl}}{(N_2 : Ar)_{std}} \times (N_2)_{sat} \quad \text{(Eq. 1)}$$

where $(N_2: Ar)_{spl}$ is the measured ratio of the water sample, $(N_2: Ar)_{std}$ is the measured ratio of the standard (both corrected for instrument drift) and $(N_2)_{sat}$ is the N_2 concentration at saturation *in situ*. Standards consisted of air-equilibrated, continuously stirred distilled water maintained at constant temperature in a water bath for 72 h prior to analysis. Standards were measured at the beginning of the analysis and after every 12 samples to estimate and correct for instrument drift.

The partial pressure of N_2O (p N_2O) was measured by headspace equilibration at ambient temperature (Cole and Caraco 2001). A volume of 1.1 L of water taken at the surface was equilibrated in a gastight bottle with ambient air (120 mL), by shaking vigorously for 2 minutes. After equilibration, triplicate 9 mL samples of headspace gas were injected into pre-evacuated vials with a thick butyl stopper and an aluminum ring. Ambient air concentration samples were also collected and injected into pre-evacuated vials. N_2O was measured by gas chromatography using an ECD detector on a Schimadzu 2014 GC with a Tekmar 7050 autosampler. We used a Poropaq Q (80/100) column to separate gases with P5 (95% argon and 5% methane) as the carrier gas. Standards consisted of vials treated exactly as above with N_2O concentrations of 0.22, 1.2 and 2.4 ppm. We corrected the measured value of N_2O from the equilibration for the introduction of

120 mL of air. N_2O concentrations were then calculated using the solubility tables of Weiss and Price (1980).

Water samples for dissolved nutrients (NO_3^-+ NO_2^- referred to as NO_3^- only for the rest of the text, NH_4^+, DOC and PO_4) were collected hourly at each site and filtered immediately in the field using 25-mm Gelman A/E filters in filter holders (Swinnex) and water samples for total analyses (Total N and P) were taken directly. All samples were kept in a cold, dark cooler in the field. In the laboratory, samples were acidified to a pH < 2 using 1 mL of 1N H_2SO_4 per 100 mL of sample. Nutrients and DOC were analysed following procedures described in Lampman et al (1999). Water samples for chlorophyll *a* were filtered through Whatman GF/F filters and then filters were frozen prior to analysis. Chlorophyll *a* was measured after methanol extraction (Holm-Hansen and Riemann 1978).

Modeling N_2 production

"Denitrification" and "N_2 production" are used interchangeably in the text although we recognize that some of the N_2 could have been produced via the anammox pathway. N_2 production was estimated as the deviation in the concentration of N-N_2 (ΔN_2) in the *Trapa* bed relative to concentration in the river channel (considered to be the initial conditions in the bed) over a specific time interval. Indeed all of the delta values of the variables of interest (ΔDO, ΔO_2 and ΔNO_3^-) represent a difference between concentrations in *Trapa* bed relative to concentrations in the main channel.

All data analyses were done using language R. To create predictive models of N_2 excess (ΔN_2), we performed simple and multiple regression models using ordinary least squares (OLS) of measured variables preselected by stepwise regression (ΔDO, ΔO_2 and ΔNO_3^-, and temperature). OLS approach does not take into account possible autocorrelation in time series data. We found no time series autocorrelation at any of the sites, for any of the dates using a Durbin Watson (DW) test (DW > 2 in all cases with a *P* between 0.11 and 0.9). Furthermore, we repeated models analysis using a generalised least square (GLS) approach that accounts for any random effect which could create within group correlation of regression errors not necessarily apparent using

DW. OLS and GLS gave very similar results, however we choose to report the OLS models on the basis of parsimony and familiarity.

Models were compared using adjusted R^2 (referred as R^2) and the Akaike's Information Criterion (AIC, Anderson et al. 1998), which incorporates the log-likelihood with a penalty for added parameters. The latter selects for the most parsimonious statistical model that provides the most amount of information out of all possible combinations of our preselected variables. The best model of ΔN_2 was estimated using ΔDO and temperature in *Trapa* beds. ΔN_2 was modelled using measured O_2 and temperature data from inner and channel sites taken at a 15-min time interval by the moored sondes during ebbing tide for 53 days from 1 July 2006 to 25 September 2006. Data were not available between July 11 and 25 and between August 26 and September 10. Average and range of N_2 production rates over the summer period during ebbing tide, could then be estimated from modeled ΔN_2.

N Mass Balance

We used a mass balance approach to evaluate the relative importance of N_2 production to N loading from the channel in areas of the TFH occupied by large *Trapa* beds (total surface of 4 km^2). N_2 production was determined two ways. First, using the model described above that combines ΔDO and temperature to determine an average summertime loss estimate during ebb period. Secondly, we used the ratio of N_2 produced per unit O_2 consumed (0.303, equivalent to the slope for our linear regression model between ΔN_2 and ΔDO only) combined with a previously measured rate of areal respiration (233 mmol-O_2 m^{-2} day^{-1} for sediment, submersed *Trapa* leaves, stems and roots) in *Trapa* beds (Caraco and Cole 2002). A range of N_2 production was determined to ways: by using long-term monitoring of O_2 concentrations in the beds as compared to the channel, measured with sondes during ebb tide and secondly by using estimates of total system metabolism in the beds. For the latter estimate, we used areal respiration data rather than site specific sonde measurements because areal rates of respiration by *Trapa* were a better and more conservative estimate of integrated DO changes at the scale of the whole bed.

Tidal inputs to beds (N_{in}) as NO_3^-, NH_4^+ and organic N were calculated as followed:

$$N_{in} = [N] \times TD \times 1.92 \quad \text{(Eq. 2)}$$

where [N] represents the average N concentrations of the various N species (in µmol N/L with TN=55, NO_3^- =30 and NH_4^+=2) in the channel for the study period, TD represents the measured tidal amplitude (in meter) and 1.92 is the average number of tides in 24 hours. We then used the correct conversion factor to obtain the load in kg per day for the 4 km^2 area covered by large *Trapa* beds.

Given the gradual change in N species concentration during the ebb tide, N exiting the bed as tidal outputs (N_{out}) to the channel needed to be accounted for at more refined intervals (15 minutes). This was calculated using the following approach:

$$N_{out} = \frac{\sum_{t=1}^{n} [N_t \times (Z_t - Z_{t-1})]}{D_{tot}} \quad \text{(Eq. 3)}$$

where N_t is the modeled N concentration (TN, NO_3^- or NH_4^+ in µmol/L) in *Trapa* beds at time *t*, Z is the measured water depth in the bed with Z_t- Z_{t-1} representing the change in depth owing to tides and D_{tot} is the total number of days used to estimate N_{out}.

Change in nutrient concentrations in the bed were measured over the ebb tide at 3 sampling dates and were found to be moderately well predicted from O_2 concentrations, with the exception of NH_4^+ which was always found at low concentrations (**Table 4.1**). We therefore modeled concentrations of the different N forms at the time of exit (N_t) as a function the O_2 concentration (µmol/L) using the continuous DO measurements at the inner *Trapa* bed site. Here again calculations were made only during ebb tide. To complete the mass balance, standing stocks of N in the beds were also determined for sediment and plants. In order to estimate sediment N standing stock, we assumed a N content of 1.2% in the first 2 cm of sediments (Templer et al. 1998) and an areal weight of 1000 g/m^2 scaled up to 4 km^2 for *Trapa* beds. N bound in plant biomass was calculated based on *Trapa* density (50 plants per m^2), plant N content (between 1.5 and 3% depending on the plant parts), and weight (Caraco and Cole 2002).

4.4. Results

Changes in gas and nutrient concentrations during ebb tide among sites

Oxygen and nitrogen concentrations varied differently between *Trapa* and *Vallisneria* beds during ebb tide (**Table 4.2**). In the main channel and the *Vallisneria* bed, changes in DO, NO_3^- and TN concentrations during ebb tide were not significant and their concentrations were considerably higher than the concentrations measured in the *Trapa* bed site. In the *Trapa* site DO, NO_3^- and NH_4^+ concentrations declined rapidly, reaching near zero during ebbing tide (Table 10) while N_2 increased by up to 45 μmol N/ L (**Figure 4.2**A, B and C). N_2O concentrations in the *Trapa* site decreased to well below saturation after an initial increase during the early phase of the ebb (Fig 2 D), while in the *Vallisneria* site changes in N_2 and N_2O concentrations were negligible during ebbing tide (**Figure 4.2**C and D). DOC, PO_4, TP and Chl*a* showed little change in concentration in both beds (**Table 4.2**). However mean values of phosphorus concentrations (PO_4 and TP), Chl*a* and TN were generally higher in the *Vallisneria* site as compared with *Trapa*.

Relationships to predict changes in N_2

In *Trapa* beds, changes in N_2 were strongly and linearly related to changes in NO_3^- and DO concentrations. The relationships between ΔN_2 and ΔNO_3^- were typically very strong and highly significant, where ΔNO_3^- could explain up to 96% of the variance in ΔN_2 on a given date. The slopes of the relationships varied significantly among sampling dates (ANCOVA: $R^2 = 0.95$; $F = 76.36$; $d.f. = 2, 27$; $p < 0.001$; **Figure 4.2A**). The shallowest slope of -0.79 was observed in July 2006 and was the closest to a 1:1 relationship where N from nitrate reduction alone could account for all the N_2 produced. In July 2007 and September 2006, slopes were substantially steeper at -2.53 and -4.60 respectively, suggesting that considerably more N_2 was produced per unit NO_3^- consumed. The relationships between ΔN_2 and ΔDO were also very strong and highly significant, where ΔDO explained between 56 – 97 % of the observed change in N_2. Again, relationships varied among sampling dates, but less so than with ΔNO_3^-. The relationships determined for September 2006 and July 2007 were not significantly different from one another with slopes of -0.42 and -0.49 respectively. However the relationship was different in July 2006 where substantially less N_2 was produced with the same change in DO (ANCOVA: $R^2 = 0.95$; $F = 42.22$;

d.f. = 2, 35; $p < 0.001$). We found that the different trends observed among sampling dates for both relationships could in part be explained by a significant interaction with temperature (ANCOVA interaction terms: Temperature × ΔNO_3^-; $p < 0.001$ and Temperature × ΔDO $p < 0.001$). Thus in July 2006 when water temperatures were warmer, less N_2 was produced for the same change in DO and NO_3^- as compared to the two other sampling dates.

To estimate N_2 production from the *Trapa* sites at a larger spatial scale, we used a simple and a multiple regression approach using OLS to develop different predictive models (**Table 4.3**). The overall relationship between ΔN_2 and ΔNO_3^- was significant but weak with an adjusted R^2 of 0.20. This is not surprising given the variability observed among dates. The global relationship between ΔN_2 and ΔDO was much stronger with an adjusted R^2 of 0.56, suggesting that overall change in N_2 was more tightly coupled with changes in DO regardless of timing. ΔN_2 was also negatively related with temperature suggestion that some of the observed change in N_2 was a function of a change in physical solubility and not necessarily biological production. However the best and most parsimonious model to predict ΔN_2 used both temperature and ΔDO as predictor variables (AIC = 274.17, **Table 4.3**).

To estimate the variability in N_2 production over time during the course of a summer, we used continuous DO and temperature data taken during ebbing tide. Variation in temperature and DO concentrations are reported in **Figure 4.4**A and B. Temperatures changed daily, varying in some cases from 2 to 4°C in a single day which would influence gas solubility. When compared to a sonde stationed in the main channel, DO values in *Trapa* bed were clearly lower and more variable. Because of tidal exchange and rapid depletion during tidal ebb DO concentrations in *Trapa* oscillated from main channel DO concentrations to near zero (**Figure 4.4B**). These measured changes in temperature and DO over the course of the ebb tide were used to predict changes in N_2 production. N_2 production was highly variable within a single day and during summer (**Figure 4.4C**), with excess N_2 varying throughout the summer from 1 to greater than 150 µmol N/L. Based on this model, N_2 production was on average 47 µmol N/L per ebbing tide or around 7 µmol N L^{-1} h^{-1} considering a 6.5 hour- long ebb tide.

