UNIVERSITÉ DU QUÉBEC À MONTRÉAL

SEDIMENT REACTIVITY AND ITS IMPACT ON DISSOLVED ORGANIC MATTER FLUXES AND NITROGEN ISOTOPE DYNAMICS DURING EARLY SEDIMENTARY DIAGENESIS IN THE ST. LAWRENCE ESTUARY AND GULF

DISSERTATION

PRESENTED AS PARTIAL REQUIREMENT

OF THE DOCTORATE OF BIOLOGY

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LA RÉACTIVITÉ DES SÉDIMENTS ET SON IMPACT SUR LES FLUX DE LA MATIÈRE ORGANIQUE DISSOUTE ET LES ISOTOPES D'AZOTE DANS L'ESTUAIRE ET LE GOLF DU SAINT-LAURENT

THÈSE

PRÉSENTÉE COMME EXIGENCE PARTIELLE

DU DOCTORAT EN BIOLOGIE

PAR

MOHAMMAD ALKHATIB

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DEDICATION

This thesis is dedicated to the souls of my parents for their love, endless support, and encouragement since the beginning of my study life.

Also, this thesis is dedicated to my sons Ather and Umar-Baipars, and to Suha who have been a great source of motivation and inspiration.

Finally, this thesis is dedicated to the "women in science" for their contributions and sacrifices to science and to all those who believe in the richness of learning.

FOREWORD/AVANT PROPOS

During my doctorate I benefited from the expertise, advice, training, facilities, and outstanding intellectual communities provided by different research institutes from Canada and Switzerland: the GEOTOP-UQAM-McGill research center (Canada), Département des Sciences Biologiques, Université du Québec a Montréal (Canada), the Swiss Federal Institute of Aquatic Science and Technology (Switzerland), and the department for Environmental Science, Universität Basel, Switzerland. This thesis is composed of 3 papers, two of them have already been accepted by international scientific journals and the third is in preparation for submission in the near future. Each paper will be presented in this thesis as a separate chapter.

Alkhatib, M., Schubert, C. J., del Giorgio, P. A., Gelinas, Y., Lehmann M. F., Organic matter reactivity indicators in sediments of the St. Lawrence Estuary. In press for "Estuarine, Coastal and Shelf Sciences".

Alkhatib, M., Lehmann M. F., del Giorgio, P. A., Gelinas, Y., Distribution of dissolved organic nitrogen in sediments of the Lower St. Lawrence Estuary: Constraints on benthic dissolved organic matter fluxes and selective organic matter degradation. In preparation.

Alkhatib, M., Lehmann, M. F., del Giorgio P. A., The nitrogen isotope effect of benthic remineralization-nitrification-denitrification coupling in an estuarine environment. In press for "Biogeosciences".

The first article is entitled "Organic matter reactivity indicators in sediments of the St. Lawrence Estuary", and constitutes the first chapter of the thesis. The sediment samples collected from the St. Lawrence Estuary and Gulf of St. Lawrence were analysed for their chlorin and amino acid contents and composition at the EWAG (Switzerland), while the bulk sediment parameters such as the percentage of organic carbon and total

nitrogen in the sediment, as well as the nitrogen stable isotopic composition were analysed in the laboratories of GEOTOP, Montreal, Canada. The organic carbon isotopic composition was determined in Basel, Switzerland. Dr. C. Schubert hosted me at EAWAG where I have received training on the methods for extracting chlorins and amino acids. It should be pointed out that the chlorin index I use to quantify OM reactivity was originally developed and proposed by Dr. Schubert. My co-supervisors Dr. del Giorgio and Dr. Lehmann corrected earlier drafts of this article, and Dr. Schubert and Dr. Gelinas have as well reviewed this paper in their capacity as coauthors, prior to its submission.

The second chapter in this thesis is entitled "Distribution of dissolved organic nitrogen in sediments of the Lower St. Lawrence Estuary: Constraints on benthic dissolved organic matter fluxes and selective organic matter degradation", and is being prepared for submission to an international scientific journal shortly. All the dissolved organic matter analyses were done in the GEOTOP labs, and the Environmental Science Department labs based at the UQAM University. The sediment interstitial porewaters (similar to all other samples) were collected during two sampling campaigns in the St. Lawrence Estuary and the Gulf of St. Lawrence in June and August 2006. This article will be coauthored by Dr. Lehmann and Dr. del Giorgio.

The third chapter is composed of the article entitled "The nitrogen isotope effect of benthic remineralization-nitrification-denitrification coupling in an estuarine environment". The isotopic analysis of nitrate and total dissolved nitrogen was carried out in the biogeochemistry and stable isotopes lab at the GEOTOP research center, UQAM. This chapter is the one that involved the largest analytical and lab efforts. The isotopic analysis of total dissolved nitrogen in particular required considerable investment of time to test and adapt the method. The coauthors in this paper, which by now is also in press in Biogeosciences, are Dr. Lehmann and Dr. de Giorgio, who helped to revise the manuscript.

ACKNOWLEDGEMENTS

The completion of this doctoral dissertation would not have been possible without the guidance, support, and help of many individuals. Of all the people that have contributed to this intellectual endeavour there are two who, for very different reasons, I owe the most, Moritz Lehmann and Paul de Giorgio. Their continued support and eagerness to allow me the freedom and flexibility to pursue new aspects of my research, learn new methodologies and participate in the intellectual settings of different research centers have truly made this doctorate an incredibly enriching experience. Despite often great geographic distance, I always felt that Moritz was present to guide, advice, and discuss. Paul has supported my research from day one after he became my adoptive supervisor at UQÀM after Moritz left for Switzerland. I am very grateful for the input by supervisors, the often challenging questions and the frequent fruitful discussions.

I wish to extend a heartfelt thanks to Carsten Schubert, at the EAWAG, who helped me with the organic geochemical analyses during my "mini-sabbatical" in Switzerland. The members of GEOTOP: researchers, technicians, and students were wonderful colleagues and friends during my doctoral studies. I would also like to thank all members of the GRIL research group at UQAM and their students, as I benefited a lot from being part of this scientific community. I am very grateful to Bassam Ghaleb, Tawfik Radi, and Cristian Teodoru, for their encouragement, motivation, and constant support.

Many thanks also to Ginette Lozeau-Lacroix (Département des sciences biologiques), Josée Savard, and Nicole Turcot (UQAM, GEOTOP).

Finally, I thank my family, Suha, Ather, and Umar-Baipars, who shared with me all the difficult moments and the challenges. I am truly grateful for having them share this moment of completion with me.

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RÉSUMÉ

Des conditions d'hypoxie sont couramment rencontrées dans les eaux profondes de l'estuaire du Saint-Laurent, dû à une forte dégradation de matière organique particulaire (MOP) hautement réactive de même qu'à une eutrophisation généralisée de l'estuaire au cours des dernières décennies. On en connaît par contre peu sur la distribution et la composition de la MOP retrouvée dans les sédiments, ainsi que sur les facteurs qui gouvernent sa réactivité dans les différentes zones de l'estuaire. Les nutriments azotés, incluant l'azote organique dissous (AOD), sont un facteur limitant courant pour la productivité primaire estuarienne, et pourtant, l'AOD est presque systématiquement absent des budgets de nutriments estuariens et marins. Dans le Saint-Laurent, les sources et les puits de l'AOD ne sont pas bien définis, et les facteurs qui régulent le relargage benthique de l'AOD, en particulier, sont incertains. La mesure de la composition isotopique des différentes espèces d'azote est utile pour comprendre et quantifier les différents processus liés au cycle de l'azote, dans les sédiments et dans la colonne d'eau (incluant les processus qui affectent l'AOD), mais l'effet de la composition isotopique sur les dits processus, en contrepartie, doivent être éclaircis. En ce moment, aucune étude n'a documenté les liens existant entre la composition isotopique des différentes espèces d'azote et les échanges de nutriments entre les milieux benthique et pélagique estuariens et marins. Dans ce contexte, l'objectif général de cette thèse est de déterminer la composition et la réactivité de la matière organique (MO) dans l'estuaire du Saint-Laurent, d'évaluer son impact sur les flux et les budgets d'azote, puis d'évaluer comment les propriétés isotopiques des espèces d'azote échangées lors des différents flux affecteront le pool d'azote dans la colonne d'eau. Les objectifs spécifiques sont donc, dans un premier temps, de déterminer la distribution de la MOP réactive au long de l'estuaire et du golf du Saint-Laurent, ainsi que d'explorer les facteurs qui contrôlent cette distribution, tels que les sources de MOP, l'oxygène dissous et les régimes de déposition. Dans un deuxième temps, j'explore ici la distribution et les flux d'azote et de carbone organique dissous (AOD; COD) dans l'eau interstitielle entre les sédiments et la colonne d'eau, ainsi que les différences stœchiométriques qui en découlent, lesquelles sont probablement régulées par la réactivité de la MOP et les conditions environnementales. Le dernier objectif est de suivre la transformation des différentes espèces d'azote organique et inorganique dissous (AOD+AID) dans les eaux interstitielles, puis de déterminer l'effet qu'aura la séquence de reminéralisation-nitrification-dénitrification de l'azote sur le contenu en ^{15}N du pool réactif d'azote dans la colonne d'eau. Pour répondre à ces objectifs, des échantillons provenant de l'eau et des sédiments de l'estuaire du Saint-Laurent ont été collectés lors de deux campagnes au long d'un gradient depuis l'estuaire vers l'océan. La composition en isotopes stables (N et C), le carbone organique total, l'azote total, les acides aminés et le contenu en chlore ont été mesuré dans les sédiments. Ces analyses sont à la base des calculs d'indices de réactivité de la matière organique, de l'indice de dégradation (ID) et de l'indice de

chlore (IC) des sédiments. De plus, les nitrates (et nitrites), l'ammonium, le carbone organique dissous (COD) et l'AOD seront mesurés dans les eaux interstitielles des sédiments. Ces variables seront ensuite utilisées pour estimer les flux diffusifs à l'interface eaux-sédiments. Finalement, les compositions isotopiques des NO_3^- et de l'azote total dissous (ATD) ont été déterminées, et l'effet isotopique associé à leurs flux à l'interface eau-sédiment a été calculé. Les résultats de ce présent travail montrent clairement un gradient de la réactivité de MOP des sédiments et un début d'altération diagénétique au long de l'estuaire du golfe du Saint-Laurent, avec plus de MO réactive dans la partie peu profonde de l'estuaire moyen et dans la zone hypoxique et très productive de l'estuaire maritime. Les analyses de stéréo-isomères des acides aminés ont révélé un couplage entre l'ampleur de la diagénèse et l'accumulation et la préservation sélective de la matière dérivée des membranes de cellules bactériennes dans les sédiments. Le temps d'exposition à l'oxygène des sédiments semble déterminer fortement la réactivité de MO des sédiments dans l'estuaire du Saint-Laurent, plutôt que la source de la MOP. Dans l'ensemble de la zone d'étude, les sédiments représentent une source d'AOD dans la colonne d'eau (de 0,11 à 0,43 mmol m^{-2} j^{-1}). Les flux d'AOD comptent pour 30 à 64% de la dénitrification benthique totale, et étaient significativement positivement corrélés avec la réactivité de la MO et négativement corrélés avec l'oxygène dissous des eaux recouvrant les sédiments. Les flux de COD étaient relativement constants (2,1 ± 0,1 mmol m^{-2} j^{-1}) et n'ont pas démontré de patron de variation au long du gradient estuaire-océan, impliquant une partition des éléments prononcée et contrôlée par l'environnement durant l'hydrolyse et la reminéralisation de la MOP durant la diagénèse hâtive des sédiments. L'azote réduit dissous (ARD) des eaux interstitielles et les nitrates étaient tous les deux significativement enrichis en ^{15}N dans les sédiments. Comme dans les autres environnements marins, le fractionnement isotopique biologique de la perte nette d'N fixé due à la dénitrification était à peine exprimé à l'échelle des échanges entre les sédiments et l'eau. Intégrer, pour la première fois, les flux d'ARD (AOD + NH_4^+) dans les calculs de l'effet isotopique de l'N total des sédiments génère des effets isotopique d'N légèrement plus élevé (4,6 ± 2 ‰), qui semblent être contrôlés par la réactivité et la profondeur de pénétration de l'oxygène dans les sédiments. Les valeurs des effets isotopiques de l'N total des sédiments reportés ici sont plus élevés que les valeurs présumées, et occasionnent des incohérences dans le cycle global des isotopes d'N, car ils impliquent un plus grand ratio de dénitrification entre les sédiments et la colonne d'eau et, ainsi un plus grand débalancement du budget global de l'N qu'il n'est actuellement considéré. En résumé, cette étude souligne les liens étroits existant entre le régime de condition de préservation et de sédimentation, la composition et la réactivité de la MO et le remaniement bactérien, ainsi que les échanges de solutés à l'interface eau/sédiment, avec des implications à grand échelle pour l'effet des processus benthiques sur les éléments et le budget isotopique de l'N dans la colonne d'eau des estuaires marins.