Nitrogen Mass balance in *Trapa* beds

Mass balance revealed more than 7000 kg N/d on average enters the *Trapa* beds of the TFH (**Figure 4.5**) during ebb tide. Tidal inputs were mainly in the form of DIN representing 57% of the total N tidal input. Groundwater N inputs were considered negligible at 98 kg N/d (Cooper et al. 1988, Nystrom 2010). Half of N input (48%) was exported by tidal outputs from the beds to the main channel. Tidal outputs were mainly in the form of organic N with DIN representing less then a quarter of total N outputs. The majority of N entering the beds (55-82%) was transformed to N_2 gas and permanently eliminated from the ecosystem. Transformation from the DIN pool to N_2 gas was the main loss term, where N_2 production accounted for 96 to 143% of DIN inputs. Despite the close correspondence between the N input and output terms on average, an estimated 205 to 2150 kg of extra N per day would be required to fuel our estimates of N_2 production. We calculate a sediment standing stock of 96 000 kg N in the *Trapa* beds, that would in theory be able to supply up to 1000 kg of N per day for 90 days. Moreover the standing sock of N in sediment could be partially replenished each year by senescing *Trapa* representing an estimated standing stock of approximately 37 000 kg N.

Table 4.1: Simple linear regression relationships between the concentration of the different N forms (in µmol/L) and DO concentrations in *Trapa* beds (in µmol/L).

Predictive model	*N*	*p*	*F*	R^2
TN= 0.106 (DO) + 23.468	25	0.0001	43.91	0.64
NO_3^-= 0.109 (DO) + 0.106	25	0.0001	127.44	0.84
NH_4^+= 0.003 (DO) + 1.027	25	0.004	4.47	0.13

Table 4.2: Average and extreme values observed (minimum - maximum) of various physical chemical properties in monospecific *Trapa* and *Vallisneria* beds for N samples in the Hudson River during ebbing tide on different sampling dates.

	July 2006		September 2006	
	Trapa (*N*=10)	*Vallisneria* (*N*=6)	*Trapa* (*N*=11)	*Vallisneria* (*N*=6)
DO (mg/L)	4.02 (0.53–7.64)	7.03 (6.73 – 7.58)	5.66 (0.48 – 9.39)	8.46 (8.16 –8.78)
Temperature (°C)	26.42 (25.66 – 27.44)	26.29 (25.77 – 27.65)	19.72 (18.05 – 20.82)	20.35 (20.18 –20.70)
NO_3^- (µmol N/L)	16.83 (0.84 – 36.41)	30.32 (29.45 –34.00)	18.05 (1.21 – 26.33)	26.24 (24.84 –27.67)
NH_4^+ (µmol N/L)	1.92 (0.98 – 3.58)	2.47 (2.09 – 2.82)	1.14 (0.44 – 1.58)	0.79 (0.15 – 1.20)
TN (µmol N/L)	35.32 (17.07 –54.34)	49.64 (47.15 – 57.43)	45.01 (24.88 –67.65)	45.79 (40.54 – 49.59)
DOC (mg/L)	4.64 (3.58 – 3.82)	4.34 (3.73 – 4.89)	3.82 (3.67 – 4.13)	3.69 (3.41 – 3.91)
PO_4 (µmol P/L)	0.49 (0.16 – 0.90)	0.80 (0.77 – 0.84)	0.33 (0.09 – 0.54)	0.44 (0.27 – 0.52)
TP (µmol P/L)	1.54 (0.90 – 2.56)	2.21 (1.88 – 3.24)	1.74 (0.76 – 4.82)	1.21 (1.13 – 1.29)
Chl*a* (µg/L)	2.07 (0.69 – 3.51)	2.98 (2.48 –3.41)	3.97 (1.49 – 6.80)	4.12 (3.27 – 5.08)

Table 4.3: Results of simple regressions and multiple regressions of change in N_2 (ΔN_2 in µmol N/L) with change in oxygen (ΔDO in µmol/L), change in nitrate (ΔNO_3^- in µmol N/L), and temperature (Temp in °C). In bold, the best model determined using the Akaike information criterion (AIC).

	N	*p*	*F*	R^2	AIC
Simple regression models					

$\Delta N_2 = -0.30(\Delta DO) + 2.22$	41	<0.0001	52.03	0.56	317.17
$\Delta N_2 = -7.30(Temp) + 201.33$	41	<0.0001	24.40	0.37	321.73
$\Delta N_2 = -1.31(\Delta NO_3^-) + 14.95$	35	0.0044	9.33	0.20	332.32
Multiple regression models					
$\Delta N_2 = -0.28(\Delta DO) - 6.51(Temp) + 154.06$	41	<0.0001	132.70	0.87	**274.17**
$\Delta N_2 = -9.45(Temp) - 1.98(\Delta NO_3^-) + 222.45$	35	<0.0001	88.39	0.84	279.20
$\Delta N_2 = -0.51(\Delta DO) + 1.75(\Delta NO_3^-) + 3.01$	35	<0.0001	32.34	0.65	309.34
$\Delta N_2 = -0.21(\Delta DO) - 7.35(Temp) - 0.58\ (\Delta NO_3^-) + 171.34$	35	<0.0001	84.24	0.88	274.20

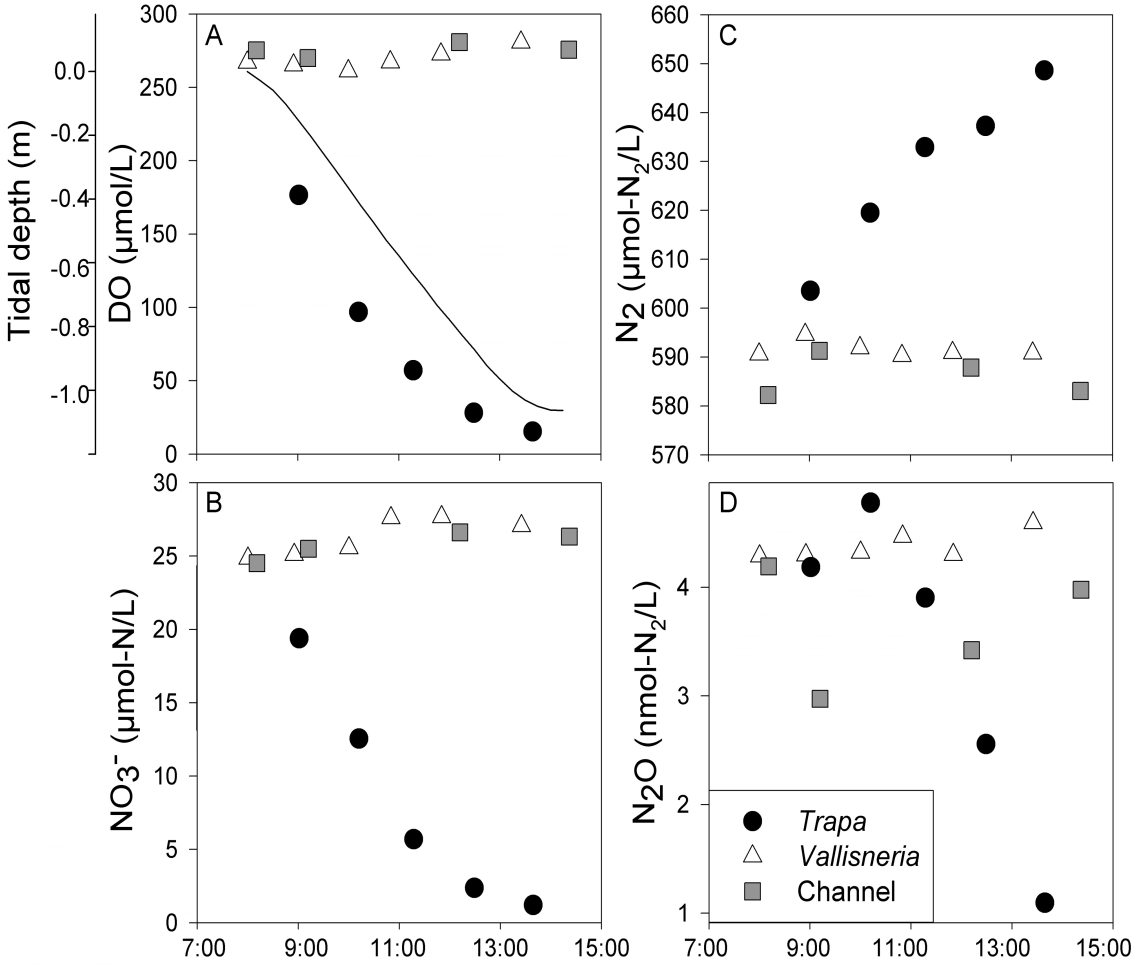


Figure 4.2: Dynamics of A) DO (mg/L) and delta tidal depth (m) represented by the symbols and the line respectively, B) NO_3^- (µmol-N/L), C) N_2 (µmol-N/L) and D) N_2O (nmol-N/L) measured in September 2006 during ebbing tide at different sites.

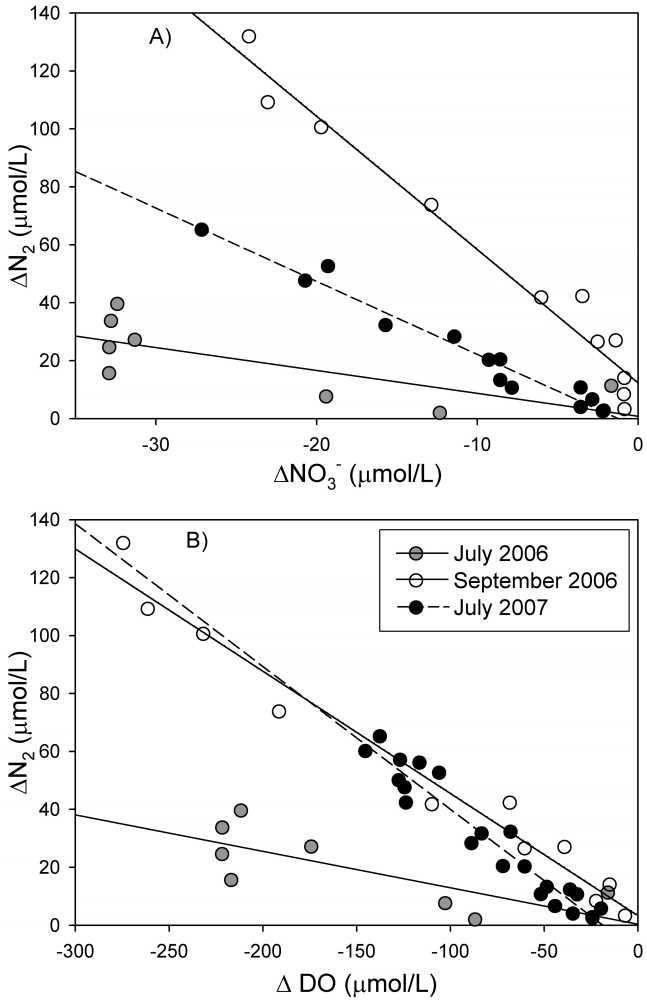


Figure 4.3: A) Relationships between ΔNO_3^- (µmol-N/L) and ΔN_2 (µmol-N/L) for each sampling date: July 2006, $\Delta N_2 = (-0.79 \times \Delta NO_3^-) + 0.83$ with $R^2 = 0.55$, n = 9, F-test: $p \leq 0.01$; September 2006, $\Delta N_2 = (-4.60 \times \Delta NO_3^-) + 12.47$ with $R^2 = 0.96$, n =11, F-test: $p \leq 0.0001$ and in July 2007: $\Delta N_2 = (-2.53 \times \Delta NO_3^-) - 3.14$ with $R^2 = 0.89$, n =13, F-test: $p \leq 0.0001$; B) Relationships between ΔDO (µmol/L) and ΔN_2 (µmol-N L^{-1}) for each sampling date: July 2006: $\Delta N_2 = (-0.13 \times \Delta DO) + 0.39$ with $R^2 = 0.56$, n = 9, F-test: $p \leq 0.01$; September 2006: $\Delta N_2 = (-0.42 \times \Delta DO) + 3.48$ with $R^2 = 0.97$, n =11, F-test: $p \leq 0.0001$ and July 2007: $\Delta N_2 = (-0.49 \times \Delta NO_3^-) - 9.25$ with $R^2 = 0.93$, n =21, F-test: $p \leq 0.0001$.

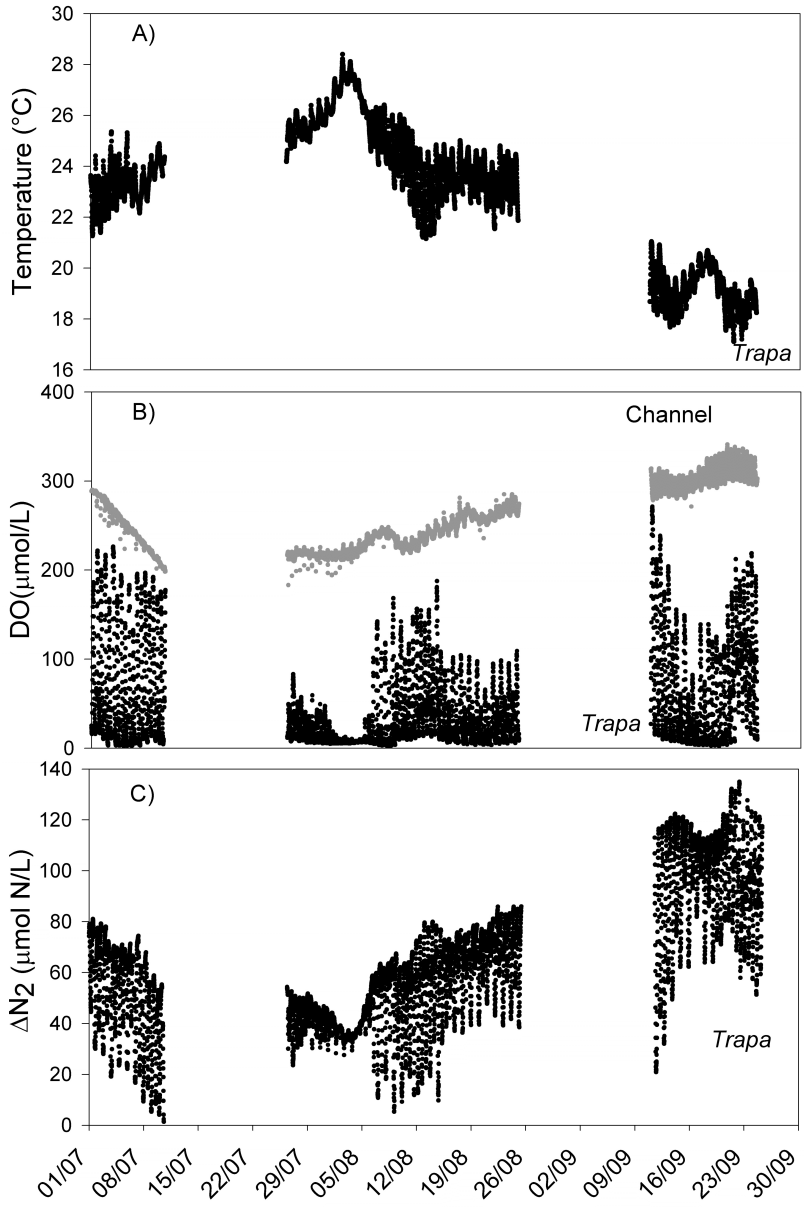
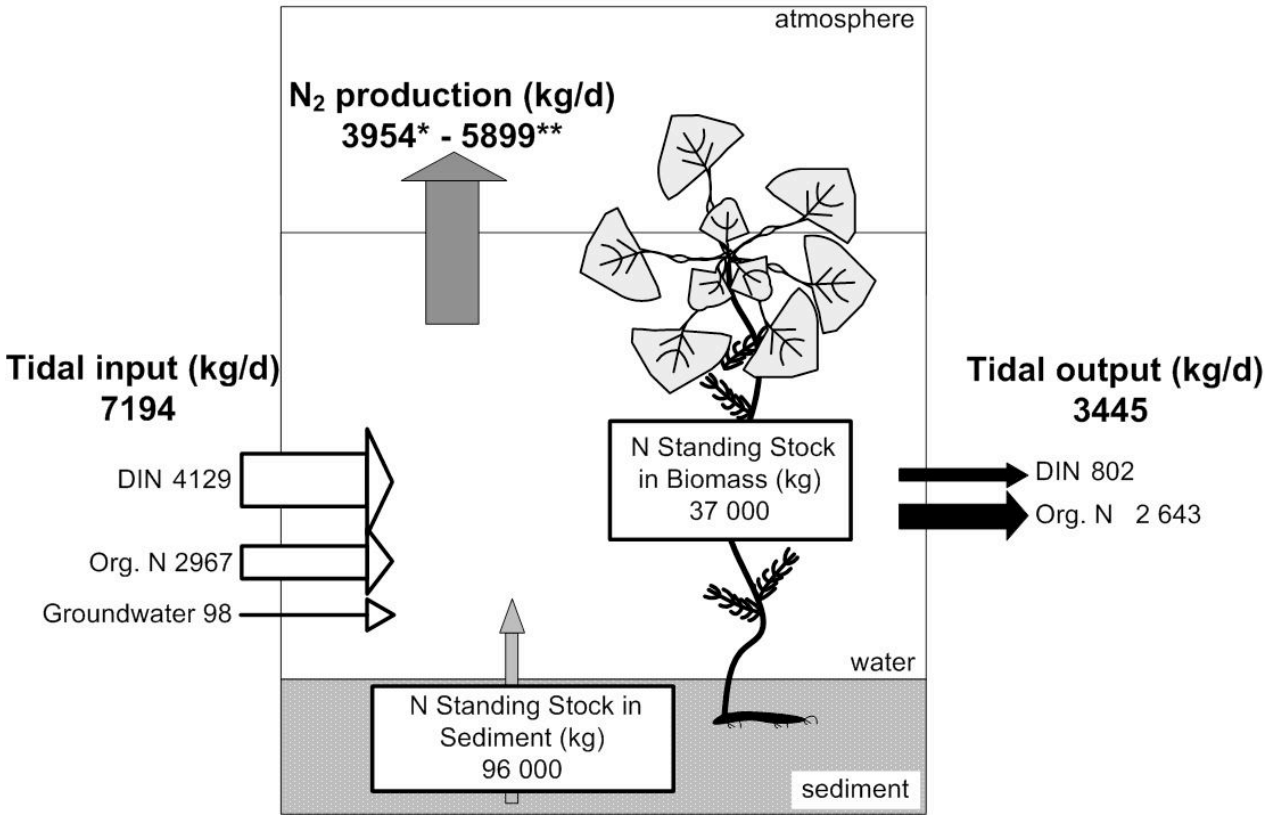


Figure 4.4: A) Temperature change in the inner *Trapa* bed site and B) DO values for the inner *Trapa* bed site (black dots) and channel site (gray dots) both measured at 15 min intervals in summer 2006 during periods of ebb tide. C) Represents modeled N_2 production using measured DO and temperature as predictor variables during ebb tide. These midsummer to fall measurements cover the period of maximum floating leaved *Trapa* biomass in the river.



* estimated using areal system respiration

** estimated using measured O_2 during ebb tide

Figure 4.5: Fate of N in the 4 km^2 *Trapa* vegetated shallows of the TFH during the summer months represented using a mass balance approach. Arrows represent the amount of N entering or exiting the beds via different processes in kg N per day. Tidal inputs represent N entering bed with the water during rising tide. Tidal outputs represent N flushed out of the beds with ebbing tide. N_2 production represents N permanently lost to the atmosphere in gas form. N stored in *Trapa* and sediments (kg N) are reported.

4.5. Discussion

Trapa beds as hotspots

Results from our study clearly demonstrate that large beds of the exotic macrophyte *Trapa natans* are hot-spots for denitrification losses within the TFH. Estimated rates of N_2 production in *Trapa* ranged from 1 to 154 µmol N/L, with an average of 47 (± 26) µmol N/L per ebb tide, in contrast to negligible changes in N_2 concentrations in native *Vallisneria* beds. Our average daily rate estimates of N_2 production of 88 (±51) µmol N L^{-1} day^{-1} inside the *Trapa* beds is in the high range of what was reported in a review of denitrification in aquatic systems (Piña-Ochoa and Alvarez-Cobelas 2006), with our highest rate being among the highest ever observed for aquatic ecosystems. It should also be noted that these daily values assume N_2 production was occurring during ebbing tide only when rates were actually measured. The average hourly rate of N_2 production during ebb was extremely high (7 µmol N L^{-1} $hour^{-1}$ ranging from 0.2 to 24), making periods of ebb tide a critically important moment for denitrifying activity, in these *Trapa* bed hotspots.

We found that N_2 production in *Trapa* beds was intimately linked with localized O_2 consumption and system metabolism. The contrasting O_2 dynamics in exotic *Trapa* beds during ebb tide as compared to native *Vallisneria* has been previously described in great detail (Caraco and Cole 2002, Goodwin et al. 2008). Briefly, when the rosette leaves of *Trapa* reach the surface, the plant vents O_2 to the atmosphere, depleting O_2 in the surrounding water during ebb tide; during rising tide, O_2 and nutrients from the main channel replenish the beds due to the physical exchange of water. The cycle of O_2 loss begins anew upon ebbing tide. Suboxic conditions created directly by *Trapa* under ebb tide favour microbial transformations that remove inorganic N species and produce N_2 gas (canonical denitrification and anaerobic ammonium oxidation). Although the loss of nitrate and a decrease in the N: P ratio had been previously reported in these beds (Caraco and Cole 2002), our study provides conclusive evidence that the N loss observed during ebb tide was a function of N_2 production, thus representing permanent N loss from the ecosystem.

Denitrification losses were clearly the dominant fate of N in the beds during ebb tide whereby gaseous production of N_2 represented between 55 and 82% of total N inputs to the beds

(**Figure 4.5**). A positive relationship between N availability and denitrification habe been observed in a range of aquatic systems (Saunders and Kalff 2001, Seitzinger et al. 2006). A recent evaluation of denitrification losses in streams found that high rates of N loss were closely associated with elevated concentrations of N (Mulholland et al. 2008). Our stronger link of N_2 production with O_2 may better reflect the dynamics that would influence the N available for denitrification beyond NO_3^- concentration in the system. Indeed in a sediment denitrification review by Fennel et al. (2009), the authors suggest that sediment oxygen demand is a more useful metric to predict denitrification in bottom waters than NO_3^- concentration because of the multiple microbial N transformations influenced by O_2 concentration that supply the substrates and create the optimal conditions required for N_2 production.