ABSTRACT

The widespread hypoxic/low dissolved oxygen conditions (DO) in the bottom waters of the St. Lawrence Estuary has been partly ascribed to the increased degradation of reactive particulate organic matter (POM) in the sediments with enhanced eutrophication over the last decades, yet there is a lack of knowledge on the distribution and composition of sedimentary POM and the factors that govern its reactivity along the St. Lawrence estuarine-marine system. Nitrogenous nutrients (including dissolved organic nitrogen, (DON)) are limiting estuarine and marine primary productivity, however, DON is neglected in most marine and estuarine nitrogen budgets. In the St. Lawrence system, sources and fate of DON are poorly constrained, and the controls on benthic DON release to the water column are uncertain. To trace and quantify the different N cycling processes within sediments and in the water column (including those that affect DON), measuring the N isotopic composition of N species has proven to be helpful, but the N isotope effects that are associated with specific N transformations need to be known. To date, observational data that elucidate the isotopic impact of benthic-pelagic exchange of reduced dissolved N (RDN) do not exist in the literature. This thesis aims at assessing the organic matter (OM) composition and reactivity of sediments along the Laurentian Channel, its impact on solute fluxes and the N budget of the St. Lawrence Estuary and Gulf, and in turn, the isotope effects these fluxes have on the water column N pool. The main objectives were (1) to determine the distribution of reactive POM along the St. Lawrence Estuary (Upper Estuary and Lower Estuary) and Gulf, and to investigate the factors that affect their distribution, such as OM sources, environmental conditions (water column oxygenation), and depositional regime, (2) to investigate the distribution and fluxes of dissolved organic nitrogen and carbon (DON and DOC) in porewaters and at the sediment water interface, as well as to study the C/N elemental partitioning imparted by DOM production/consumption processes during early diagenetic reactions, likely controlled by POM reactivity and environmental conditions, and (3) to trace the dissolved organic and inorganic nitrogen (DON+DIN) cycling inside the sediment porewater pool and to assess the N isotope effect of coupled remineralization-nitrification-denitrification-on the ^{15}N content of the reactive N pool in the overlying water column. To achieve these objectives, sediment and water samples were collected along the estuarine-marine gradient from the St. Lawrence Estuary during two scientific cruises. Solid sediments were analysed for its N and C stable isotope composition, total organic carbon, total nitrogen, amino acids, and chlorin contents. These data represented the basis for the calculation of sediment OM reactivity indices, the degradation index (DI) and the chlorin index (CI). Porewaters were analysed for nitrate (+nitrite), ammonium, [DOC], and [DON], and these were used to estimate the diffusive flux of these components at the sediment water interface. Finally, the isotopic composition of NO_3^- and total dissolved nitrogen (TDN) was determined, and the isotopic effect associated with their flux across the SWI was calculated. The results

of my thesis work show a clear gradient in the sediment POM reactivity and early diagenetic alteration along the St. Lawrence Estuary and Gulf, with more reactive OM in the shallow Upper Estuary and in the hypoxic, highly-productive Lower Estuary. Amino acid stereoisomer analysis revealed a close coupling between the extent of diagenesis and the accumulation and selective preservation of bacterially-derived cell wall material in the sediments. Oxygen exposure time of the sediments appears to strongly determine sediment OM reactivity in the St. Lawrence Estuary, rather than the source of POM. Throughout the study area, sediments represent a significant source on DON to the water column (0.11 to 0.43 mmol m^{-2} d^{-1}). DON fluxes accounted for 30% to 64% of the total benthic denitrification, and were significantly correlated with OM reactivity (positively) and with the [DO] in the overlying bottom water (inversely). DOC fluxes were relatively constant and did not show any consistent pattern of variation along the estuarine-marine gradient (2.1 ± 0.1 mmol m^{-2} d^{-1}), implicating pronounced and environmentally-controlled element partitioning during the hydrolysis and remineralization of POM during early sedimentary diagenesis. Both porewater RDN and nitrate were significantly enriched in ^{15}N in the sediment. As in other marine environments, the biological nitrate isotope fractionation of net fixed N loss due to denitrification was barely expressed at the scale of sediment-water-exchange. Integrating, for the first time, the RDN (DON+NH_4^+) fluxes in calculations of the total N total sedimentary N isotope effect yielded slightly higher N isotope effects 4.6 ± 2 ‰, which seem to be controlled by the sediment reactivity and oxygen penetration depth in the sediments. Here reported values of the total sedimentary N isotope effect are larger than previously assumed, and result in inconsistencies with respect to the global N isotope budget, because they imply a greater sediment-to-water column denitrification ratio and, thus a greater overall imbalance in the global N budget than currently considered. In summary, this study highlights the close links between preservation conditions/sedimentation regime, the composition and reactivity of OM and bacterial reworking, as well as the solute exchange at the sediment water interface, with large-scale implications for the effect of benthic processes on the elemental and N isotopic budgets in the estuarine/ocean water column.

INTRODUCTION

Rationale: the ecological health of the lower estuary and prime motivation for this study

Biogeochemical processes in estuarine sediments are known to be highly heterogeneous due to several factors, including differences in the contributions of marine versus terrestrial organic matter (OM) to the sediments, the rate and composition of particulate organic matter (POM) vertical flux to the sediments, the dissolved oxygen (DO) concentrations in the overlying waters, exposure time and depth of oxic zone, and the presence and density of benthic macro-fauna. As a result, sediment POM reactivity and porewater dissolved organic matter (DOM) concentration and composition tend to vary along the estuarine gradients. Eutrophication in coastal environments that arises from an increase in nutrient and DOM concentrations over natural levels, leads to a greater production of POM in the water column and enhanced POM flux (Gray et al., 2002). The sedimenting organic particles are degraded largely in the water column and later in sediments, by both oxic and anoxic processes (Plourde and Therriault, 2004; Niggemann et al., 2007). However, if oxygen is not supplied by advective and vertical mixing, and/or the consumption rate exceeds re-supply, oxygen concentrations may decline beyond the point that sustains most forms of animal life ([O2] <62µmol l-1; Rabalais et al., 2002 ; Gilbert et al., 2007, 2005). This condition of low dissolved oxygen is known as hypoxia.

An example of widespread hypoxia can be found in the Saint Lawrence River Estuary, which together with the Saint Lawrence Gulf forms the world's largest estuary. The bottom waters along its mains channel (the Laurentian Channel, LC; Fig. 1 & 2) are characterized by consistently low dissolved oxygen (DO) conditions, especially in the Lower Estuary where year-round hypoxic conditions occur, with [DO] dropping to as low as 51.2 $\mu molL^{-1}$ recorded in 2003 (Gilbert et al., 2005).

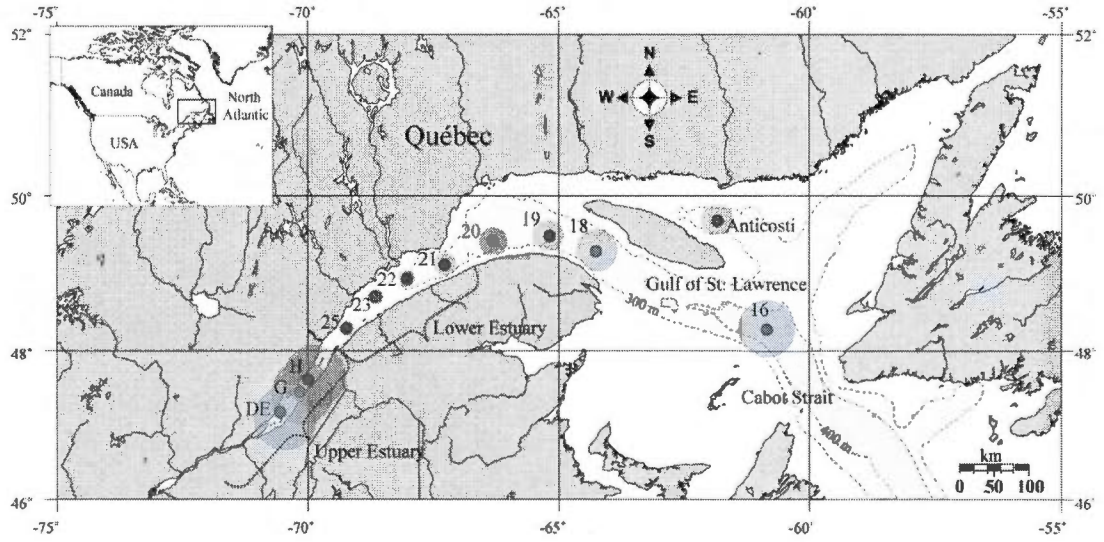

Figure 1. A map showing the sampling locations in the St. Lawrence Estuary (Upper and Lower) and the Gulf of St. Lawrence. Bathymetric contours outline the Laurentian Channel along the 300 and 400 m isobaths. The size of shadowed circles around study sites denotes bottom water DO concentrations.

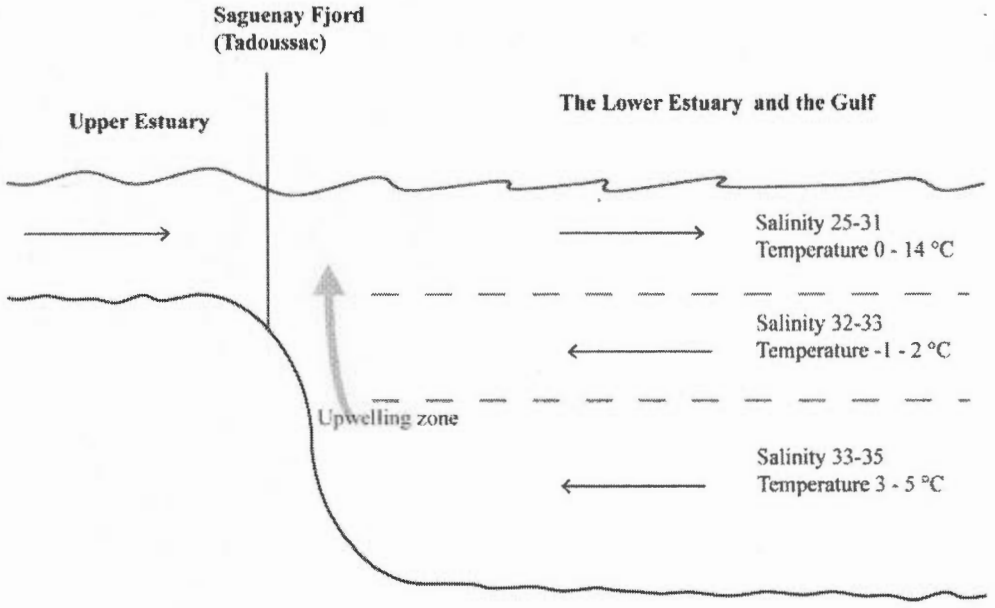

Figure 2. A schematic drawing representing the water circulation and the different water masses along the St. Lawrence Estuary and Gulf.

Oxygen in the bottom waters of the Lower Estuary declined by 60μmol L^{-1} between the 1930s and 1990s, over the same period of time, the temperature of the bottom waters warmed by 1.65°C, suggesting that the recent decline in bottom water O_2 can, at least in part, be ascribed to oceanographic/climatic changes in the Lower Estuary (Gilbert et al., 2005). However, the temperature difference between bottom waters of Lower Estuary and waters at 250m depth in Cabot Strait did not change from the 1970s to the 1990s, yet, the oxygen depletion increased by 30μmol L^{-1} over the same period. In this regard, Gilbert et al. (2005) have suggested that sediment oxygen demand in the Lower

Estuary may be responsible for this change along the LC oxygen gradient. Further supporting evidence for the important role of sediments in oxygen consumption came from a modelling study by Benoit et al. (2006). In a micropaleontological study by Thibodeau et al. (2006), a significant increase of benthic foraminifera tests and their organic linings (up to one order of magnitude over the last three decades) has been interpreted as being indicative of a generally increased flux of labile organic carbon from surface waters due to enhanced primary productivity, providing a conceptual link between estuarine eutrophication and hypoxia in the Lower Estuary (via the respiration of organic material resulting from microbial degradation and early diagenesis; (see below). Indeed, a 70% increase in the use of natural and artificial fertilizers between 1970 and 1988 parallels the decrease in dissolved oxygen content of the Lower Estuary (Thibodeau at al., 2006).