Average daily N input and output estimates for the 4 km^2 patches occupied by large *Trapa* beds were well balanced, with an estimated 7194 kg N/d entering these shallow beds and between 7399-9344 kg N/d exiting, 3954 to 5899 kg N/d of it as N_2 gas. Surprisingly most of the N_2 production could have been fuelled by DIN loading to the beds, when comparing the lower estimate of N_2 production to our mass balance terms. Although we saw strong relationships between ΔN_2 and ΔNO_3^- in the *Trapa* beds by date, the slopes of these relationships suggested that depending on the date, change in NO_3^- concentration alone was unable to account for all N_2 produced (Figure 16). Given the variability in the mole to mole relationships of NO_3^- versus N_2, NO_3^- must have been internally produced, likely via nitrification. Nitrification in oxic sediments can be an important source of NO_3^- fuelling coupled nitrification-denitrification (Seitzinger 1988) and the rapid cycling of N stored in the sediment was the most likely source to fuel these reactions. The huge variability in N_2 production can also be linked to the variable rates of O_2 loss in *Trapa* beds (Goodwin et al. 2008, **Figure 4.3B**). Gradual O_2 loss would promote nitrification and enhance N_2 production beyond NO_3^- concentration. However a rapid loss of O_2 caused by enhanced respiratory losses at very high temperatures or an incomplete replenishment of O_2 to the bed would hinder the nitrification-denitrification coupling and N_2 losses would likely reflect the available NO_3^- concentrations only.

Although other studies have shown direct N uptake by *Trapa* plants to be a significant N sink, removing between 15 to 85% of available dissolved inorganic nitrogen (Tsuchiya and Iwakuma 1993), direct uptake by plants in the TFH was by comparison a small and temporary N

loss term. *Trapa* N uptake represents approximately 7% of the total N removed from the TFH. Furthermore this would be only a temporary N storage term as plants would likely release this N during their decay and serve to partially replenish the *Trapa* bed sediment with N.

Contrary to expectation, high rates of N_2O production were not observed in *Trapa* beds. In fact, we measured a decrease in N_2O concentration with increasing N_2 production suggesting that N_2O produced in the beds was ultimately reduced to N_2. Observations of net N_2O consumption in aquatic systems remain rare (Beaulieu et al 2008, Baulch et al. submitted). However a review by Chapuis-Lardy et al. (2007) reported that soils could be an important N_2O sink in conditions of low mineral N and large moisture content. One possible mechanistic explanation for this N_2O consumption is that the enzyme NOR, responsible for N_2O reduction to N_2, is more sensitive to oxygen than other denitrification enzymes (Knowles 1982) and hypoxic-anoxic conditions in *Trapa* beds could have enhanced its efficiency at reducing N_2O.

Species can matter in ecosystem function

When compared to native vegetation, invasive plant species are known to strongly influence N dynamics by either altering rates of key microbial processes or modifying standing stocks (Ehrenfeld 2003), but impacts vary widely among species. For example, *Phragmites australis*, an invasive perennial wetland grass is reported to have 60% more N bound in its biomass, and its dominance accelerates the rate of N mineralization as compared to native vegetation (Windham and Ehrenfeld 2003). This species apparently can access the dissolved organic N more effectively and has higher affinity for DIN than native vegetation (Modzer et al 2010). Alternatively, *Microstegium vimineum,* another invasive wetland grass, has lower N requirements and reduced N remineralization rates when compared to a diverse native community (DeMeester and Richter 2010). This invasive plant lowered the redox potential of the soils, thereby reducing the rates of soil decomposition. N-fixing invasive species are also well known for their impacts on altering N cycling dynamics through their capacity to increase inorganic N pools, influencing overall mineralization and nitrification rates (D'Antonio and Corbin 2003). *Myrica faya*, an exotic N-fixing shrub has completely modified ecosystem properties in Hawaii and is now the largest N source to this once N-limited system (Vitousek and Walker 1989). Although this input of new N to an N-limited system may be perceived as positive, negative impacts may be

observed at larger scales. Invasive N-fixing Kudzu and *M. faya* have been reported to double or triple N_2O emissions per unit area as compared to native vegetation (Hall and Asner 2007, Hickman et al. 2010) resulting in a decrease of air quality.

Our study clearly shows that the presence of an exotic and invasive macrophyte significantly enhances the permanent loss of N from the TFH, thereby playing a positive role in whole ecosystem function. Sites invaded by large *Trapa* beds were found to be hotspots of N removal, whereas beds of native *Vallisneria* did not demonstrate significant rates of N loss. The TFH reach is estimated to remove 2000 mT N/yr or 5480 kg N/d (Lampman et al. 1999). Although the *Trapa* area represents only 2.7% of this 110 km^2 reach of the TFH, our study suggests that around 70 to greater than 100% of this daily removal occurred in the *Trapa* vegetated shallows during the summer months. Furthermore, if we consider that *Trapa* rosette leaves are emergent for only 90 days in a year, *Trapa* beds could remove between 331 and 556 mT, an impressive 18 to 27% of the annual N retention, making the summer months a serious "hot-moment" of N removal.

Species functional characteristics enable consideration of species effects on ecosystem processes (Hooper et al. 2005) and the bigger the difference between an invasive's and a native species' functional trait, the bigger should be the impact of the invasive on ecosystem functioning. The striking difference in growth form between *Trapa* (floating leaves) and the dominant resident species (submerged leaves) is most likely the key factor in *Trapa*'s strong impact on O_2 and N cycling, whereby *Trapa* vents O_2 to the atmosphere. However a difference in this trait alone may not be sufficient enough to result in a major functional ecosystem impact between the invader and the native species. The physical structure of the ecosystem may also be an important determining factor in this case, one that works synergistically to facilitate the impact of the trait. In the case of the Hudson River, it is the combined tidal action and atmospheric venting of O_2 by *Trapa* that makes these beds permanent N removal hotspot sites during the summer in the TFH. The continuous replenishment of *Trapa* beds with oxygenated water rich in nutrients and its subsequent export downstream amplify the impact of N removal at the TFH scale. In the case of a non-tidal system, dense beds of *Trapa* would create large zones of water depleted with O_2 where the removal of N is limited to the amount of N originally present in the bed. This would still result in a significant difference in function between this particular invasive and non-native species, but with a lesser impact on whole ecosystem function.

The percent N removed in the TFH is consistent with the proportion predicted from riverine N removal models, approximately 20% of total N input (Alexander et al 2000, Seitzinger et al 2002). Our data suggest that a large portion of that N removal occurred in *Trapa* beds. However these models typically do not take into account the spatial heterogeneity and variability in N removal within the system, such as the presence of large mono-specific macrophyte beds. Reduction of anthropogenic N-loading to aquatic ecosystems is essential to improve water quality, protect drinking-water supplies and minimize export to N-limited coastal zones (Conley et al. 2009). Introduced species like *Trapa* are known to alter patterns of ecosystem processes (Chapin et al. 2000), but these exotic species are classically perceived as having negative impacts on ecosystems. However in the case of the Hudson River, N removal by *Trapa* can be described as a positive impact, an ecosystem service, defined as a function useful to humans (Kremen 2005). Indeed the N removed by *Trapa* in the TFH is equivalent to the amount loaded to this River as sewage from the city of Albany (Lampman et al. 1999). The strategic location of the *Trapa* below this city works to reduce anthropogenic N load to the coastal environment, thus performing an essential ecosystem service.

Literature cited

Alexander, R. B., J. K. Bohlke, E. W. Boyer, M. B. David, J. W. Harvey, P. J. Mulholland, S. P. Seitzinger, C. R. Tobias, C. Tonitto, and W. M. Wollheim. 2009. Dynamic modeling of nitrogen losses in river networks unravels the coupled effects of hydrological and biogeochemical processes. Biogeochemistry 93:91-116.

Alexander, R. B., R. A. Smith, and G. E. Schwarz. 2000. Effect of stream channel size on the delivery of nitrogen to the Gulf of Mexico. Nature 403:758-761.

Anderson, D. R., K. P. Burnham, and G. C. White. 1998. Comparison of Akaike information criterion and consistent Akaike information criterion for model selection and statistical inference from capture-recapture studies. Journal of Applied Statistics 25:263-282.

Arrigoni, A., S. Findlay, D. Fischer, and K. Tockner. 2008. Predicting carbon and nutrient transformations in tidal freshwater wetlands of the Hudson River. Ecosystems 11:790-802.

Beaulieu, J. J., C. P. Arango, S. K. Hamilton, and J. L. Tank. 2008. The production and emission of nitrous oxide from headwater streams in the Midwestern United States. Global Change Biology 14:878-894.

Bernot, M. J., and W. K. Dodds. 2005. Nitrogen retention, removal, and saturation in lotic ecosystems. Ecosystems 8:442-453.

Caraco, N. F., and J. J. Cole. 2002. Contrasting impacts of a native and alien macrophyte on dissolved oxygen in a large river. Ecological Applications 12:1496-1509.

Chapin, F. S., E. S. Zavaleta, V. T. Eviner, R. L. Naylor, P. M. Vitousek, H. L. Reynolds, D. U. Hooper, S. Lavorel, O. E. Sala, S. E. Hobbie, M. C. Mack, and S. Diaz. 2000. Consequences of changing biodiversity. Nature 405:234-242.

Chapuis-Lardy, L., N. Wrage, A. Metay, J. L. Chotte, and M. Bernoux. 2007. Soils, a sink for N_2O? A review. Global Change Biology 13:1-17.

Cole, J. J., and N. F. Caraco. 2001. Emissions of nitrous oxide (N_2O) from a tidal, freshwater river, the Hudson River, New York. Environmental Science & Technology 35:991-996.

Conley, D. J., H. W. Paerl, R. W. Howarth, D. F. Boesch, S. P. Seitzinger, K. E. Havens, C. Lancelot, and G. E. Likens. 2009. Controlling Eutrophication: Nitrogen and Phosphorus. Science 323:1014-1015.

Cooper, J. C., F. R. Cantelmos, and C. E. Newton. 1988. Overview of the Hudson River estuary. American Fisheries Society Monograph 4:11:24.

Costanza, R., R. d'Arge, R. de Groot, S. Farber, M. Grasso, B. Hannon, K. Limburg, S. Naeem, R. V. O'Neill, J. Paruelo, R. G. Raskin, P. Sutton, and M. van den Belt. 1997. The value of the world's ecosystem services and natural capital. Nature 387:253-260.

D'Antonio, C. M., and J. D. Corbin. 2003. Effects of plant invaders on nutrient cycling: Using models to explore the link between invasion and development of species effects.

DeMeester, J. E., and D. D. Richter. 2010. Differences in wetland nitrogen cycling between the invasive grass *Microstegium vimineum* and a diverse plant community. Ecological Applications 20:609-619.

Ehrenfeld, J. G. 2003. Effects of exotic plant invasions on soil nutrient cycling processes. Ecosystems 6:503-523.

Fennel, K., D. Brady, D. Di Toro, R. W. Fulweiler, W. S. Gardner, A. Giblin, M. J. McCarthy, A. Rao, S. Seitzinger, M. Thouvenot-Korppoo, and C. Tobias. 2009. Modeling denitrification in aquatic sediments. Pages 159-178.

Galloway, J., E. Cowling, and E. Kessler. 2002. Reactive nitrogen. Ambio 31:59-59.

Goodwin, K., N. Caraco, and J. Cole. 2008. Temporal dynamics of dissolved oxygen in a floating-leaved macrophyte bed. Freshwater Biology 53:1632-1641.

Hall, S. J., and G. P. Asner. 2007. Biological invasion alters regional nitrogen-oxide emissions from tropical rainforests. Global Change Biology 13:2143-2160.

Hickman, J. E., S. L. Wu, L. J. Mickley, and M. T. Lerdau. 2010. Kudzu (*Pueraria montana*) invasion doubles emissions of nitric oxide and increases ozone pollution. Proceedings of the National Academy of Sciences of the United States of America 107:10115-10119.

Holm-Hansen, O., and B. Riemann. 1978. Chlorophyll *a* determination: Improvement in methodology. Oikos 30:438-447.

Hooper, D. U., F. S. Chapin, J. J. Ewel, A. Hector, P. Inchausti, S. Lavorel, J. H. Lawton, D. M. Lodge, M. Loreau, S. Naeem, B. Schmid, H. Setala, A. J. Symstad, J. Vandermeer, and D. A. Wardle. 2005. Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. Ecological Monographs 75:3-35.

Howarth, R. W. 1998. An assessment of human influences on fluxes of nitrogen from the terrestrial landscape to the estuaries and continental shelves of the North Atlantic Ocean. Nutrient Cycling in Agroecosystems 52:213-223.

Kana, T. M., C. Darkangelo, M. D. Hunt, J. B. Oldham, G. E. Bennett, and J. C. Cornwell. 1994. Membrane inlet mass spectrometer for rapid high precision determination of N_2, O_2 and Ar in environmental water samples Analytical Chemistry 66:4166-4170.

Knowles, R. 1982. Denitrification. Microbiological Reviews 46:43-70.

Kremen, C. 2005. Managing ecosystem services: what do we need to know about their ecology? Ecology Letters 8:468-479.

Lampman, G. G., N. F. Caraco, and J. J. Cole. 1999. Spatial and temporal patterns of nutrient concentration and export in the tidal Hudson River. Estuaries 22:285-296.

McClain, M. E., E. W. Boyer, C. L. Dent, S. E. Gergel, N. B. Grimm, P. M. Groffman, S. C. Hart, J. W. Harvey, C. A. Johnston, E. Mayorga, W. H. McDowell, and G. Pinay. 2003. Biogeochemical hotspots and hot moments at the interface of terrestrial and aquatic ecosystems. Ecosystems 6:301-312.

Mozdzer, T. J., J. C. Zieman, and K. J. McGlathery. 2010. Nitrogen Uptake by Native and Invasive Temperate Coastal Macrophytes: Importance of Dissolved Organic Nitrogen. Estuaries and Coasts 33:784-797.

Mulholland, P. J., A. M. Helton, G. C. Poole, R. O. Hall, S. K. Hamilton, B. J. Peterson, J. L. Tank, L. R. Ashkenas, L. W. Cooper, C. N. Dahm, W. K. Dodds, S. E. G. Findlay, S. V. Gregory, N. B. Grimm, S. L. Johnson, W. H. McDowell, J. L. Meyer, H. M. Valett, J. R. Webster, C. P. Arango, J. J. Beaulieu, M. J. Bernot, A. J. Burgin, C. L. Crenshaw, L. T. Johnson, B. R. Niederlehner, J. M. O'Brien, J. D. Potter, R. W. Sheibley, D. J. Sobota, and S. M. Thomas. 2008. Stream denitrification across biomes and its response to anthropogenic nitrate loading. Nature 452:202-205

Nieder, W. C., E. Barnaba, S. E. G. Findlay, S. Hoskins, N. Holochuck, and E. A. Blair. 2004. Distribution and abundance of submerged aquatic vegetation and *Trapa natans* in the Hudson River estuary. Journal of Coastal Research 45:150-161.

Nystrom, E.A. 2010. Groundwater quality in the Lower Hudson River Basin, New York, 2008: U.S. Geological Survey Open-File Report 2010-1197, 39 p., available only at http://pubs.usgs.gov/of/2010/1197/.

Paerl, H. W. 1997. Coastal eutrophication and harmful algal blooms: Importance of atmospheric deposition and groundwater as "new" nitrogen and other nutrient sources. Limnology and Oceanography 42:1154-1165.

Peterson, B. J., W. M. Wollheim, P. J. Mulholland, J. R. Webster, J. L. Meyer, J. L. Tank, E. Marti, W. B. Bowden, H. M. Valett, A. E. Hershey, W. H. McDowell, W. K. Dodds, S. K. Hamilton, S. Gregory, and D. D. Morrall. 2001. Control of nitrogen export from watersheds by headwater streams. Science 292:86-90.

Piña-Ochoa, E., and M. Alvarez-Cobelas. 2006. Denitrification in aquatic environments: A cross-system analysis. Biogeochemistry 81:111-130.

Raymond, P. A., N. F. Caraco, and J. J. Cole. 1997. Carbon dioxide concentration and atmospheric flux in the Hudson River. Estuaries 20:381-390.

Rooney, N., Kalff J., C. Habel. 2003. The role of submerged macrophyte beds in phosphorus and sediment accumulation in Lake Memphremagog, Quebec, Canada. Limnology and Oceanography. 48 (5): 1927-1937.

Saunders, D. L., and J. Kalff. 2001. Nitrogen retention in wetlands, lakes and rivers. Hydrobiologia 443:205-212.

Schlesinger, W. H. 2009. On the fate of anthropogenic nitrogen. Proceedings of the National Academy of Sciences of the United States of America 106:203-208.

Seitzinger, S. P. 1988. Denitrification in freshwater and coastal marine ecosystems - Ecological and geochemical significance. Limnology and Oceanography 33:702-724.

Seitzinger, S., J. A. Harrison, J. K. Bohlke, A. F. Bouwman, R. Lowrance, B. Peterson, C. Tobias, and G. Van Drecht. 2006. Denitrification across landscapes and waterscapes: A synthesis. Ecological Applications 16:2064-2090.

Seitzinger, S. P., R. V. Styles, E. W. Boyer, R. B. Alexander, G. Billen, R. W. Howarth, B. Mayer, and N. Van Breemen. 2002. Nitrogen retention in rivers: model development and application to watersheds in the northeastern USA. Biogeochemistry 57:199-237.

Stanley, E. H., and J. T. Maxted. 2008. Changes in the dissolved nitrogen pool across land cover gradients in Wisconsin streams. Ecological Applications 18:1579-1590.

Tank, J. L., E. J. Rosi-Marshall, M. A. Baker, and R. O. Hall. 2008. Are rivers just big streams? A pulse method to quantify nitrogen demand in a large river Ecology 89:2935-2945.

Templer, P., S. E. G. Findlay, and C. Wigand. 1998. Sediment chemistry associated with native and non-native emergent macrophytes of a Hudson River marsh ecosystem. Wetlands 18:70-78.

Tsuchiya, T., and T. Iwakuma. 1993. Growth and leaf life-span of floating-leaved plant, *Trapa natans* L., as influenced by nitrogen flux Aquatic Botany 46:317-324.

Vitousek, P. M., and L. R. Walker. 1989. Biological invasion by *Myrica faya* in Hawaii - Plant demography, nitrogen-fixation, ecosystem effects. Ecological Monographs 59:247-265.

Weiss, R. F., and B. A. Price. 1980. Nitrous oxide solubility in water and seawater. Marine Chemistry 8:347-359.

Wigand, C., Stevenson, J.C., and J.C. Cornwell. 1997. Effects of different submersed macrophytes on sediment biogeochemistry. Aquatic Botany 56:233-244.

Windham, L., and J. G. Ehrenfeld. 2003. Net impact of a plant invasion on nitrogen-cycling processes within a brackish tidal marsh. Ecological Applications 13:883-896.

Wollheim, W. M., C. J. Vorosmarty, B. J. Peterson, S. P. Seitzinger, and C. S. Hopkinson. 2006. Relationship between river size and nutrient removal. Geophysical Research Letters 33:4.

5. Conclusion Générale

« L'Homme a bouleversé le cycle de l'azote en créant des quantités industrielles de N réactif afin que l'agriculture puisse soutenir la croissance de la population. Les mécanismes de rétroaction naturels réalisés par les micro-organismes (dénitrification et autres transformations de N produisant du N_2) seraient susceptibles de rétablir un nouvel équilibre sur des échelles de temps de plusieurs décennies. (...) Ainsi l'excès de N serait éliminé à des taux comparables aux taux d'addition de N. Cependant les prévisions d'augmentation de la population humaine suggèrent que la demande en N destinée à la production agricole sera aussi à la hausse. Cette escalade permanente de l'utilisation de N provoquera une hausse continue des flux de N vers les rivières. »

Traduit librement de l'article « The Evolution and Future of Earth's Nitrogen Cycle » par Canfield et al. (2010) paru dans la revue Science.

Au moment de remettre cette thèse, la lecture de l'article cité plus haut et paru récemment (Canfield et al. 2010), confirme toute la pertinence au niveau régional et global du sujet et des hypothèses que nous avons testés. Cette thèse de recherche porte sur la rétention de N dans les grandes rivières et les facteurs hydrologiques et biogéochimiques qui affectent cette rétention. Pour ce faire, nous avons voulu donner un portrait complet du processus de rétention dans les rivières en nous penchant sur la complexité spatiale des transformations de N et en donnant un aperçu du rôle de divers organismes impliqués dans ces transformations. Nous avons, tout d'abord, étudié les émissions de N_2O et les ratios N_2O: N_2 qui bien que mesurés à une échelle locale ont un non seulement régional mais probablement global. Nous avons ensuite évalué le rôle des tapis de cyanobactéries benthiques sur le budget de N en extrapolant des mesures locales à l'ensemble de l'écosystème. Et enfin nous avons démontré l'importance d'une plante invasive pour la dénitrification en mettant l'accent sur les services écosystémiques apportés par une espèce exotique.

Dans le reste de ce chapitre les principaux résultats de cette recherche sont discutés en mettant l'accent sur leurs originalités et sur leur importance dans la compréhension globale de la dynamique de N dans les rivières.

5.1. Les émissions de N_2O et la dénitrification : l'hydrologie un facteur-clé

L'étude des émissions de N_2O dans le lac Saint-Pierre (LSP) est la première à mesurer et à rapporter des taux d'émissions de N_2O dans le fleuve Saint-Laurent. C'est aussi la première à estimer les taux de dénitrification dans la zone du fleuve précédant l'estuaire fluvial. Les réseaux de rivières sont largement reconnus comme étant des émetteurs de N_2O (Seitzinger et Kroeze 1998) mais les données sur les grandes rivières sont encore peu nombreuses. Les résultats de ce chapitre aident donc à mieux définir les limites des émissions de N_2O dans les fleuves en fournissant de nouvelles données mesurées sur le terrain et à comprendre les facteurs qui contrôlent ces émissions.

Nous avons créé un modèle conceptuel inspiré du modèle HIP de Firestone et Davidson (1989) décrit dans le chapitre 1. Ce modèle a été adapté aux écosystèmes de rivières et il a été baptisé le « Leaky-Split- Pipe Model » (**Figure 5.1**). La grande différence entre le modèle HIP et notre modèle, est que le modèle du « Leaky-Split-Pipe » permet l'advection de N inorganique dissous (DIN = NH_4^+ et NO_3^-) vers l'aval du système. Avec notre modèle, nous pouvons donc estimer une valeur de rétention dans le système, alors que le modèle HIP suppose que tout le DIN qui entre dans un système est transformé en gaz (N_2O et N_2). Dans le modèle conceptuel du « Leaky-Split pipe », les tuyaux représentent les flux de N qui entrent, sortent et sont émis sous la forme de N_2. Le trou représente la fuite sous forme de N_2O. La taille de tuyaux et des trous est proportionnelle à la quantité de N qui y est transformée ou qui y transite. La rétention correspond à la charge de N moins la sortie de N ce qui prend en compte les différents mécanismes de rétention de N (dénitrification, sédimentation et assimilation).

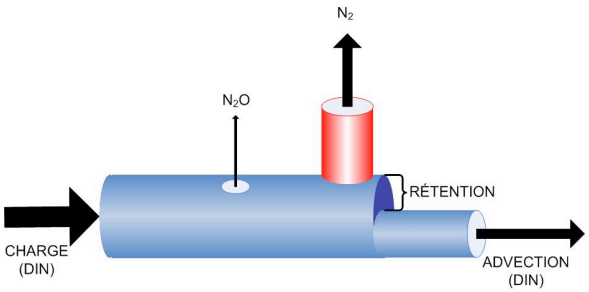


Figure 5.1 : Le modèle conceptuel du « Leaky-Split-Pipe » illustre les flux d'azote inorganique et leurs transformations dans les systèmes lotiques.