While pelagic primary production and OM fluxes in the Lower Estuary are probably largely controlled by the input of nutrients and OM from terrestrial/anthropogenic sources, the recycling of nutrients and processing of OM at the sediment water interface most likely has an important effect on the availability of nitrogenous compounds and elemental ratios of dissolved species in the overlying water column, which in turn impacts rates of primary productivity. For example enhanced remineralization of OM may exacerbate eutrophication, while enhanced burial of OM or microbial elimination of nutrients (e.g., via denitrification) may counterbalance it.

The main hypothesis this study is based on is that the diagenetic state of organic matter (or OM reactivity) in the sediments largely controls the exchange of DIN and DON between the sediments and the bottom water column. To test this hypothesis, we assessed OM reactivity in the sediments along the LC using state-of-the-art OM reactivity bioindicators, as well as its control on solute exchange at the sediment water interface, and the biogeochemical reactions that control this exchange. Since the main scope of this study is the cycling of nitrogen species in the sediments, and the assessment of the role of sediments as a source or sink of nitrogen, I focused on dissolved organic and inorganic N

species and their N-isotopic composition. In order to trace the different N cycling processes in sediments that might add or eliminate bioavailable N to/from the system, I have performed isotopic measurements of the different N compounds (NO_3^- and DON/TDN). The data set and results that evolved in this study provide the basis for a comprehensive nitrogen budget for the whole St. Lawrence estuarine system.

Background

Ecological significance of nitrogen and the nitrogen cycle

In most marine environments, the extent and duration of phytoplankton production is constrained by the availability of essential plant macronutrients, of which nitrogen (mainly NO_3^- and NH_4^+, but also urea, amino acids and shorter peptides) is often considered limiting (e.g., Hecky and Kilham, 1988; Antia et al., 1991; Seitzinger and Sanders, 1999). Human activity has significantly altered the natural biogeochemical cycle of nitrogen by increasing the inputs of N to the estuaries and coastal waters in general (Nixon, 1995; Howarth et al., 1996; Galloway et al., 1998; Diaz, 2001; Gray et al., 2002). Although changes are most evident on a regional scale, the global cycle of nitrogen has been altered by human activities to a greater extent than most other bioactive elements (Nixon, 1995; Codispoti et al., 2001; Zehr and Ward; 2002).

Nitrogen in marine and fresh waters comprises several different forms. The dominant N species (excluding molecular N_2) are particulate organic N (PON), dissolved organic N (DON) and dissolved inorganic N (DIN), including NO_3^-, NO_2^- and NH_4^+. Aquatic N transformations that can alter the redox state of N are primarily microbiologically-mediated and often redox dependent. The most important N-cycling reactions are indicated schematically in Fig. 3. These involve assimilation, nitrogen fixation, nitrification, remineralization, anammox, denitrification and nitrate ammonification (DNRA).

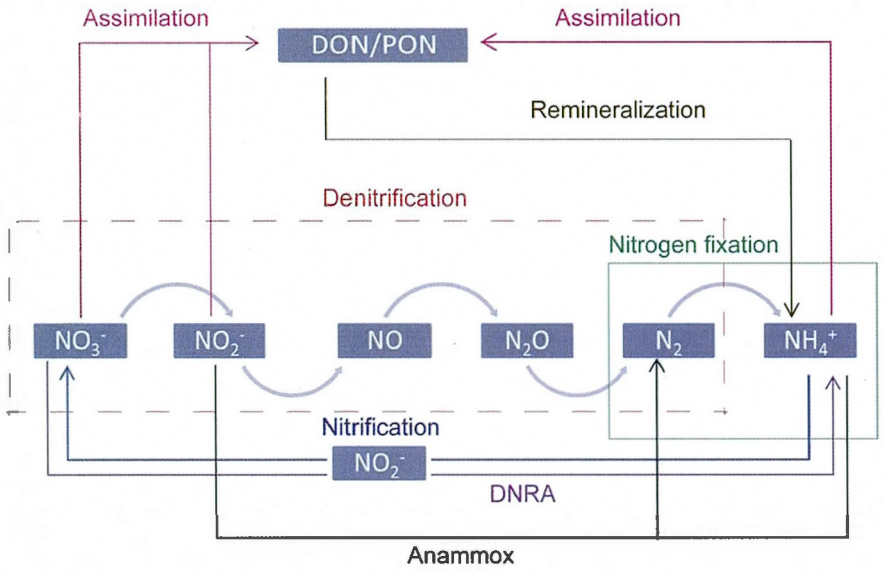

Figure 3. General schematic diagram of the N cycle in the Ocean

Assimilation is the incorporation of fixed forms of N into organisms during biosynthesis. N_2 is essentially unavailable to most marine planktonic organisms that lack the nitrogenase enzymes (Howarth et al., 1988), however, the most important source of N to pristine environments is N_2 fixation, an assimilatory process where N_2 gas is reduced to biologically available NH_4^+ by a wide variety of prokaryotes (e.g., marine cyanobacteria; Howarth et al., 1988). This transformation of N_2 to reduced N forms (e.g., nucleic acids) results in the addition of new available nitrogen to the ocean or other aquatic environments. During the remineralization of OM, i.e. the breakdown of OM into dissolved organic forms of N and ammonium, and subsequent nitrification of remineralized ammonium to nitrate, organic N is returned to the dissolved inorganic N pool (Fig. 3). In coastal and estuarine environments, external/anthropogenic sources of

fixed N (e.g., riverine- and atmospheric input, and anthropogenic N-fixation) are often a much more important source of fixed N.

Denitrification, a dissimilatory process in which nitrate rather than oxygen is used as electron acceptor by microorganisms, is generally acknowledged as the main sink for available N in marine environments (e.g., Seitzinger, 1988; Zehr and Ward, 2002). During denitrification, dissolved NO_3^- is reduced to N_2 gas through a series of intermediates (NO_2^-, NO, and N_2O). In marine sediments, the coupled process of remineralization-nitrification-denitrification represents a sink that shunts N away from recycling pathways (Jenkins and Kemp, 1984). These coupled processes are quantitatively important in the N budgets of estuaries, where N loss via denitrification may account for half of the terrestrial inputs (Seitzinger, 1988; Thibodeau et al., 2010), providing a partial buffering against the global trend of coastal eutrophication.

On a global scale, it is uncertain whether total elimination by denitrification and sediment burial exceeds the total amount of fixed N or not (Codispoti, 1995; Codispoti et al., 2001; Middelburg et al., 1996). According to recent revisions, total inputs appear to be significantly smaller than estimates of nitrogen removal (Codispoti et al., 2001). Findings of additional, alternative N reaction pathways seem to further exacerbate the imbalance as they introduce additional routes of N removal. For example, there are indications that previously unknown mechanisms of anaerobic ammonium oxidation (ANAMMOX) by nitrite to N_2 may be significant for N cycling and N removal in some environments (Thamdrup and Dalsgaard, 2002; Ward, 2003).

Controls on organic matter degradation

Through processes mentioned in the previous chapter, marine N fluxes are intrinsically linked to the biosynthesis and degradation of OM. Denitrification, for example, can be limited by the bioavailability of the organic substrate. The susceptibility of bulk particulate OM, and of specific organic components, to degradation during transport and burial in sediments under variable depositional conditions is receiving

increasing attention (Burdige 2007; Niggemann et al., 2007). Although the most important factors that appear to govern OM degradation and preservation are relatively well known (e.g., water column depth, nature and origin of OM, sedimentation rate, oxygenation conditions, and average grain size), a mechanistic understanding of the pathways that determine the quality and quantity of OM in sediments is still lacking. This understanding is necessary to develop predictive models of coastal sediment processes, as well as for paleoceanographic or paleoclimatic reconstructions (e.g., Lehmann et al., 2002).

The source of the OM supplied to the sediments is one of the factors that determine the quality of sedimentary OM (Burdige 2005; Bourgoin and Tremblay 2011). Whereas the production and degradation of authochthonous OM occurs entirely within the marine environment, terrestrial OM is produced and transported on land and may be already significantly altered before entering the marine system (Hedges et al., 1997). Nevertheless, OM from both sources may contain both labile and refractory compounds for bacterial degradation, depending on their initial molecular structure and composition (Burdige, 2007).

The deposition conditions control the bulk composition of OM falling through the water column, and affect further sedimentary OM decomposition processes (Niggemann et al., 2007). Depositional conditions are critical factors determining the quality and quantity of detrital particles reaching the sediments. For example, Niggemann et al. (2007) compared depositional conditions, OM composition, and organic carbon turnover in sediments from two different depositional systems along the Chilean continental margin. They found that, with increasing water depth, chlorin concentrations decrease, C/N-ratios increase and OM reactivity decreases. Another factor that influences sedimentary OM diagenesis is the ambient redox conditions. The efficiency of different respiration pathways (e.g., oxic vs. anoxic) with regards to the rates and degree of OM degradation is subject to an ongoing debate (e.g., Lehmann et al., 2002; Pantoja et al., 2009). However, it is likely that at least to some degree dissolved oxygen (DO) availability modulates the extent of OM alteration and of OM accumulation rates in sediments (Hartnett and Devol, 2003). For example, bulk organic carbon (C_{org}) preservation along continental margins has

been shown to be directly related to the oxygen exposure time (OET) of sinking and sedimented particles (Keil et al., 1994; Hartnett et al., 1998; Hedges et al., 1999), and redox oscillations have been found to enhance OM degradation largely by promoting symbiosis of aerobic and anaerobic microorganisms (Aller, 1994).

Indicators of OM preservation

Novel developments in analytical chemistry, especially chromatography, have enabled researchers to unravel the chemical composition of labile organic material to a high degree. As we are incapable to characterize all the organic molecules that make up the organic matrix in natural systems (e.g., due to the fact that not all compounds can be analyzed using standard chromatographic techniques because they are not hydrolysable), we have to select certain individual compounds, or biomarkers, that can provide us with specific information, e.g. on organism groups, or specific (bio) chemical and ecological processes (Boschker and Middelburg, 2002). In this thesis I focus on key groups of biomarkers, such as phytopigments (chlorins) and amino acids. Although these individual compounds or compound groups represent only a minor fraction of the total sedimentary OM pool, and thus caution is advised when using them to extract information on the degradational state of sedimentary OM, the underlying assumption of my work is that they still provide useful information on bulk sediment properties, especially when several of these biomarkers are combined to inform on different facets of this pool.

Biomarkers

Chlorins - During early diagenetic transformations of OM in the water column, chlorophylls are transformed into what is collectively called "chlorins" (Brown et al., 1991). The transformations include (1) the loss of the central magnesium atom to yield phaeophytins, (2) the loss of the phytyl ester group to yield chlorophyllides, or (3) loss of both the Mg^{2+} and the phytyl group to yield phaeophorbides (see Fig. 4). Thus, chlorins comprise the immediate diagenetic products of chlorophylls (Schubert et al., 2005).

Further degradation in sediments involves oxidative cleavage of the macrocyclic ring, the break-down of chlorins into non-fluorescing, colourless compounds (Matile et al., 1996). The extent of chlorin loss depends on the degree of degradation during sinking and early sedimentary diagenesis, and is most likely dependent on redox conditions (Leavitt, 1993).