Nous avons ainsi testé plusieurs hypothèses majeures concernant l'effet des changements hydrologiques en nous basant sur ce modèle conceptuel. Le modèle du «Leaky-Split-Pipe» suggère que l'augmentation de la charge en DIN entraîne : 1) une augmentation de la quantité de N dénitrifié (taux plus élevé); 2) une augmentation du ratio N_2O: N_2 et 3) une baisse du pourcentage de DIN retenu. Dans le cas contraire où la charge en DIN diminue en raison d'une baisse du débit des tributaires, notre modèle prévoit : 1) une plus grande proportion de N dénitrifié en raison du temps de résidence de l'eau prolongé ; 2) un ratio N_2O: N_2 plus bas car la dénitrification serait complète et produirait moins de N_2O.

Nos résultats indiquent que LSP est une source de N_2O pour l'atmosphère. Cependant les émissions de N_2O dans LSP sont faibles. LSP fait partie des systèmes avec les plus faibles émissions mesurées lorsque comparées à celles d'autres rivières et ruisseaux (Cole et Caraco 2001, Dong et al. 2002, Beaulieu et al. 2008, Beaulieu et al. 2010).

Les flux estimés au LSP durant deux années aux conditions hydrologiques très différentes (bas débits en 2005 et débits plus élevés en 2006) permettent d'étayer certaines hypothèses de notre modèle conceptuel. Les faibles débits enregistrés en 2005 ont causé une faible charge en N dans LSP. Au niveau de la rétention de N, nos résultats montrent que la quantité de N retenu dans LSP est plus élevée lorsque la charge en N est élevée. Par contre, la proportion de cette charge qui est effectivement retenue diminue lorsque la charge augmente. Le rôle de la dénitrification dans le processus de rétention est variable. L'importance relative de la dénitrification augmente lorsque la charge augmente. En effet, dans les situations où le débit de l'eau et la charge en N diminuent, les écosystèmes fluviaux peu profonds comme LSP connaissent de fortes hausses de leur biomasse de producteurs primaires. L'assimilation de N devient alors le principal mécanisme de rétention de N.

Étonnamment, nous avons trouvé une augmentation significative du ratio N_2O: N_2 (dix fois plus élevée) dans les conditions de faibles charges en N et de bas niveaux d'eau. Ces résultats vont à l'encontre des hypothèses de notre modèle mais aussi des conclusions tirés de la littérature (Firestone et Davidson 1989). Cependant, un ratio N_2O: N_2 plus élevé

pourrait être dû aux émissions de N_2O par la nitrification. En effet, lorsque la charge en NO_3^- est basse, la dénitrification peut être couplée à la nitrification qui fournit les NO_3^- nécessaires (Firestone et Davidson 1989, Garnier et al. 2006)

Comme cela a été prouvé dans d'autres rivières (Stow et al. 2005, Beaulieu et al. 2009), les flux de N_2O dans LSP sont liés à la concentration de NO_3^- dans l'eau. En généralisant cette relation à 16 rivières dans le monde pour lesquelles les données étaient accessibles nous avons démontré que les variations d'émissions de N_2O entre les rivières s'expliquent en majorité par le lien positif avec les concentrations en NO_3^- et le lien négatif avec la charge hydraulique du système. Ce dernier point confirme donc que les charges hydrauliques plus élevées sont associées à des émissions de N_2O plus grandes et comme le prouve, le cas du LSP, probablement à des ratios N_2O: N_2 plus élevés.

Dans un contexte global, nos résultats suggèrent que l'utilisation croissante de fertilisants azotés associés à des épisodes de réductions de niveaux d'eau de plus en plus fréquents dans les rivières (Hodgkins et Dudley 2006, Boyer et al. 2010) pourraient stimuler la production de N_2O et modifier les ratios N_2O: N_2 dans ces milieux.

5.2. Les tapis de cyanobactérie : une source importante de N dans le le fleuve Saint-Laurent?

Alors que la dénitrification est reconnue comme un processus dominant dans les rivières, la fixation de N dans ces écosystèmes a jusqu'à présent été considérée comme un processus mineur dans le fonctionnement et dans le budget en N des écosystèmes lotiques (Howarth et al. 1988, Galloway et al. 2004). La fixation importante de N dans des tapis de cyanobactéries ou des herbiers marins a surtout été démontrée dans des eaux peu profondes, oligotrophes ou dans des écosystèmes tropicaux (Howarth et al. 1988). Notre estimation des flux nets de N_2 dans un tapis de cyanobactéries est donc une contribution scientifique originale car elle permet de déterminer laquelle de la dénitrification ou de la fixation de N est le processus dominant dans les tapis de cyanobactéries des lacs Saint-Louis (LSL) et Saint-Pierre (LSP), deux élargissements du fleuve Saint-Laurent. En effet, le déséquilibre

entre ces deux processus est considéré comme le principal mécanisme de contrôle de la limitation en N dans les systèmes aquatiques (Vitousek et al. 2002, Codispoti 2007). Nous avons ensuite extrapolé nos résultats à la grandeur de LSP, un des deux écosystèmes étudiés pour lequel des estimés de la couverture de ces tapis existaient (Hudon et Carignan 2008, Vis et al. 2008).

Dans ces tapis, nous nous attendions à observer une alternance entre des périodes de flux de N_2 positifs (dénitrification nette) et des flux de N_2 négatifs (fixation nette). Notre hypothèse était que les flux positifs seraient liés à une forte concentration de NO_3^- et les flux négatifs à de faibles concentrations ou à l'absence de NO_3^-.

Nos résultats indiquent que les tapis de cyanobactéries dans le fleuve Saint-Laurent connaissent en moyenne des flux négatifs et sont donc des fixateurs nets de N pendant la période de l'étude de juin à octobre. Ces flux négatifs de N_2 sont très élevés et ils sont comparables aux taux de fixation nets mesurés dans les sédiments de la baie de Narragansset (Fulweiler et al. 2007). Bien que les flux fussent très variables, nous n'avons pas mesuré de flux de N_2 positifs dans les tapis de cyanobactéries.

Les flux de N_2 dans les tapis de cyanobactéries du fleuve Saint-Laurent, sont liés principalement à la concentration et aux flux d'oxygène. Contrairement à notre hypothèse, il n'y a pas de relation significative entre les flux de N_2 et les concentrations de NO_3^-. Cette absence de relation avec les NO_3^- est sûrement due à la faible concentration des NO_3^- et à la gamme étroite de variations mesurée durant nos expériences (5,1 – 14,8 μmol L^{-1}). Les relations entre les flux de N_2 et les flux d'oxygène indiquent que la fixation est accompagnée d'une importante respiration dans le système. Nos résultats suggèrent donc que la fixation dans les tapis de cyanobactéries est probablement effectuée en partie par d'autre diazotrophes non-photosynthéhiques et que la dénitrification et la fixation sont réalisées simultanément. En effet, la fixation de N et la dénitrification ont déjà été observées simultanément dans des eaux peu profondes, des écosystèmes subtropicaux et dans des sédiments estuariens (Gardner et al. 2006). Cependant, la méthode de ratio N_2: Ar (Kana et al. 1994) pour mesurer les concentrations en N_2 que nous avons utilisée ne permet

pas de mesurer les taux bruts de chacun des processus mais seulement les taux nets de dénitrification ou de fixation de N.

Ces tapis de cyanobactéries représentent une source de N non négligeable durant l'été et l'automne où ils peuvent occuper de grandes surfaces de l'écosystème et ainsi compenser pour le N perdu par la dénitrification (plus de 30% du N dénitrifié, chapitre 3). La capacité de ces consortiums microbiens d'exécuter plusieurs transformations et de fonctionner suivant différents types de métabolismes (autotrophe ou hétérotrophe) leur donne un avantage certain pour résister aux changements de conditions du milieu. Dans des conditions de limitation en N, les tapis de cyanobactéries pourraient donc réaliser en équipe, la fixation de N au taux maximum permis par les conditions environnementales. Nous suggérons donc que le facteur limitant de la fixation ou de la dénitrification, dans ces milieux pourrait être le carbone organique labile fournit par la photosynthèse comme l'a prouvé Fulweiler et al. (2008) avec des expériences d'additions de carbone dans les sédiments de la baie de Narragansset.

5.3. Les lits de macrophytes exotiques : des zones actives de dénitrification

Trapa natans, une macrophyte originaire d'Asie, a été introduite dans l'état de New-York au début du 19ème siècle. Aujourd'hui elle est la 2ème plante la plus abondante dans le fleuve Hudson après la Vallisnérie américaine, une espèce indigène (Caraco et Cole 2002). Contrairement à la Vallisnérie qui est entièrement submergée dans l'eau, *Trapa* a des feuilles flottantes. Cette dernière caractéristique combinée à une forte production de matière organique crée un déficit en oxygène dans son environnement immédiat. Les lits très denses de *Trapa* sont des puits d'oxygène mais aussi de NO_3^-. L'hypothèse de la dénitrification pour expliquer les pertes importantes de NO_3^- dans ces sites a été proposée à plusieurs reprises (Caraco et Cole 2002, Arrigoni et al. 2008) mais elle n'avait jamais été testée avant notre étude.

Nous avons donc mesuré la production de N_2 dans un vaste lit de macrophytes pendant deux étés consécutifs. Nos résultats indiquent une forte production de N_2 par la dénitrification. L'hypoxie dans les lits de *Trapa* stimule la dénitrification. Ces hauts taux de dénitrification ont été reliés principalement à la respiration métabolique des sites. Curieusement, les fortes productions de N_2O, le corollaire habituel des taux élevés de dénitrification, n'ont pas été observées. Au contraire, les lits de *Trapa* agissent comme des puits pour ce gaz à effet de serre très puissant.

Nous avons pu modéliser la production de N_2 dans les lits de *Trapa* pour une portion du fleuve Hudson. Les performances des lits de *Trapa* son impressionnantes. En effet, alors que ces sites occupent moins de 3% de la portion de fleuve étudiée, ils peuvent éliminer de 70 à 100% de la charge de N retenue par l'ensemble de l'écosystème durant l'été et jusqu'à 35% sur une base annuelle.

Cette étude est un exemple de l'influence que peut avoir une plante exotique envahissante sur le cycle de N. Les exemples similaires (Vitousek et Walker 1989, Hickman et al. 2010) décrivent généralement les effets négatifs de l'espèce exotique. Dans le cas de *Trapa*, l'élimination de N constitue un service écosystémique précieux pour le fleuve Hudson qui est un écosystème qui reçoit des apports importants en nutriments.

5.4. Perspectives

Les résultats de cette thèse participeront à une meilleure compréhension des facteurs qui contrôlent la rétention de N, la dénitrification et les émissions de N_2O dans les grandes rivières. Cependant, nos résultats illustrent aussi la grande variabilité spatiale (inter et intra écosystèmes), temporelle (inter et intra annuelle) et la diversité des organismes biologiques qui peuvent influencer ces différents processus. Nos résultats permettent de lier la rétention de N, la dénitrification et les émissions de N_2O dans les grandes rivières au métabolisme (respiration), à l'hydrologie et à la chimie des écosystèmes dans lesquels ils sont mesurés.

Il apparaît important de continuer à réaliser des études empiriques qui mesurent les processus sur le terrain et font le lien entre ces processus et les facteurs contrôlant à l'échelle de l'écosystème particulièrement pour les fleuves et les grandes rivières où les données sont peu nombreuses. En effet, ces données sont au cœur de la construction des modèles mécanistiques et pour le moment, les modèles globaux d'émissions de N_2O ou de rétention de N contiennent beaucoup d'incertitudes dans les valeurs estimées pour les rivières principalement par manque de données exploitables (Davidson et Seitzinger 2006, Tank et al. 2008).

Dans les rivières, la dénitrification peut être plus ou moins complète et le rendement en N_2O associé est variable. De plus, le N_2O peut aussi provenir du processus de nitrification. Une approche à privilégier pour évaluer le rendement de la dénitrification dans un système, est l'utilisation des ratios N_2O: N_2. Il existe cependant peu d'études qui rapportent des ratios N_2O: N_2 dans les systèmes aquatiques et encore moins dans les rivières (Mulholland et al. 2004, Beaulieu et al. 2008). Cette lacune est probablement due à la difficulté technique associée à la mesure de la dénitrification et de la production de N_2. Il existe de nombreuses méthodes pour mesurer la dénitrification mais elles s'avèrent souvent onéreuses ou inadaptées dans le cas de grandes rivières (Groffman et al. 2006). Nous en avons fait personnellement l'expérience en tentant de mesurer directement les concentrations de N_2 dans le lac Saint-Pierre. Les concentrations que nous avons obtenues pour les sept mois où nous avons échantillonné se sont révélées sous-saturées en N_2 par rapport à l'air rendant impossible l'estimation d'une production de N_2 et donc de la dénitrification. Les méthodes de mesures directes de N_2 in situ (Kana et al. 1994) ne sont pas adéquates dans tous les milieux car les concentrations élevées de N_2 dissous dans l'eau ainsi que les processus physiques d'échanges air-eau très dynamiques empêchent parfois de détecter la production de N_2 par la dénitrification sans l'utilisation d'incubation.

Au Canada, les effets de l'excès de N sur les systèmes aquatiques sont moins dramatiques que dans la plupart des pays industrialisés ou émergents (Schindler et al. 2006). Cependant, plusieurs signes tels que l'augmentation de l'hypoxie dans l'estuaire du Saint-Laurent (Lehmann et al. 2009), les épisodes récents de fleurs d'eau dans les lacs, la

contamination croissante des eaux souterraines (Schindler et al. 2006) suggèrent que la qualité des eaux canadiennes déclinent et que certains des ces problèmes pourraient être liés au cycle de N (par exemple la prolifération d'algues toxiques). En effet, des politiques de gestions de la qualité de l'eau existent déjà mais l'accent a toujours été mis sur l'importance du phosphore pour contrôler la production primaire des systèmes aquatiques canadiens et par conséquent il y a peu de connaissances sur le cycle aquatique de N au Canada. Par ailleurs, il y a maintenant un débat à savoir quel est le facteur le plus limitant dans les systèmes d'eaux douces entre N et P car les transformations de N dans les systèmes eutrophes en particulier, peuvent conduire au maintien de la limitation en N. Il s'avère désormais primordial que des études soient entreprises pour améliorer nos connaissances des facteurs contrôlant ces phénomènes car la gestion d'un cycle aussi complexe que celui de l'azote est très ardue.

Bibliographie générale

Aber, J. D., A. Magill, S. G. McNulty, R. D. Boone, K. J. Nadelhoffer, M. Downs, et R. Hallett. 1995. Forest biogeochemistry and primary production altered by nitrogen saturation. Water Air and Soil Pollution 85:1665-1670.

Alexander, R. B., J. K. Bohlke, E. W. Boyer, M. B. David, J. W. Harvey, P. J. Mulholland, S. P. Seitzinger, C. R. Tobias, C. Tonitto, et W. M. Wollheim. 2009. Dynamic modeling of nitrogen losses in river networks unravels the coupled effects of hydrological and biogeochemical processes. Biogeochemistry 93:91-116.

Alexander, R. B., P. J. Johnes, E. W. Boyer, et R. A. Smith. 2002. A comparison of models for estimating the riverine export of nitrogen from large watersheds. Biogeochemistry 57:295-339.

Arrigo, K. R. 2005. Marine microorganisms and global nutrient cycles. Nature 437:349-355.

Arrigoni, A., S. Findlay, D. Fischer, et K. Tockner. 2008. Predicting carbon and nutrient transformations in tidal freshwater wetlands of the Hudson River. Ecosystems 11:790-802.

Basso, B., et J. T. Ritchie. 2005. Impact of compost, manure and inorganic fertilizer on nitrate leaching and yield for a 6-year maize-alfalfa rotation in Michigan. Agriculture Ecosystems & Environment 108:329-341.

Beaulieu, J. J., C. P. Arango, S. K. Hamilton, et J. L. Tank. 2008. The production and emission of nitrous oxide from headwater streams in the Midwestern United States. Global Change Biology 14:878-894.

Beaulieu, J. J., C. P. Arango, et J. L. Tank. 2009. The effects of season and agriculture on nitrous oxide production in headwater streams. Journal of Environmental Quality 38:637-646.

Beaulieu, J. J., W. D. Shuster, et J. A. Rebholz. 2010. Nitrous oxide emissions from a large, impounded river: The Ohio River. Environmental Science & Technology 44:7527-7533.

Bergman, B., J. R. Gallon, A. N. Rai, et L. J. Stal. 1997. N_2 fixation by non-heterocystous cyanobacteria. Fems Microbiology Reviews 19:139-185.

Bohlke, J. K., R. C. Antweiler, J. W. Harvey, A. E. Laursen, L. K. Smith, R. L. Smith, et M. A. Voytek. 2009. Multi-scale measurements and modeling of denitrification in streams with varying flow and nitrate concentration in the upper Mississippi River basin, USA. Biogeochemistry 93:117-141.

Boyer, C., D. Chaumont, I. Chartier, et A. G. Roy. 2010. Impact of climate change on the hydrology of St. Lawrence tributaries. Journal of Hydrology 384:65-83.

Burk, D. 1930. The influence of oxygen gaz upon the organic catalysis of nitrogen fixatin by *Azobacter*. Journal of Physical Chemistry 34:1195-1209.

Camargo, J. A., A. Alonso, et A. Salamanca. 2005. Nitrate toxicity to aquatic animals: a review with new data for freshwater invertebrates. Chemosphere 58:1255-1267.

Canfield, D. E., A. N. Glazer, et P. G. Falkowski. 2010. The Evolution and Future of Earth's Nitrogen Cycle. Science 330:192-196.

Cantor, K. P. 1997. Drinking water and cancer. Cancer Causes & Control 8:292-308.

Caraco, N. F., et J. J. Cole. 1999. Human impact on nitrate export: An analysis using major world rivers. Ambio 28:167-170.

Caraco, N. F., et J. J. Cole. 2002. Contrasting impacts of a native and alien macrophyte on dissolved oxygen in a large river. Ecological Applications 12:1496-1509.

Carpenter, S. R., N. F. Caraco, D. L. Correll, R. W. Howarth, A. N. Sharpley, et V. H. Smith. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecological Applications 8:559-568.

Codispoti, L. A. 2007. An oceanic fixed nitrogen sink exceeding 400 Tg N a^{-1} vs the concept of homeostasis in the fixed-nitrogen inventory. Biogeosciences 4:233-253.

Cole, J. J., et N. F. Caraco. 2001. Emissions of nitrous oxide (N_2O) from a tidal, freshwater river, the Hudson River, New York. Environmental Science & Technology 35:991-996.

Davidson, E. A., et S. Seitzinger. 2006. The enigma of progress in denitrification research. Ecological Applications 16:2057-2063.

Dong, L. F., D. B. Nedwell, G. J. C. Underwood, D. C. O. Thornton, et I. Rusmana. 2002. Nitrous oxide formation in the Colne estuary, England: The central role of nitrite. Applied and Environmental Microbiology 68:1240-1249.

Dumont, E., J. A. Harrison, C. Kroeze, E. J. Bakker, et S. P. Seitzinger. 2005. Global distribution and sources of dissolved inorganic nitrogen export to the coastal zone: Results from a spatially explicit, global model. Global Biogeochemical Cycles.

Elser, J. J., M. E. S. Bracken, E. E. Cleland, D. S. Gruner, W. S. Harpole, H. Hillebrand, J. T. Ngai, E. W. Seabloom, J. B. Shurin, et J. E. Smith. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecology Letters 10:1135-1142.

Ensign, S. H., et M. W. Doyle. 2006. Nutrient spiraling in streams and river networks. Journal of Geophysical Research-Biogeosciences 111.

Eriksson, P. G. 2001. Interaction effects of flow velocity and oxygen metabolism on nitrification and denitrification in biofilms on submersed macrophytes. Biogeochemistry 55:29-44.

Fay, P. 1992. Oxygen relations of nitrogen-fixation in cyanobacteria. Microbiological Reviews 56:340-373.

Firestone, M. K., et E. A. Davidson. 1989. Microbial basis of NO and N_2O production and consumption in soil. Pages 7-21 *in* M. O. Andreae and D. S. Schimel, editors. Exchange of trace gases between terrestrial ecosystems and the atmosphere. John Wiley, New York.

Francoeur, S. N. 2001. Meta-analysis of lotic nutrient amendment experiments: detecting and quantifying subtle responses. Journal of the North American Benthological Society 20:358-368.

Fulweiler, R. W., S. W. Nixon, B. A. Buckley, et S. L. Granger. 2007. Reversal of the net dinitrogen gas flux in coastal marine sediments. Nature 448:180-182.

Galloway, J., E. Cowling, et E. Kessler. 2002. Reactive nitrogen. Ambio 31:59-59.

Galloway, J. N., F. J. Dentener, D. G. Capone, E. W. Boyer, R. W. Howarth, S. P. Seitzinger, G. P. Asner, C. C. Cleveland, P. A. Green, E. A. Holland, D. M. Karl, A. F. Michaels, J. H. Porter, A. R. Townsend, et C. J. Vorosmarty. 2004. Nitrogen cycles: past, present, and future. Biogeochemistry 70:153-226.

Garcia-Ruiz, R., S. N. Pattinson, et B. A. Whitton. 1999. Nitrous oxide production in the river Swale-Ouse, North-East England. Water Research 33:1231-1237.

Gardner, W. S., M. J. McCarthy, S. M. An, D. Sobolev, K. S. Sell, et D. Brock. 2006. Nitrogen fixation and dissimilatory nitrate reduction to ammonium (DNRA) support nitrogen dynamics in Texas estuaries. Limnology and Oceanography 51:558-568.

Garnier, J., A. Cebron, G. Tallec, G. Billen, M. Sebilo, et A. Martinez. 2006. Nitrogen behaviour and nitrous oxide emission in the tidal Seine River estuary (France) as influenced by human activities in the upstream watershed. Biogeochemistry 77:305-326.

Giles, J. 2005. Nitrogen study fertilizes fears of pollution. Nature 433:791-791.

Groffman, P. M., M. A. Altabet, J. K. Bohlke, K. Butterbach-Bahl, M. B. David, M. K. Firestone, A. E. Giblin, T. M. Kana, L. P. Nielsen, et M. A. Voytek. 2006. Methods for measuring denitrification: Diverse approaches to a difficult problem. Ecological Applications 16:2091-2122.

Heffernan, J. B., M. J. Cohen, T. K. Frazer, R. G. Thomas, T. J. Rayfield, J. Gulley, J. B. Martin, J. J. Delfino, et W. D. Graham. 2010. Hydrologic and biotic influences on nitrate removal in a subtropical spring-fed river. Limnology and Oceanography 55:249-263.

Hickman, J. E., S. L. Wu, L. J. Mickley, et M. T. Lerdau. 2010. Kudzu (*Pueraria montana*) invasion doubles emissions of nitric oxide and increases ozone pollution. Proceedings of the National Academy of Sciences of the United States of America 107:10115-10119.

Hobbs, R. J., et L. F. Huenneke. 1992. Disturbance, diversity and invasion - implication for conservations. Conservation Biology 6:324-337.

Hodgkins, G. A., et R. W. Dudley. 2006. Changes in the timing of winter-spring streamflows in eastern North America, 1913-2002. Geophysical Research Letters 33.