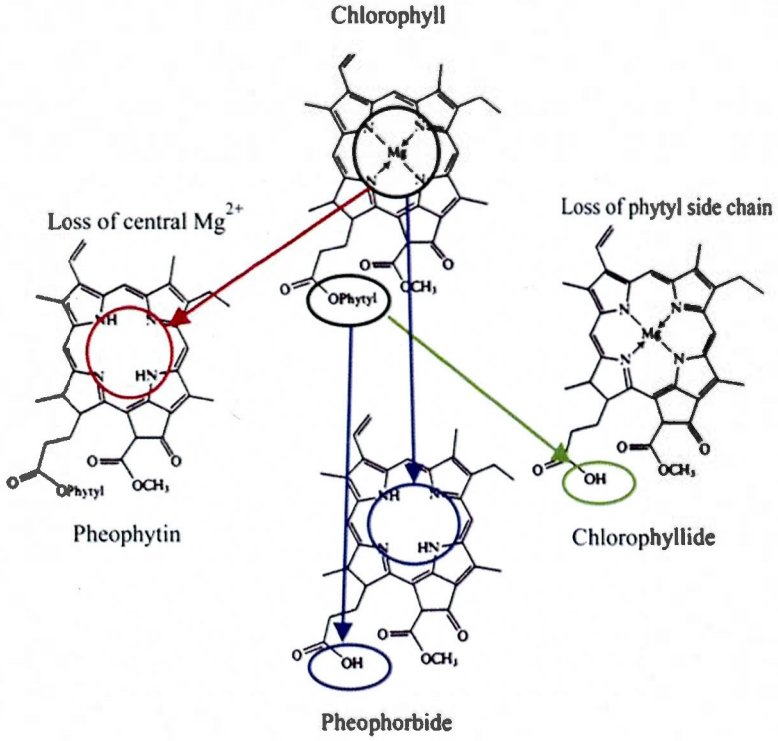

Figure 4. Major early diagenetic (water column and very early sedimentary) pathways for chlorophyll transformation

Amino Acids - Amino acids, constituents of proteins, are N-rich compounds, and generally assumed to degrade faster than N-poor compounds (e.g. lipids). Due to the preferential degradation of amino acids, the contribution of the total hydrolysable amino acids (THAA) to the percentage of total organic carbon and total nitrogen (THAA-C and THAA-N) has been reported to drop as diagenesis progresses (Lee, 1988; Cowie and

Hedges, 1994). In addition, several studies investigating amino acids in sediment trap material (Lee, 1988; Nguyen and Harvey, 1997) and sediments (Cowie and Hedges, 1992; Dauwe and Middleburg, 1998) have indicated that the relative contribution of individual amino acids to THAA changes during mineralization. Thus, the amino acid composition is a valuable tool to assess the OM degradation state (Dauwe and Middleburg, 1998). In addition, the contribution of the non-protein amino acids β-Alanine (BALA) and γ-Aminobutyric acid (GABA) to the THAA have been shown to increase during OM degradation, relative to their more abundant protein counterparts (Cowie and Hedges, 1994; Keil et al., 2000). BALA and GABA represent degradation products of protein amino acids and their percentage represents a reliable biochemically-based indicator of organic matter degradation. Another type of amino acid degradation biomarker is based on the analysis of D-amino acids (D-AA). Since bacteria are the only organisms to incorporate D-AA into their biomass, the D to L ratio of some (e.g. alanine) sedimentary amino acids can be used as bacterial biomass tracer and, indirectly, as OM diagenesis biomarker (e.g. Udea et al., 1989; Plez et al., 1998; Lomstein et al., 2006).

Degradation Indices

A series of molecular diagenetic indicators have been developed and used to estimate the relative degradation state of the OM (e.g., Schubert et al., 2005; Dauwe and Middelburg, 1998; Cowie and Hedges, 1994; Wakeham et al., 1997). Broadly applicable degradation state indicators should be based on major components that are widely distributed geographically and that are omnipresent in most organic matter sources, such as chlorophylls and their degradation product pigments and protein amino acids (Dauwe et al., 1999).

Chlorin Index (CI) - The CI reflects the degradation state of chlorins in sediments. Under certain depositional conditions where most of the organic material is derived from authochthonous algal production, it is a good indicator for the reactiveness of the bulk

sediment. The CI has been suggested to be a versatile tool applicable to a wide range of aquatic environments, e.g., the upwelling region off Namibia and Peru, the shelf off Chile, the Arabian Sea (Schubert et al., 2005), in sediments along the Chilean coast (Niggemann et al., 2007), and in Lake Zug in Switzerland (Meckler et al., 2004). The CI is basically the ratio between the fluorescence intensity of a sediment extract before and after acidification with hydrochloric acid. The untreated extract can include different chlorin species, as well as chlorophyll remnants (Schubert, C. pers. comm.). Upon acidification and the chemical transformation/destruction of the labile pigments, their optical properties change, with a lower fluorescent yield (Schubert et al., 2005). Therefore, an extract of fresh OM will considerably change its fluorescence, while an extract of more degraded sediments will only slightly change its fluorescence. The CI ranges from 0.2 for chlorophyll to 1 for a chemically inert material.

Degradation Index (DI) - The DI is based on the distribution of common protein-derived amino acids present in sediments (Dauwe and Middleburg, 1998). During degradation, some amino acids become relatively enriched, whereas others are preferentially metabolized/remineralized (Dauwe and Middelburg, 1998). This results in compositional changes that can be used to determine the degradation state of the (AA-containing) organic material. The DI was originally proposed by Dauwe and Middleburg, (1998) and has been adapted later by Dauwe et al. (1999). The DI is a robust indicator of degradation state, in part because it is based on a large number of single measurements. It has successfully been applied to both freshwater (Meckler et al., 2004; Unger et al., 2005), and marine environments (Niggemann and Schubert, 2006; Dauwe et al., 2001).

Stable nitrogen isotopes

There are two stable isotopes of N: ^{14}N and ^{15}N, with a natural abundance $^{15}N/^{14}N$ ratio of 1/272 (Junk and Svec, 1958). Nitrogen isotope ratios are generally reported in per mil (‰) relative to N_2 in atmospheric air, according to the standard delta notation: $\delta^{15}N_x = \{[(^{15}N/^{14}N)_x/ (^{15}N/^{14}N)_{AIR}] -1\} \cdot 1000$, where x = sample and AIR = N_2 in air. In general,

during biogeochemical transformations, the molecules containing the lighter isotope react more readily, resulting in reaction products that are "lighter" than the reactants (Kendall, 1998). The extent to which heavy isotopes are discriminated against in a unidirectional biological reaction can be quantified in terms of the kinetic isotope effect, ε, which can be characteristic for specific N-cycle transformations. Thus, isotopic signatures in the modern ocean and estuaries, but also in sedimentary archives, can potentially be used as geochemical fingerprints that allow the tracing of these transformations, today and in the past.

Processes affecting N isotopic compositions of dissolved N

Nitrate is the dominant form of fixed N in most marine environments (Gruber, 2005); the mean $\delta^{15}N$ of oceanic nitrate (or basins at the more regional scale) reflects the N isotope effect of the major N-cycle processes. Determination of the relation between nitrate concentrations in the water column and the quantity of nitrate introduced from a particular source is complicated by the occurrence of multiple possible sources of nitrate and reactions that transform or eliminate the various forms of DIN. Isotope ratios in these N species can potentially offer a direct means of source identification because different sources of nitrate often have isotopically distinct nitrogen isotopic compositions (Kendall, 1998). As indicated schematically in Figure 5, biological cycling of nitrogen often changes isotopic ratios in predictable and recognizable directions that can be reconstructed from the isotopic compositions.

Nitrification - Nitrification is a multi-step oxidation process mediated by different microbes. *Nitrosomonas* oxidize ammonia to NO_2^-, while *Nitrobacter* subsequently oxidise the NO_2^- to NO_3^-. Because the oxidation of NO_2^- to NO_3^- is quantitative, fractionation is caused by the rate-determining step of ammonium oxidation by *Nitrosomonas*. Field study by Horrigan et al. (1990) in Chesapeake Bay shows N isotope

effects of 12‰ - 16‰ during nitrification, while Casciotti et al. (2003) reported a higher variability for the N isotope effects associated with ammonium oxidation to nitrite, with a range of 14‰ to 19‰ for marine and saltwater strains and a range of 24‰ to 38‰ for the freshwater strains. The variation in the N isotope fractionation by nitrifying bacteria has been explained in terms of differences in the ammonia mono-oxygenase enzyme that are involved in nitrification.

Denitrification - Denitrification is the second most important respiration process after aerobic (oxic) respiration for microbes involved in organic matter degradation. Denitrification is a multi-step process with various nitrogen oxides (e.g., N_2O, NO) as intermediate compounds resulting from the chemical or biologically mediated reduction of nitrate to N_2. As other kinetic reactions, denitrification strongly discriminates against nitrate molecules containing the heavier isotope, leading to a marked increase of ^{15}N in the residual nitrate. In suboxic ($[O_2]< 5\mu M$] water columns, such as the oxygen minimum zones of the Arabian sea and the eastern subtropical north and south Pacific, the biological isotope fractionation during water column denitrification seems to be fully expressed with isotope effects (organism-level; ε_{cell}) between 20-30‰ (Cline and Kaplan, 1975; Voss et al., 2001), on the same order of magnitude as estimates for biological isotope effects for denitrification in laboratory experiments (Granger et al., 2008). Denitrification in sediments seems to follow the same isotopic patterns with similar organism-scale N isotope effects for benthic denitrification ranging between 11‰ and 30‰ (Lehmann et al., 2007). However, the N-isotope enrichment of porewater nitrate does not necessarily find its expression at the scale of sediment-water column exchange. Previous work by Lehmann et al. (2004; 2007) and Brandes and Devol (1997) indicated a strong suppression of the N-isotope effect in the water column nitrate pool with apparent nitrate N-isotope effects (ε_{app} , the nitrate N isotope effect at the scale of sediment-water exchange which takes into account flux of nitrate) generally below 3‰. This observation has been explained by diffusion limitation resulting in the almost complete consumption of nitrate in the reaction zone. It is important to note that it is the apparent (ε_{app}), and not the

organism-level (ε_{cell}) isotope effect that is relevant to trace fixed N-elimination within sediments, and which has to be considered in N-isotope balances.

An important point raised by previous work, but not directly addressed using measured data, is the potential effect of dissolved N-forms other than nitrate (ammonium and DON) on the overall isotope effect of benthic N-cycling. Thus far, ammonium and DON fluxes out of the sediments have not been investigated in terms of their N-isotopic composition. Based on model simulations, Lehmann et al. (2007) argued that partial nitrification can lead to porewater ammonium that is strongly enriched in ^{15}N, and, if escaping to the water column, can shunt significant amounts of "heavy" fixed N to the water column, an aspect that has, until today, been largely ignored in global or regional N isotope balances. In a related process, DON effluxing from sediments has a high potential to be remineralized in the overlying water, and as a result may impact the isotopic composition of the DIN pool in the water column, and this process has seldom been investigated before.

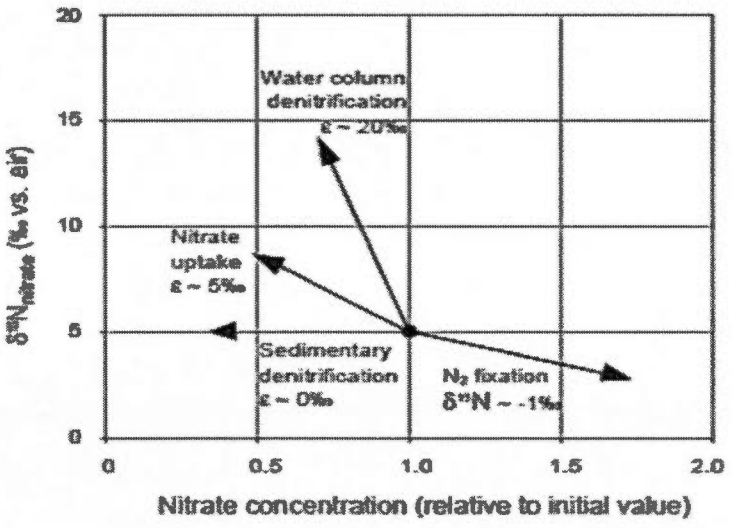

Figure 5. Schematic diagram indicating the isotopic effect associated with the indicated addition or removal of nitrogen from an initial nitrate pool (Adapted from Galbraith, 2006)

AIMS AND HYPOTHESES

A resurgent interest in sediment processes within coastal zones has resulted from the realization of the key role they play in controlling and determining coastal biogeochemistry, which in turn exerts a major influence on marine chemical cycles. It is clear now that the benthos plays an important role in the consumption of oxygen and as a provider/eliminator of N species in estuaries, and this is also true for the St. Lawrence marine system. The first objective of this study was to investigate the quality (e.g., amino acid biogeo- and stereochemistry) as well as the reactivity and preservation status of St. Lawrence sediments along the salinity gradient (terrestrial-marine transition zone) and in the Laurentian Channel (LC) using various OM degradation status indices (e.g., Chlorin Index, Dauwe Index). The main hypothesis was that the OM preservation and reactivity change as a function of OM fluxes, the sources of OM, and exposure to $[O_2]$ along the St. Lawrence River estuary.