Howarth, R., et E. L. Gene. 2009. Nitrogen. Pages 57-64 Encyclopedia of Inland Waters. Academic Press, Oxford.

Howarth, R. W. 1998. An assessment of human influences on fluxes of nitrogen from the terrestrial landscape to the estuaries and continental shelves of the North Atlantic Ocean. Nutrient Cycling in Agroecosystems 52:213-223.

Howarth, R. W., G. Billen, D. Swaney, A. Townsend, N. Jaworski, K. Lajtha, J. A. Downing, R. Elmgren, N. Caraco, T. Jordan, F. Berendse, J. Freney, V. Kudeyarov, P. Murdoch, et Z. L. Zhu. 1996. Regional nitrogen budgets and riverine N and P fluxes for the drainages to the North Atlantic Ocean: Natural and human influences. Biogeochemistry 35:75-139.

Howarth, R. W., R. Marino, et J. J. Cole. 1988a. Nitrogen fixation in freshwater, estuarine and marine ecosystems 2. Biogeochemical controls. Limnology and Oceanography 33:688-701.

Howarth, R. W., R. Marino, J. Lane, et J. J. Cole. 1988b. Nitrogen-fixation in fresh-water, estuarine, and marine ecosystems .1. Rates and Importance. Limnology and Oceanography 33:669-687.

Hudon, C., et R. Carignan. 2008. Cumulative impacts of hydrology and human activities on water quality in the St. Lawrence River (Lake Saint-Pierre, Quebec, Canada). Canadian Journal of Fisheries and Aquatic Sciences 65:1165–1180

Joye, S. B., et H. W. Paerl. 1993. Contemporaneous nitrogen fixation and denitrification in intertidal microbial mats - Rapid response to runoff events. Marine Ecology-Progress Series 94:267-274.

Kana, T. M., C. Darkangelo, M. D. Hunt, J. B. Oldham, G. E. Bennett, et J. C. Cornwell. 1994. Membrane inlet mass spectrometer for rapid high precision determination of N_2, O_2 and Ar in environmental water samples Analytical Chemistry 66:4166-4170.

Knobeloch, L., B. Salna, A. Hogan, J. Postle, et H. Anderson. 2000. Blue babies and nitrate-contaminated well water. Environmental Health Perspectives 108:675-678.

Knowles, R. 1982. Denitrification. Microbiological Reviews 46:43-70.

Kremen, C. 2005. Managing ecosystem services: what do we need to know about their ecology? Ecology Letters 8:468-479.

Lehmann, M. F., B. Barnett, Y. Gelinas, D. Gilbert, R. J. Maranger, A. Mucci, B. Sundby, et B. Thibodeau. 2009. Aerobic respiration and hypoxia in the Lower St. Lawrence

Estuary: Stable isotope ratios of dissolved oxygen constrain oxygen sink partitioning. Limnology and Oceanography 54:2157-2169.

Lewis, W. M., et W. A. Wurtsbaugh. 2008. Control of Lacustrine Phytoplankton by Nutrients: Erosion of the Phosphorus Paradigm. International Review of Hydrobiology 93:446-465.

Luther, G. W., B. Sundby, B. L. Lewis, P. J. Brendel, et N. Silverberg. 1997. Interactions of manganese with the nitrogen cycle: Alternative pathways to dinitrogen. Geochimica Et Cosmochimica Acta 61:4043-4052.

Mack, M. C., et C. M. D'Antonio. 1998. Impacts of biological invasions on disturbance regimes. Trends in Ecology & Evolution 13:195-198.

Metz, B., O. R. Davidson, P. R. Bosch, R. Dave, et L. A. Meyer, editors. 2007. Contribution of working group III to the fourth assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge.

Mulholland, P. J., A. M. Helton, G. C. Poole, R. O. Hall, S. K. Hamilton, B. J. Peterson, J. L. Tank, L. R. Ashkenas, L. W. Cooper, C. N. Dahm, W. K. Dodds, S. E. G. Findlay, S. V. Gregory, N. B. Grimm, S. L. Johnson, W. H. McDowell, J. L. Meyer, H. M. Valett, J. R. Webster, C. P. Arango, J. J. Beaulieu, M. J. Bernot, A. J. Burgin, C. L. Crenshaw, L. T. Johnson, B. R. Niederlehner, J. M. O'Brien, J. D. Potter, R. W. Sheibley, D. J. Sobota, et S. M. Thomas. 2008. Stream denitrification across biomes and its response to anthropogenic nitrate loading. Nature 452:202-205.

Mulholland, P. J., H. M. Valett, J. R. Webster, S. A. Thomas, L. W. Cooper, S. K. Hamilton, et B. J. Peterson. 2004. Stream denitrification and total nitrate uptake rates measured using a field N-15 tracer addition approach. Limnology and Oceanography 49:809-820.

Palmer, M., E. Bernhardt, E. Chornesky, S. Collins, A. Dobson, C. Duke, B. Gold, R. Jacobson, S. Kingsland, R. Kranz, M. Mappin, M. L. Martinez, F. Micheli, J. Morse, M. Pace, M. Pascual, S. Palumbi, O. J. Reichman, A. Simons, A. Townsend, et M. Turner. 2004. Ecology for a crowded planet. Science 304:1251-1252.

Peterson, B. J., W. M. Wollheim, P. J. Mulholland, J. R. Webster, J. L. Meyer, J. L. Tank, E. Marti, W. B. Bowden, H. M. Valett, A. E. Hershey, W. H. McDowell, W. K.

Dodds, S. K. Hamilton, S. Gregory, et D. D. Morrall. 2001. Control of nitrogen export from watersheds by headwater streams. Science 292:86-90.

Piña-Ochoa, E., et M. Alvarez-Cobelas. 2006. Denitrification in aquatic environments: A cross-system analysis. Biogeochemistry 81:111-130.

Prather, M., R. Derwent, D. Ehhalt, P. Fraser, E. Sanhueza, et X. Zhou. 1995. Other trace gases and atmospheric chemistry.*in* J. Houghton, L. G. Meira, E. Haites, N. Harris, et K. Maskell, editors. Climate change 1995: Radiative Forcing of Climate Changes and an Evaluation of the IPCC IS92 Emission Scenarios. Cambridge University Press, New York.

Pysek, P., et D. M. Richardson. 2006. The biogeography of naturalization in alien plants. Journal of Biogeography 33:2040-2050.

Rabalais, N. N. 2002. Nitrogen in aquatic ecosystems. Ambio 31:102-112.

Sarathchandra, S. U. 1978. Nitrification Activities of Some New-Zealand Soils and the Effect of Some Clay Types on Nitrification. New Zealand Journal of Agricultural Research 21:615-621.

Saunders, D. L., et J. Kalff. 2001. Nitrogen retention in wetlands, lakes and rivers. Hydrobiologia 443:205-212.

Schindler, D. W., P. J. Dillon, et H. Schreier. 2006. A review of anthropogenic sources of nitrogen and their effects on Canadian aquatic ecosystems. Biogeochemistry 79:25-44.

Scott, J. T., R. D. Doyle, et C. T. Filstrup. 2005. Periphyton nutrient limitation and nitrogen fixation potential along a wetland nutrient-depletion gradient. Wetlands 25:439-448.

Scott, J. T., et M. J. McCarthy. 2010. Nitrogen fixation may not balance the nitrogen pool in lakes over timescales relevant to eutrophication management. Limnology and Oceanography 55:1265-1270.

Seitzinger, S., J. A. Harrison, J. K. Bohlke, A. F. Bouwman, R. Lowrance, B. Peterson, C. Tobias, et G. Van Drecht. 2006. Denitrification across landscapes and waterscapes: A synthesis. Ecological Applications 16:2064-2090.

Seitzinger, S. P., et C. Kroeze. 1998. Global distribution of nitrous oxide production and N inputs in freshwater and coastal marine ecosystems. Global Biogeochemical Cycles 12:93-113.

Seitzinger, S. P., R. V. Styles, E. W. Boyer, R. B. Alexander, G. Billen, R. W. Howarth, B. Mayer, et N. Van Breemen. 2002. Nitrogen retention in rivers: model development and application to watersheds in the northeastern USA. Biogeochemistry 57:199-237.

Smil, V. 2001. Enriching the Earth: Fritz Haber, Carl Bosch, and the transformation of world food production. Massashussets Institute of Technology, Cambridge.

Stanley, E. H., et J. T. Maxted. 2008. Changes in the dissolved nitrogen pool across land cover gradients in Wisconsin streams. Ecological Applications 18:1579-1590.

Stenstrom, M. K., et R. A. Poduska. 1980. The effect of dissolved-oxygen concentration on nitrification. Water Research 14:643-649.

Sterner, R. W., et J. J. Elser. 2002. Ecological stoichiometry: The biology of elements from molecules to biosphere. Princeton University Press, New Jersey.

Stow, C. A., J. T. Walker, L. Cardoch, P. Spence, et C. Geron. 2005. N_2O emissions from streams in the Neuse River watershed, North Carolina. Environmental Science & Technology 39:6999-7004.

Strauss, E. A., et W. K. Dodds. 1997. Influence of protozoa and nutrient availability on nitrification rates in subsurface sediments. Microbial Ecology 34:155-165.

Tank, J. L., E. J. Rosi-Marshall, M. A. Baker, et R. O. Hall. 2008. Are rivers just big streams? A pulse method to quantify nitrogen demand in a large river Ecology 89:2935-2945.

Thamdrup, B., et T. Dalsgaard. 2002. Production of N_2 through anaerobic ammonium oxidation coupled to nitrate reduction in marine sediments. Applied and Environmental Microbiology 68:1312-1318.

Vis, C., A. Cattaneo, et C. Hudon. 2008. Shift from chlorophytes to cyanobacteria in benthic macroalgae along a gradient of nitrate depletion. Journal of Phycology 44:38-44.

Vitousek, P. M., J. D. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. W. Schindler, W. H. Schlesinger, et D. G. Tilman. 1997. Human alteration of the global nitrogen cycle: Sources and consequences. Ecological Applications 7:737-750.

Vitousek, P. M., K. Cassman, C. Cleveland, T. Crews, C. B. Field, N. B. Grimm, R. W. Howarth, R. Marino, L. Martinelli, E. B. Rastetter, et J. I. Sprent. 2002. Towards an ecological understanding of biological nitrogen fixation. Biogeochemistry 57:1-45.

Vitousek, P. M., et R. W. Howarth. 1991. Nitrogen Limitation on Land and in the Sea - How Can It Occur. Biogeochemistry 13:87-115.

Vitousek, P. M., et L. R. Walker. 1989. Biological invasion by *Myrica faya* in Hawaii - Plant demography, nitrogen-fixation, ecosystem effects Ecological Monographs 59:247-265.

Weisner, S. E. B., P. G. Eriksson, W. Graneli, et L. Leonardson. 1994. Influence of Macrophytes on Nitrate Removal in Wetlands. Ambio 23:363-366.

Welsh, D. T., M. Bartoli, D. Nizzoli, G. Castaldelli, S. A. Riou, et P. Viaroli. 2000. Denitrification, nitrogen fixation, community primary productivity and inorganic-N and oxygen fluxes in an intertidal Zostera noltii meadow. Marine Ecology-Progress Series 208:65-77.

Wetzel, R. G. 2001. Limnology. Lake and River Ecosystems. 3rd edition. Academic Press, San Diego.

Wollheim, W. M., C. J. Voosmarty, B. J. Peterson, S. P. Seitzinger, et C. S. Hopkinson. 2006. Relationship between river size and nutrient removal. Geophysical Research Letters 33.

Zehr, J. P., et B. B. Ward. 2002. Nitrogen cycling in the ocean: New perspectives on processes and paradigms. Applied and Environmental Microbiology 68:1015-1024.

Zumft, W. G. 1997. Cell biology and molecular basis of denitrification. Microbiology and Molecular Biology Reviews 61:533-616.