There are increasing concerns about eutrophication and the deterioration of the St. Lawrence marine system water quality, partly related to the mobility of various key nutrients and their cycling within the system. The dissertation's second aim was to elucidate changes in the benthic fluxes of DIN, TDN (and DON by their difference), as a function of OM reactivity. Previous results from the St. Lawrence Estuary suggest that most of the porewater ammonium is oxidized within the sediments and denitrification rates are relatively high. On this basis, I hypothesized that benthic N fluxes out of the sediments into the water column are dominated by the organic forms of N (DON), while coupling between nitrification-denitrification consumes most the inorganic forms. As a consequence, net fluxes of TDN should depend on 1) the total denitrification rates (controlling NO_3^- fluxes into the sediments), and 2) the reactivity of the sediment OM (controlling DON fluxes out of the sediments as well as the oxygen penetration depth, which in turn will affect nitrate fluxes and denitrification rates).

The role of estuarine sediments in system-wide N-budgets, as well as in shaping the isotopic signature of the N species is poorly understood, and this is true as well for the

St. Lawrence estuary. The third aim was to analyze the $\delta^{15}N$ of the total dissolved N (TDN), as well as nitrate and ammonium in the St. Lawrence marine system sediment porewater, and to study the distribution of $^{15}N/^{14}N$ ratios of DIN and DON in the porewater, in order to 1) elucidate the biogeochemical reactions that produce the observed distributions, and 2) to study the N-isotope effect of sedimentary N-elimination (including the combined effects of remineralization, nitrification, and denitrification) on the water column. The latter will be carried out by assessing the N isotope composition of nitrate, ammonium and DON fluxes at the sediment water interface, as a function of environmental condition (i.e., sediment quality and bottom water $[O_2]$). The main hypotheses that I tested in this third component of the thesis were: 1) Due to the organism-scale N-isotope effect during sedimentary denitrification, the porewater should be enriched in $^{15}N\text{-}NO_3^-$, while across the sediment-water interface, this enrichment is not fully expressed, 2) the expression of N isotope fractionation within the sediments at the sediment-water interface scale (i.e., the apparent N isotope effect) varies as a function of bottom water oxygen concentrations and of sediment OM reactivity, and $\delta^{15}N$ DON controls the TDN isotopes composition of effluxing N.

CHAPTER I

ORGANIC MATTER REACTIVITY INDICATORS IN SEDIMENTS OF THE ST. LAWRENCE ESTUARY

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Organic matter reactivity indicators in sediments of the St. Lawrence Estuary

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ABSTRACT

Here we report multiple parameters used to describe the diagenetic state of sediments, including total hydrolysable amino acid (THAA), amino acid enantiomer, chlorin (CI) and amino acid degradation (DI, RI) indices, along a transect between the Upper St. Lawrence Estuary and the Gulf of St. Lawrence, Canada. The study area is characterized by gradients in water oxygen concentration, water depth, organic matter (OM) source, primary productivity, and sedimentation rate. Both CI and DI indicate a decline in OM reactivity with the transition from a more terrestrial to a more marine-dominated sedimentation regime as one moves from the shallow Upper Estuary (23-95m) to the hypoxic, mid-depth Lower Estuary and to the deep (>400m), well-oxygenated Gulf. Whereas the CI more accurately reflected OM reactivity in surface sediments and sediments down to 5cm, the amino acid-based degradation indices (DI and RI) better described degradation in sediments down to 35 cm. Systematic variations in the amino acid composition along the Laurentian Channel confirmed the increased diagenesis of OM with distance from the Upper St. Lawrence Estuary. The ratio of D/L stereoisomers of alanine increased along the transect, and the co-variation between DI and the D/L-Ala suggest a close coupling between the extent of diagenesis and the accumulation and selective preservation of bacterially-derived cell wall material in the sediments. The same patterns that we observed along the estuarine transect were present down-core in two sediment cores, confirming the robustness of our reactivity indices. Oxygen exposure time of the sediments appears to strongly determine sediment OM reactivity in the St. Lawrence Estuary. The sediment oxygen regime itself is related to the interplay between water column depth, vertical OM flux, and reactivity of settling OM.

1.1. INTRODUCTION

The susceptibility of individual components of particulate organic matter (OM) to degradation during transport and burial in estuarine sediments under variable depositional conditions has long been discussed (e.g., Burdige, 2007; Niggemann et al., 2007). Key factors that influence particulate OM degradation and preservation include water column depth, redox conditions, particulate OM fluxes, sedimentation rate, sediment physical properties and microbial activity. The latter depends mainly on the nutritional quality and availability for microbes, generally defined as the OM bioreactivity (Gray et al., 2002). There is no single explanation for what exactly controls the turn-over of bulk OM in general, and single components in particular, in estuaries (Hopkinson and Smith, 2005). The coincidence of spatial variations of several environmental factors can make it very difficult to separate the influence of individual factors at any given location.

The origin of the OM supplied to the sediments is one of the main factors that determines the composition and reactivity of sedimentary OM (Burdige, 2007). Whereas the production and degradation of autochthonous OM occurs entirely within the marine environment, terrestrial OM is produced and transported on land and may already be significantly altered before entering the marine system (Hedges et al., 1995). Shifts in the relative importance (marine vs. terrestrial) of the particulate OM flux at the sediment surface may influence OM reactivity and degradation in sediments. The general perception is that terrestrially-derived OM is rather recalcitrant. As a consequence, sediments dominated by terrestrial inputs can be expected to be less reactive than sediments that contain mostly autochthonous algal OM, at least if early diagenetic processes are of secondary importance.

Another factor that influences sedimentary OM diagenesis is the local redox condition. The efficiency of different respiration pathways (e.g., oxic vs. anoxic) with regards to the rates and degree of OM degradation has been investigated in laboratory experiments and the field (e.g., Lehmann et al., 2002; Pantoja et al., 2009). Furthermore, bulk organic carbon (C) preservation has been shown to be directly related to the oxygen

exposure time of sinking and settling particles (Hartnett et al., 1998; Hedges et al., 1999), and redox oscillations have been found to enhance OM degradation largely by promoting symbiosis of aerobic and anaerobic microorganisms (Aller, 1994).

A major challenge in our understanding of OM dynamics in estuarine sediments is the actual description and quantification of the diagenetic state or reactivity of the sedimentary OM pool. Generally, bulk descriptors of sediments such as organic carbon content or carbon-to-nitrogen atomic ratio, explain little of the variation in benthic heterotrophic OM degradation rates at neither local nor regional scale (Zimmerman and Canuel, 2001; Hopkinson and Smith, 2005). Therefore, a number of bioindicators have been proposed to determine the relative degradation state of OM (e.g., Cowie and Hedges, 1994; Dauwe et al., 1999; Schubert et al., 2005). In particular, protein amino acids (AA) and chlorins (i.e., chlorophyll and its early degradation products), have been used as indicators for the overall state of OM degradation (Dauwe et al., 1999; Schubert et al., 2005). Also, the relative abundance (mole %) of the non-protein amino acids β-Alanine (BALA) and γ-Aminobutyric acid (GABA) increase as OM is degraded, thus providing additional information on the degradation state of total OM (Cowie and Hedges, 1994; Keil et al., 2000).

Other diagenetic indicators are directly related to microbial processes in the sediments. Bacterial cell death produces bacterial remnants consisting of a variety of components that have variable susceptibilities towards degradation (Lomstein et al., 2009). One of the more refractory components is peptidoglycan, a unique constituent of bacterial cell walls containing D-amino acids (Grutters et al., 2002). D-AAs in aquatic systems, usually reported relative to their respective ubiquitous L-stereoisomers as D/L-AA ratios can thus be used as indicator of bacterial biomass (Grutters et al., 2002; Veuger et al., 2005). Indeed, the D-alanine to L-alanine (D/L-Ala) ratio of sediments generally increases during early diagenesis as bacterially produced OM accumulates (Lomstein et al., 2006, 2009).

These various indices of sedimentary OM target related but distinct facets of sediment OM quality and diagenetic state, and most likely provide complementary

information. Yet the links that exist between them, and with other environmental factors, are still not well understood. The Laurentian Channel of the Saint Lawrence Estuary (Fig. 1.1) displays pronounced spatial variations in OM source, water column depth, surface water productivity, and water column DO concentration, and hence provides an excellent test case to study the environmental controls on sediment OM reactivity. In this study we combined the indicators described above, with bulk chemical and isotopic measures of sediment OM, to explore patterns in diagenetic state OM along the St. Lawrence Estuary and their links to OM sources and depositional regime.

1.2. SAMPLING AND METHODS

1.2.1. Study site and sampling

The Lower St. Lawrence Estuary and the Gulf form a semi-enclosed sea connected to the Atlantic by the south-eastern Cabot Strait (Fig. 1.1). The estuary is divided into the Lower Estuary and the Upper Estuary near the mouth of the Saguenay Fjord, where average water depth drops suddenly from ~ 100m to ~300m. The morphology of the Lower Estuary and the Gulf is dominated by the Laurentian Channel, a 1200 km long submarine canyon that stretches from the mouth of the Saguenay Fjord through the Gulf of St. Lawrence and the Cabot Strait to the edge of the continental shelf.

The Upper Estuary is characterised by extremely low net sedimentation, with less than 10% of its total surface area covered with fine sediment deposits (d'Anglejan, 1990). On average, the suspended particulate matter load discharged by the St. Lawrence River to the Lower Estuary and the Gulf of the St. Lawrence amounts to 6.5×10^6 t yr^{-1} (Rondeau et al., 2000). The Lower Estuary is characterized by elevated sedimentation rates and a comparatively high primary productivity due to the upwelling of nutrient-rich deep water masses. Both surface water primary productivity and sedimentation rates decrease eastward along the Laurentian Channel (the latter from 0.45 g cm^{-2} yr^{-1} at the head of the Laurentian Channel to 0.04 g cm^{-2} yr^{-1} in the Gulf; Roy et al., 2008; see Table 1.1).

Recent studies of the Laurentian Channel revealed that approximately 1300 km^2 of the seafloor are perennially overlain by severely hypoxic waters (<20% saturation) since

the mid-1980s, in part due to high OM input (Gilbert et al., 2005; Lehmann et al., 2009). The oxygen-deficient area is confined within the bottom waters of the Lower Estuary in the Laurentian Channel (Table 1.1), and is isolated from the oxic upper layers by a steep density gradient that only allows weak diffusion of oxygen through its boundary (Gilbert et al., 2005).

Surface sediments were collected with a Van Veen grab in the Upper Estuary (Sta. DE, G, and H), and a multicorer was used in the Lower Estuary (Sta. 25-21) and Gulf (Sta. 20-16, and Anticosti) during two summer 2006 cruises (June and August) aboard the *R/V Coriolis II* (Fig. 1.1; Table 1.1). Sediment cores were sub-sampled by slicing at 1-cm intervals within the upper 10 cm, 2-cm intervals between 10 and 20 cm, and 3-cm intervals below 20 cm. Sediment samples were kept frozen in sterilized plastic bags prior to lyophilisation, homogenization, and analysis in the laboratory. In the following sections we distinguish between down-core records from multicores, and surface sediments. For surface sediments in the Lower Estuary and the Gulf, measured parameters represent the average of the uppermost 4 cm of the sediment column.

1.2.2. Elemental and isotope analysis

C_{org} and nitrogen (N) contents of sediments were determined using an elemental analyzer (NC Instruments). Prior to organic carbon analyses, inorganic carbon was removed by acidification of sediment sub-samples with concentrated HCl fumes overnight (Hedges and Stern, 1984). The C/N ratio was calculated as the molar ratio of C_{org} to N. Reproducibility based on standard deviation of replicate measurements was better than 0.01% for N and better than 0.04% for C_{org} (n=10).

The C and N isotope composition was determined using a continuous flow isotope-ratio mass spectrometer (Micromass Isoprime) coupled to a Carlo-Erba elemental analyser. C and N isotope ratios are reported as $\delta^{13}C_{org}$ and $\delta^{15}N$, respectively, where δ-values are denoted as ‰-deviation from the carbon isotopic composition of the Pee Dee Belemnite (PDB) standard, and from the isotope composition of atmospheric nitrogen gas (AIR), respectively: $\delta^{13}C_{org}$ (or $\delta^{15}N$) = $[(R_{sample}/R_{standard})-1] \times 1000$, where R is either the

$^{13}C/^{12}C$ ratio or the $^{15}N/^{14}N$ ratio. Average standard deviations based on replicate measurements were better than 0.2‰ for both $\delta^{15}N$ and $\delta^{13}C_{org}$.

1.2.3. Chlorins and Chlorin Index (CI)

Chlorins were extracted by successive (3) sonication of freeze-dried ground sediments (10-20 mg) in acetone (HPLC grade) following the procedure described by Schubert et al. (2005). Extracts were kept in dark and on ice to prevent any degradation prior to analysis, and were measured fluorometrically (Cary Eclipse 50 fluorescence spectrophotometer) at an excitation wave length of 407 nm and an emission wavelength of 662 nm (Schubert et al., 2005). Pheophytin-a from the acidification (HCl 25%) of a Chlorophyll-a (Sigma) standard was used for calibration. Chlorin concentrations are reported as μg per gram dry weight sediment (μg gdws-1). The precision of the method was better than 7% (n=5). The chlorin index (CI) is the ratio of the fluorescence of the acidified to the original extract. The CI scale ranges from 0.2 for pure chlorophyll to almost 1 for highly degraded pigments (Schubert et al., 2005).

1.2.4. Hydrolysable amino acid analysis

Total hydrolysable AA (THAA) were extracted by adding 5mL HCl (6M) to freeze dried homogenized sediment (~100mg) in pre-cleaned and muffled (450C° for 3h) glass vials, and purging the headspace with N_2. The vials were kept in an oven at 110C° for 24 h. Acid hydrolysates were then centrifuged (5000 rpm; 10 min.) and neutralized with 6N KOH. Individual AAs glycine (Gly), aspartic acid (Asp), glutamic acid (Glu), serine (Ser), threonine (Thr), arginine (Arg), alanine (Ala), β-alanine (BALA), γ-aminobutyric acid (GABA), tyrosine (Tyr), valine (Val), methionine (Met), phenylalanine (Phe), isoleucine (ILeu), and leucine (Leu) were quantified according to Lindroth and Mopper (1979) by high-performance liquid chromatography (HPLC, JASCO System) after precolumn derivatisation with OPA using a Nova-Pack C-18 column (at 25°C). Blanks showed negligible AA concentrations. THAA concentration was calculated as the sum of the individual amino acid concentrations. Concentrations of the stereoisomers D- and L-Ala

were measured following the method of Mopper and Furton (1999). Isobutyryl-L-cysteine was used as an additional chiral agent (Brückner et al., 1995). D- and L-Ala concentrations were corrected for racemization during acid hydrolysis according to Kaiser and Benner (2005). The percentage of racemization used for D-Ala was 1.2%.

1.2.5. Amino acid-based reactivity (Degradation Index)

The Degradation Index (DI) was calculated for whole depth profiles at Sta.18 and 23 only, as well as for surface sediments from all stations. DI scores for the St. Lawrence samples were calculated using the mole percentages of AAs in the following formula: $DI = \Sigma_i [var_i\text{-}AVG_i / STD_i] * FC_i$, where var_i is the mole percentage of the AA *i* (non-standardized), AVG_i and STD_i are the average and standard deviation of the var_i respectively, and FC_i is the factor coefficient assigned for var_i on the first axis of the PCA analysis, also called variable loading (Dauwe et al., 1999). Dauwe and Middelburg (1999) determined the DI scores for different OM, and found that DI for sources such as phytoplankton, bacteria, and sediment trap material have DIs between 1 and 1.5, while coastal and ocean margin sediments have scores between -0.3 and 1. Deep-sea sediments display scores less than -1.

1.2.6. Oxygen penetration depth and oxygen exposure time

Adopting the approach of Cai and Sayles (1996), O_2 penetration depths (OPD) were approximated based on benthic O_2 flux data from Thibodeau et al. (2010; Table 1.1), with $OPD = [D^s_{O2} \times O_{2\_BW}] / F_{O2}$, where F_{O2} is the benthic O_2 flux, O_{2_BW} is the bottom water O_2 concentration, and (D^s_{O2}) is the sedimentary diffusion coefficient at the *in situ* temperature corrected for sediment porosity. Sediment O_2 exposure time (OET) is calculated as the OPD divided by the corresponding sediment accumulation rate (data from Smith and Schafer, 1999; Table 1.1).

1.3. RESULTS

1.3.1 Elemental carbon and nitrogen concentrations and isotopic composition

In surface sediments, $\%C_{org}$ and %N in the Upper Estuary ranged from 2.6 to 1.5% and from 0.21 to 0.14%, respectively (Fig. 1.2a). In the Lower Estuary and the Gulf, $\%C_{org}$ showed a decreasing trend, while nitrogen concentrations increased. C/N ratios declined from >18 in the Upper Estuary to <9 in the Gulf (Fig. 1.2b). The $\delta^{13}C_{org}$ of sediments of the Upper Estuary displayed a narrow range between -25.8 and -25.2‰, while in the Laurentian Channel, $\delta^{13}C_{org}$ values increased from -24.2‰ at the head of the Channel to -22.5‰ at Anticosti and -22.0‰ at Sta. 16 (Fig. 1.2c). The observed $\delta^{13}C_{org}$ and C/N ratios found in the surface and core sediments along the upper estuary and the Laurentian Channel indicate different OM sources (see Smith and Epstein, 1971; Fry and Sherr, 1984). Terrigenous contribution to the OM was highest in the Upper Estuary sediments and decreased as a function of distance from the St. Lawrence River mouth. This trend is confirmed by decreasing lignin concentrations from the Upper Estuary towards to the Atlantic Shelf (Louchouarn et al., 1997). The $\delta^{13}C_{org}$ in the Gulf sediments shows that sedimentary OM is mainly of marine origin, with minimal contribution of terrestrial OM at Sta. 16 ($\delta^{13}C$ = -22, Fig. 1.2). An enrichment in ^{15}N was also observed along the terrestrial-marine gradient, with $\delta^{15}N$ values <5‰ in the Upper Estuary and >7‰ at Sta. 16 (Fig. 1.2c). This pattern is related to incomplete nutrient utilization as described by Thibodeau et al. (2010).

Down-core, $\%C_{org}$ and %N decrease at most sites (Fig. 1.3a,b). C/N ratios in sediments increased with depth by 1-2 at Sta. 25 and 23 in the Lower Estuary and by 0.5-1.5 at the Anticosti station and Sta. 16 in the Gulf (Fig. 1.3c). In contrast, the C/N ratios did not change significantly with depth in the sediments between Sta. 22 in the Lower Estuary and Sta. 19 in the Gulf (Fig. 1.3c). Our data indicate down-core enrichment in the $^{13}C_{org}$ in the upper 10 cm of the sediment cores at Sta. 25, 23, 22, 18, and 16, while at the other stations the $\delta^{13}C_{org}$ did not show a clear down-core trend (Fig. 1.3d).. We also observed a decrease in $\delta^{15}N$ by ~0.5 to 1‰ down-core in the uppermost 6 cm at Sta. 25,

23, 16 and Anticosti, whereas at the other stations no clear trend could be discerned (Fig. 1.3e).

1.3.2. Chlorin concentrations and index

Surface sediment chlorin concentrations decreased seaward along the investigated transect (Fig. 1.2b). In the Upper Estuary at Sta. DE, chlorin concentrations were >15 μg g^{-1} (Fig. 1.2b). Along the Laurentian Channel, they ranged between 12 μg g^{-1} in the Lower Estuary and 4-6 μg g^{-1} at Sta. 16 and the Anticosti station in the Gulf. At all stations but Sta. Anticosti, chlorin concentrations decreased with sediment depth, with 25-49% of the chlorins being lost in the upper 5 cm (Table 1.1). CI in surface sediments increased along the Laurentian Channel, with the lowest values (0.43-0.48) in the Upper Estuary (Sta. DE, Sta. G, and Sta. H), and the highest values (>0.81) at Sta. 16 (Fig. 1.4a). At most stations, the CI increased down-core in the upper sediment layer (~10 cm), whereas, no change in CI could be discerned deeper in the sediments (Fig. 1.3f).

1.3.3. Total hydrolysable amino acids

THAA concentrations in the surface sediments showed a slight decrease downstream the Laurentian Channel, with highest values in the Upper Estuary (50.2-58 μmol g^{-1} dws), intermediate concentrations in the Lower Estuary (41-51 μmol g^{-1}), and the lowest concentrations at Sta.16 in the Gulf (31 μmol g^{-1}; Fig. 1.2b). In the surface sediments, the percentage of C_{org} found in amino acids (%THAA-C_{org}) varied between 11 and 20% in the Upper Estuary, between 12 and 19% in the Lower Estuary, and ranged from 10 to 16% in the Gulf (Fig. 1.2d). Percent amino acid N (%THAA-N) varied between 42 and 69% in the Upper Estuary, between 42 and 60% in the Lower Estuary, and between 23 and 42% in the Gulf (Fig. 1.2d). Sediment profiles at Sta. 23 and 18 showed a decrease in the THAA concentration with depth, from 41 to 23 mg g^{-1} and 37 to 23 μmol g^{-1}, respectively (Fig. 1.5a). At Sta. 23 in the Lower Estuary, %THAA-N and %THAA-C_{org} decreased from 0 to 35 cm by 17% and 26%, respectively, whereas at Sta. 18 in the Gulf the %THAA-N and %THAA-C_{org} decrease was significantly more

pronounced (31% and 29%, respectively) (Fig. 1.5 b, c). At least for the Gulf stations, a stable input of OM to the sediments can be assumed (Genovesi et al., 2011) (we observe almost invariant profiles of carbon and nitrogen concentrations), and the %THAA-N and %THAA-C_{org} trends can be interpreted as the result of fractional degradation.

1.3.4. Amino acid composition and degradation index

Surface sediment mole percentages (mole%) of Asp, BALA, GABA, Gly, and, to a lesser extent, Arg and Thr, increased with distance seaward from the Upper Estuary, while those of Ala, Glu, ILeu, Lys, Leu, Phe, Tyr, and Val decreased along the same gradient. Met did not show a clear along-channel concentration gradient (Table 1.2). The relative AA abundance was similar in the Lower Estuary and Gulf sediments (Sta. 23 and 18; Fig. 1.6). Ala, Asp, Glu, Gly, Ser, and Thr were the most important AAs, comprising more than 60% of the THAA. At the two stations where we obtained AA concentration profiles (Sta. 23 and Sta. 18), Asp, Glu, Ileu, Phe, Ser, Thr, and Val decreased with depth, while BALA, GABA, Gly, and Lys increased. With regards to Ala, Arg, Leu, Met, and Tyr, only minor down-core changes were observed.

D/L-Ala increased along the Upper Estuary and the Laurentian Channel, with average values of 0.17 in the Upper Estuary, 0.22 in the Lower Estuary, and 0.32 in the Gulf (Table. 1.2). At both investigated stations, D/L-Ala ratios increased down-core from ~0.2 in surface sediments to ~0.4 in the deeper sediments (Fig. 1.5d). The DI in surface sediments ranged between -0.08 and 0.13 in the Upper Estuary, -0.4 and -0.53 in the Lower Estuary, and -0.5 and -1.0 in the Gulf (Fig. 1.4b). DI decreased down-core at both Sta. 18 and Sta. 23, (Fig. 1.5e) from ~ -0.6 to -0.8 and from -0.4 to -0.5, respectively.

1.4. DISCUSSION

1.4.1. Relative loss of chlorins and THAA

Our THAA concentrations in surface sediments along the estuary (upper and lower) and the Gulf (Fig. 1.2b) are typical for natural estuarine and coastal marine environments (Cowie and Hedges, 1992; Lomstein et al., 2006; Arnarson and Keil, 2007). Chlorin concentrations were one order of magnitude higher than those found in the Arabian Sea sediments (Schubert et al., 1998; Shankle et al., 2002), but one order of magnitude lower than values reported from Lake Zug sediments (Meckler et al., 2004). The absolute percentage of THAA and chlorins is influenced by the overall OM fluxes and, hence, is a function of primary productivity (which varies along the estuary and between the estuary and the Gulf). Chlorin and THAA concentrations normalized to N and C_{org} concentrations, on the other hand, reflect changes in degradation state (Lee et al., 2000; Lomstein et al., 2006; Schubert et al., 2005) or changes in the terrestrial-to-marine OM ratio. Normalized THAA concentrations decrease downstream (Fig. 1.2d) and down-core (Fig. 1.5b,c), consistent with the preferential degradation of AAs relative to bulk N and C_{org} with ongoing OM degradation (Burdige and Martens, 1988; Cowie and Hedges, 1992). The degree of chlorin and THAA degradation most likely reflects the intensity of early diagenesis (either in the water column or within the sediments), and the lower chlorin concentrations in the Gulf hints to a more advanced degradation. Along the same line, the comparatively high chlorin and THAA concentrations in the Upper Estuary can be explained by a better OM preservation in shallower waters. Indeed, a significant exponential relationship exists between vertical OM flux in the water column (obtained from previously published studies in the same area, see Table 1.1) with surface sediment chlorin concentration per 100 mg C_{org} (Table 1.1; r^2=0.41, n=6), as well as with surface sediment %THAA-N (r^2=0.68, n=6). The more seaward stations in the eastern Lower Estuary and the Gulf are typically nutrient-depleted leading to low productivity (Roy et al., 2008; Thibodeau et al., 2010), whereas the western landward Lower Estuary is generally more productive (Gilbert et al., 2005), and therefore sustains higher OM fluxes.

Pelagic processes (e.g., OM flux, OM decomposition and transformation) affect the biogeochemical composition and reactivity of the sinking OM that reaches the sediments; it is thus *a priori* difficult to determine whether the observed along-channel changes in the composition of the surface sediments are due to reworking in the water column or the result of post-depositional processing. In this regard, the patterns of chlorin and total OM loss down-core in the sediments can be used as an index of the intensity of post-burial diagenesis. Along the Laurentian Channel, between 16% and 50% of the surface sediment chlorin was lost in the upper 5 cm of the sediment column (Fig. 1.3g, Table 1.1). The highest chlorin loss (see Table 1.1 for definition) was observed at the head of the Laurentian Channel, in the hypoxic portion of the Lower Estuary, while minimal loss was observed at Sta. 16 in the Gulf (Table 1.1). Enhanced down-core chlorin loss at the shallower, low-DO stations (Sta. 25 to 21) suggests that, here, chlorins are less effectively degraded in the water column and quantitative degradation sets in upon deposition at the sediment surface. At the deeper stations in (e.g., Sta. 16) particle oxygen exposure time is increased and chlorins are significantly degraded already during settling through the water column, leaving less chlorin "substrate" for degradation in the sediments.

Sedimentation rates at the Lower-Estuary stations are higher than at the Gulf sites (Table 1.1). As a consequence, the integrative (4 cm) surface sediment samples have a different ages and some of the along-channel biogeochemical variations are likely attributable to those differences. However, the variability of chlorin and THAA loss in down-core sequences (representing longer timescales compared to the surface-sediment age difference between stations) is much less than in surface sediments along the Upper Estuary to Gulf transition; these age differences alone thus cannot explain the observed along-channel trends.

1.4.2. Amino acid composition changes and reactivity indices

AAs display variable susceptibility towards bacterial decomposition and reworking. Due to their comparatively labile nature and high nutritional value for bacteria,

Glu, ILeu, Leu, Tyr, and Phe are preferentially degraded in sediments during early diagenesis (Burdige and Martens, 1988; Dauwe et al., 1999; Lee et al., 2000). Lee et al. (2000) observed that Glu, Phe, and Tyr decrease as particles sink in the water column at various locations in the Central Equatorial Pacific Ocean. In surface sediments along the Laurentian Channel, a general trend of higher concentrations of these AAs upstream and lower concentrations downstream (Table 1.2) can, therefore, be interpreted as an increase in OM degradation state towards the open waters.

In surface sediments along the terrestrial-marine gradient, Lys decreased while Asp increased (Table 1.2). Cowie and Hedges (1992) have shown that Lys is enriched in vascular plant OM compared to marine phytoplankton. Therefore, the observed relative Lys concentration decrease, together with the observed C/N and $\delta^{13}C_{org}$ changes, confirm the decreasing trend in the amount of terrestrial plant material deposited in downstream sediments. Nunn and Keil (2005) found that enrichment in Asp during OM degradation is likely due to both preferential preservation of Asp-rich proteins and the production of Asp during bacterial reworking (Nunn and Keil 2005). Both processes likely contributed to the observed relative enrichment of Asp downstream.

The increase in % Gly both downstream along the Upper Estuary and the Laurentian Channel (Table 1.1) and down-core at Sta. 18 and 23 (Fig. 1.6a) suggests that Gly preferentially accumulates during OM degradation. Dauwe and Middelburg (1998) interpreted the enrichment in Gly as a consequence of its low nutritional value for benthic fauna and microbial communities, whereas Lee et al. (2000) suggested enrichment in Gly due to selective diatom cell wall preservation. Gly is also an important component found in bacterial cell walls (Lee et al., 2000; Ingalls et al., 2003). In contrast to bacterial cell walls, diatom cell walls and frustules are also enriched in Ser and Thr, in addition to Gly (Ingalls et al., 2003). Ser decreased both downstream in surface sediments and down-core at both investigated stations. Thr on the other hand decreased down-core at Sta. 18 and did neither change down-core at Sta. 23 nor downstream in surface sediments. Hence, the most plausible assumption is that it is indeed the increasing contribution of bacterial necromass to the bulk sediment OM pool with ongoing degradation (Keil et al., 2000), in

addition to diagenetic alteration of other AAs that leads to the relative enrichment of Gly within the St. Lawrence Estuary and Gulf sediments.

Progressive diagenesis in sediments is also indicated by the increased abundance of non-protein AAs (Cowie and Hedges, 1994; Hedges et al., 1999; Lee et al., 2000). We found a pronounced increase in the relative abundance of BALA and GABA, both in surface sediments along the studied transect towards the Gulf (Table 1.2), and down-core (Fig. 1.6p,q), which can be interpreted as an additional indicator of compositional changes of the sedimentary OM due to the more advanced state of degradation, respectively. The patterns of individual AAs are coherent with the values determined for the DI, both converging to suggest declining reactivity (i.e., more advanced degradation) in surface sediments along the Upper Estuary and the Laurentian Channel (Fig. 1.4b), and with depth in sediments at Sta. 18 and 23 (Fig. 1.5e).

Consistent with the DI scores, surface sediment CI values in the Upper Estuary were lower compared to those in the Lower Estuary and the Gulf, also indicating a lower degree of alteration (Fig. 1.4a). Overall, the DI and CI suggest that the most degraded and least reactive sediments occur in the Gulf. The agreement between DI and CI in surface sediments along the Laurentian Channel is better (r^2=0.89, n=12; Fig. 1.7a) than for the down-core records (r^2=0.43, n=39). Because chlorins and amino acids are molecularly different compounds, they are likely to be degraded at different rates (Meckler et al., 2004). As becomes evident from our data set, the CI is only very sensitive in surface sediments and down to the first five centimeters in the two investigated cores. Below 5 cm sediment depth, hardly any change in the CI can be discerned. This is in agreement with a previous study by Meckler et al. (2004), showing that the CI is more sensitive at early stages (i.e., years) of OM degradation.

Our interpretation of the trends in the DI and CI is further supported by a third reactivity indicator (Reactivity Index, or RI), which is based on the ratio of aromatic AA (Phe+Tyr) to non-protein AA (GABA+BALA), and which varies from 0.1 for heavily degraded OM to 3.6 for freshly deposited reactive OM (Jennerjahn and Ittekkot, 1997). As with the other indices, the RI suggests that surface sediments in the more seaward parts of

the St. Lawrence Estuary and the Gulf are more degraded than in the Upper Estuary and the estuarine channel mouth (Fig. 1.4c). The good agreement between CI and the other AA-based reactivity indices (Fig. 1.7) supports the robustness of those indices in transitional aquatic environments such as the St. Lawrence Estuary.

1.4.3. Bacterial OM

Unlike L-AAs, the D-AAs are source-specific and therefore have the potential to indicate OM origin (McCarthy et al., 1998; Amon et al., 2001). D-Ala is present in all bacteria providing a robust tracer for bacterial biomass (e.g., Amon et al., 2001; Grutters et al., 2002; Veuger et al., 2005; Jørgensen and Middelboe, 2006). An increasing proportion of D-Ala with depth and age of the sediment can, in addition to bacterial synthesis, be the result of chemical racemization. However, this possibility was tested in various coastal and continental shelf studies (Pedersen et al., 2001; Grutters et al., 2002; Lomstein et al., 2006), confirming that biological processes generally dominate the production of D-AA. The increase in D/L-Ala in surface sediments downstream (from an average of 0.17 in the Upper Estuary to 0.22 in the Lower Estuary, and to 0.32 in the Gulf; Table 1.2) suggests the enrichment of the OM pool with bacterial remnants. Moreover, D/L-Ala increases down-core at Sta. 23 and 18 (Fig. 1.5d), demonstrating the accumulation of bacterial remnants during sedimentary diagenesis. Similar observations have been made in other marine environments, such as the Aarhus Bay and Roskilde Fjord, Denmark (Pedersen et al., 2001; Jørgensen and Middelboe, 2006), the northeastern Atlantic continental slope (Grutters et al., 2002), and in coastal sediments off Chile and Peru (Lomstein et al., 2006, 2009). D/L-Ala ratios can further be used to estimate the relative contribution of bacterial remnants to bulk sedimentary OM preserved in the sediments. The strong inverse correlation between D/L-Ala and the reactivity indicator DI, both downstream (r^2=0.83, n=12) and down-core at Sta. 23 and 18, indicates a close link between the degradation state and the degree of bacterial processing of sedimentary OM. A similar correlation between DI and D-AA enrichment has been found for marine dissolved OM (Amon et al., 2001) supporting the concept that bacteria not only consume

labile organic material but replace it with OM that is more refractory in nature. Alternatively, if not refractory *per se*, bacterial OM may be continuously and efficiently recycled to maintain a stable "bacterial" OM signature in the sediments. These details aside, our observations in the St. Lawrence Estuary and Gulf are consistent with previous reports, which show that bacterial remnants are preferentially preserved both in laboratory incubations and marine sediments (Tremblay and Benner,, 2006; Veuger et al., 2005; Lomstein et al., 2009), and thus confirm that bacterial degradation and biosynthesis shape the chemical composition of OM by selectively removing bio-reactive components from the original OM and leaving behind (bio-refractory) bacterial OM components (e.g., peptidoglycan).

1.4.4. Environmental factors

Both the chlorin-based and AA-based reactivity indices suggest that the reactivity of the sedimentary OM declines along the Upper Estuary- Gulf gradient transition. The factors that control this reactivity gradient is, however, not clear. In order to explore this question in detail, we compared the OM reactivity in surface sediments to water depth, bottom water oxygen content, and oxygen exposure time.

1.4.4.1. *Water column depth*

Despite similar environmental conditions in the Upper Estuary (Sta. DE, G, and H) and at Sta. 16, OM reactivity is much greater at the shallow sites in the Upper Estuary, suggesting that water depth may be an important determinant of the quality of the sedimentary OM. In fact, we found a significant correlation between water depth and the CI (r^2=0.92, n=12; Fig. 1.8a), providing putative evidence that the settling time of organic particles and aggregates in the water column plays a crucial role in determining the quality of OM that ultimately reaches the sediments. Particle-associated bacteria degrade organic compounds from sinking particles, leading to the production of dissolved OM (Honjo et al., 2008 and references therein), while breaking the sinking particles down into smaller, suspended OM particles, thus reducing the overall OM particles flux and inducing

compositional changes. This process is drastically reduced when water depth is shallow like in the upper estuary. Co-variation of sediment OM reactivity and water column depth was observed in other environments as well (Hedges et al., 1999; Shankle et al., 2002; Lomstein et al., 2006). Niggemann et al. (2007) compared depositional conditions, OM composition, and organic carbon turnover in sediments from two different depositional systems along the Chilean continental margin, and in agreement with our observations, concluded that water depth plays an important role in the quality of sinking OM. Along the same line, Shankle et al. (2002) found a significant correlation between sediment chlorin concentration and water column depth in the Arabian Sea. Hedges et al. (1999) found that with increasing water column depth off shore the continental margin of Washington, the fraction of GABA and BALA increased in the sedimentary OM, indicating advanced degradation of the sediments. Bourgoin and Tremblay (2010) collected suspended particles at different water column depths in the St. Lawrence Estuary. Consistent with our findings of a depth-controlled reactivity of the Upper vs. Lower Estuary sediments, they found that in the Lower Estuary, bottom water particulate THAA concentrations were up to 35 times lower compared to surface water concentrations, while in the Upper Estuary THAA loss with depth in the water column was minimal.

1.4.4.2. *Dissolved oxygen and vertical OM flux*

In the Lower Estuary and the Gulf, water depth variations are less pronounced than between the Upper Estuary and Lower Estuary. Nevertheless, surface sediment reactivity appears to decrease eastward (Fig. 1.4). We found a significant negative relationship between CI in surface sediments and the C_{org} flux to the sediments (see Table 1.1 with references therein; r^2= 0.87, n=5; Fig. 1.8b), and a positive relationship with the bottom water DO content (r^2=0.86, n=9; Fig. 1.8c). This suggests that low DO conditions and enhanced fluxes of OM to the sediments combine to enhance OM preservation in the Lower Estuary sediments. Increased sedimentation rates have been hypothesized to be responsible for increased OM preservation through rapid burial (Henrichs and Reeburgh,

1987; Bertrand and Lallier-Verges, 1993). Ambient oxygen concentration also partly control the extent of OM degradation in the water column and in sediments, both on short and long time scales (Bianchi et al., 2000; Kristensen and Holmer, 2001; Bechtel and Schubert, 2009). It is important to note, however, that the initial rate of fresh bulk OM remineralization occurs at similar rates under both oxic and hypoxic to anoxic conditions, while that of older and refractory OM seems to be slower under anaerobic compared to oxic conditions (Lehmann et al., 2002; Pantoja et al., 2009; Bechtel and Schubert, 2009). Chlorins appear to be more susceptible to oxic degradation (Sun et al., 1993; Shankle et al., 2002). The strong correlation between CI (and DI, r^2=0.93, n=9) and bottom water oxygen concentration suggests that OM delivered to sediments in the Lower Estuary and Gulf may be partially degraded in the water column or in the bottom nepheloid layer, to a degree that allows the oxygen to play a role on preferential preservation of OM under low oxygen concentration condition, in agreement with the findings of Archer and Devol (1992) and Bourgoin and Tremblay (2010). We also note that oxygen concentrations only correlate with specific components (here pigments and AA) and do not correlate significantly with total $\%C_{org}$ in surface sediments (r^2=0.19, n=9). Similar uncoupling between $\%C_{org}$ and DO has been observed across the Indus margin of the Arabian Sea (Cowie et al., 2009) and across the Washington State continental shelf and slope (Archer and Devol, 1992).

The effects of DO concentrations and OM sedimentation rates combine to determine yet another factor that is likely to have close links to the reactivity of sedimentary OM: The exposure time (OET) of organic particles to oxic conditions within the sediments. The OET depends on the burial rate and the oxygen penetration depth (OPD) in the sediments, which in turn is a direct function of the DO in the overlying water and the reactivity of sediments (Hartnett et al., 1998; Hedges et al., 1999; Lehmann et al., 2009). The calculated OPD in the St. Lawrence estuarine sediments (corresponding to the thickness of the oxic sediment layer) increases from approximately 0.6 -0.9 cm in sediments at the head of the Laurentian Channel at Sta. 25 and Sta. 23 to approximately 1.5 - 1.8 cm in the Gulf at Sta. 18, 19, and Anticosti. Similar values for the OPD (1.5 to

1.7 cm) were measured for the St. Lawrence Gulf sediments by Silverberg et al. (2000) using microelectrodes. A negative correlation between OPD and OM reactivity (DI; r^2=0.73; n=6) indicates that the sedimentary OM reactivity decreases as OPD increases, yet the causal links are not obvious. OET was lowest in the Lower Estuary, ranging between 1.2-1.7 years at Sta. 25 and Sta. 23, increasing to ~7 years between Sta. 21 and Sta. 19, and reaching ~14 years at Sta. 18. As with OPD, OET correlates with OM reactivity (DI; r^2=0.88; n=5; and CI r^2=0.72; n=5). There are several components of OM that are oxygen-sensitive (Hedges et al., 1999), and have higher potential to be preserved under reducing conditions or shorter OET of years and decades (Arnarson and Keil, 2007). Both high OM flux and low bottom-water DO (Table 1.1) at the head of the Laurentian Channel are conducive to shorter OET and shallower OPD. While our data indicate that oxygen exposure time of the sediments determines OM reactivity, the sediment oxygen regime may itself be related to the reactivity of the sedimenting OM. Direct (through core incubations) and indirect (porewater DO profiles) determinations of benthic O_2 fluxes carried out by Thibodeau et al. (2010) revealed that oxic bacterial respiration rates along the Laurentian Channel are a function of the sediment reactivity rather than OM content. Hence, we argue that feedback loops can be created wherein the reactivity of the OM may influence the establishment of hypoxic conditions, which in turn, may enhance the preservation of OM in the sediment.

1.5. Conclusions

In this paper we have explored the relative importance of OM sources versus processing in determining the sediment OM reactivity along estuarine gradients. Terrestrially-derived OM has traditionally been considered recalcitrant, and thus sediments dominated by terrestrial inputs would be expected to be less reactive than those dominated by algal materials. Our results do not support this assumption. The various compositional characteristics of the studied sediments suggest a trend towards less reactive material from the Upper Estuary towards the Gulf, while geochemical evidence

highlights the transition from a more terrestrial to a more marine- dominated sedimentation regime along this transect. The agreement between chlorin- and amino acid-based reactivity indicators (CI, DI, RI) demonstrates the applicability of both types of indices to complex estuarine environments with terrestrial and marine organic matter sources. The CI better describes the initial degradation of OM (i.e. in the water column and the uppermost sediments), whereas the amino acid-based indicators also account for longer-term degradation processes in the deeper sediment. The spatial pattern in sediment reactivity along the Laurentian Channel seem to primarily result from increasing intensity of OM degradation in the water column and within the sediments. Hence, our data provide a good example where OM source seems to be secondary to early diagenetic processing in determining sediment reactivity. We provide putative evidence that water depth (which influences particle settling time), OM flux rates and bottom water DO all play a role in OM preservation and thus control the bulk OM reactivity of the sediments. The observed trends towards higher D/L Ala ratios in surface sediments downstream the Laurentian Channel (and down-core), indicate the accumulation of bacterial remnants with increasing OM degradation, and parallels the DI and CI changes. Hence the preservation and accumulation of bacterial cell wall remnants is coherent with the patterns of reactivity, confirming 1) the close link between OM degradation, in situ bacterial biosynthesis, and the preservation of OM and 2) the refractory nature of the preserved (or continuously recycled) bacterial material. While our data suggest that bottom water DO is an important constraint on OM degradation and reactivity, we speculate that OM reactivity itself likely influences oxygen flux and penetration depth in the sediments, with direct implications for the dissolved oxygen budget and hypoxia in the Lower St. Lawrence Estuary.

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Table 1.1. Sampling site characteristics along the St. Lawrence Estuary and the Gulf of St. Lawrence. Chlorin loss (in %) defined as: ($[Chlr]_{1cm}$ -$[Chlr]_{5cm}$)/[$Chlr]_{1cm}$ ×100).

Station	Depth (m)	Bottom water $[O_2]$ [a] (μmol L^{-1})	OET (years)	OPD (cm)	Sediment acc. Rate[a] (g cm^{-2} yr^{-1})	C_{org} flux (μmol cm^{-2} yr^{-1})	C_{org} burial rate (μmol cm^{-2} yr^{-1})	Chlorin loss (%)
DE	23	290	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
G	93	290	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
H	64	291	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
25	290	65	1.17	0.64	0.55	N.D.	N.D.	49
23	350	63	1.72	0.92	0.45	471 to 932 [c]	~108 to 364[c]	34
22	321	70	N.D.	N.D.	0.45	573 [d]	~158 [d]	46
21	330	75	6.57	1.46	0.22	N.D.	N.D	42
20	330	97	N.D.	N.D.	0.14	~ 22 [e]	~13 [e]	39
19	370	108	7.68	1.82	0.24	66 to 160 [f]	~46 [f]	41
18	370	123	13.57	1.56	0.12	~ 19 [e]	~ 10 [e]	25
Anticosti	283	106	N.D.	1.53	N.D.	N.D.	~ 13 [e]	29
16	420	197	N.D.	N.D.	0.04	~ 22 [e]	~ 11 [e]	17

(ND = not determined).

Data from: [a]Thibodeau et al. (2010); [b]Smith and Schafer (1999); [c]Silverberg et al. (1987); [d]Colombo et al. (1996); [e]Muzuka and Hillaire-Marcel (1999); [f]Silverberg et al. (2000)

Table 1.2. Amino acids (mole-% of THAA) in surface sediments along the St Lawrence Estuary (Sta. DE – 21) and the Gulf of St. Lawrence (Sta. 20 to 16).

Station	16	Ant	18	19	20	21	22	23	25	H	G	DE
Ala	7.4	7.5	8.6	7.2	7.5	7.4	7.8	8.9	8.3	8.1	8.2	8.1
D/L Ala	0.4	0.2	0.3	0.4	0.3	0.2	0.2	0.2	0.2	0.2	0.1	0.1
Arg	4.7	4.0	4.5	4.7	4.2	4.3	3.6	3.8	3.7	3.6	3.9	3.8
Asp	17.	16.	16.	15.	16.	15.	16.	15.	15.	13.	13.	13.
BALA	2.9	2.4	2.4	2.6	2.5	2.1	2.1	1.8	1.1	1.2	0.9	0.6
GABA	1.7	1.5	1.6	1.8	1.7	1.5	1.7	1.5	1.7	1.6	1.3	1.0
Glu	8.7	9.0	9.2	9.0	9.8	9.7	9.2	10.	10.	10.	10.	10.
Gly	19.	17.	16.	17.	16.	17.	16.	16.	15.	15.	15.	15.
His	1.5	1.4	1.7	1.6	1.9	1.7	1.8	1.4	1.4	1.9	2.1	2.0
ILeu	2.7	3.3	3.2	3.0	3.2	3.0	3.1	3.4	3.5	3.5	3.9	3.6
Leu	2.9	3.8	3.2	3.8	4.3	3.9	3.8	4.3	4.7	4.5	5.7	5.4
Lys	4.4	6.9	5.2	4.9	3.9	4.9	5.4	4.4	6.4	8.1	6.9	7.4
Met	0.4	0.5	0.6	0.7	0.7	0.6	0.4	0.5	0.6	0.6	0.4	0.4
Phe	2.3	2.5	2.6	2.3	2.2	2.6	2.5	2.8	2.4	3.0	3.2	2.7
Ser	7.5	7.7	8.9	8.0	8.3	8.5	8.4	8.9	8.1	8.2	7.9	8.0
Thr	8.1	7.6	6.6	7.9	8.3	7.8	7.7	7.3	8.0	7.4	6.9	7.8
Tyr	1.3	1.5	1.3	1.4	1.3	1.6	1.7	1.6	1.5	1.6	1.5	1.6
Val	7.0	7.0	7.5	7.6	7.1	7.3	7.3	7.6	7.1	7.4	7.5	7.6

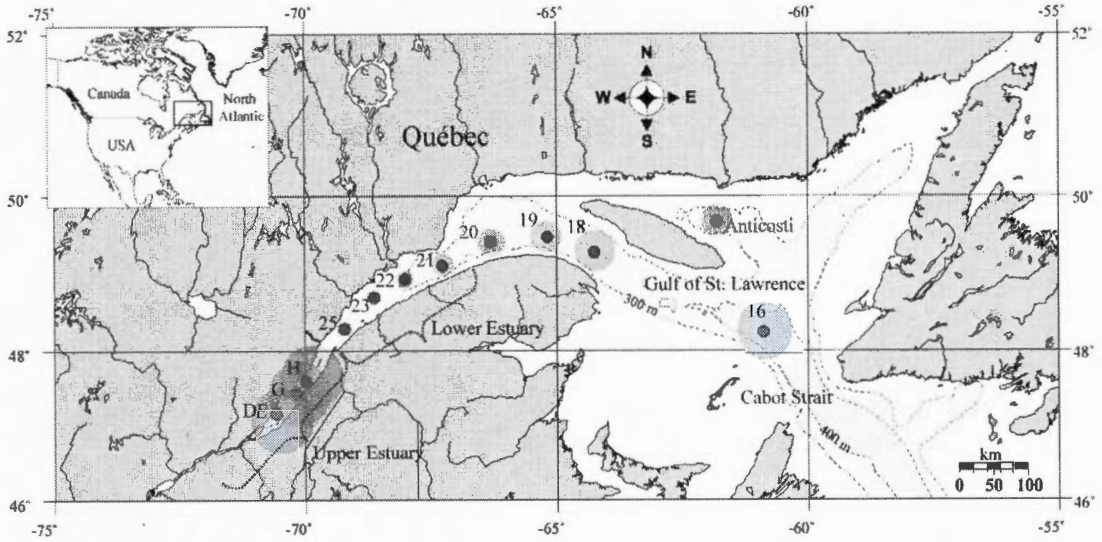

Fig. 1.1. Map showing the sampling locations in the St. Lawrence Estuary (Upper and Lower) and the Gulf of St. Lawrence. Bathymetric contours outline the Laurentian Channel along the 300 and 400 m isobaths. The size of shadowed circles around study sites denotes bottom water DO concentrations. For absolute values of bottom water DO see Table 2.1.

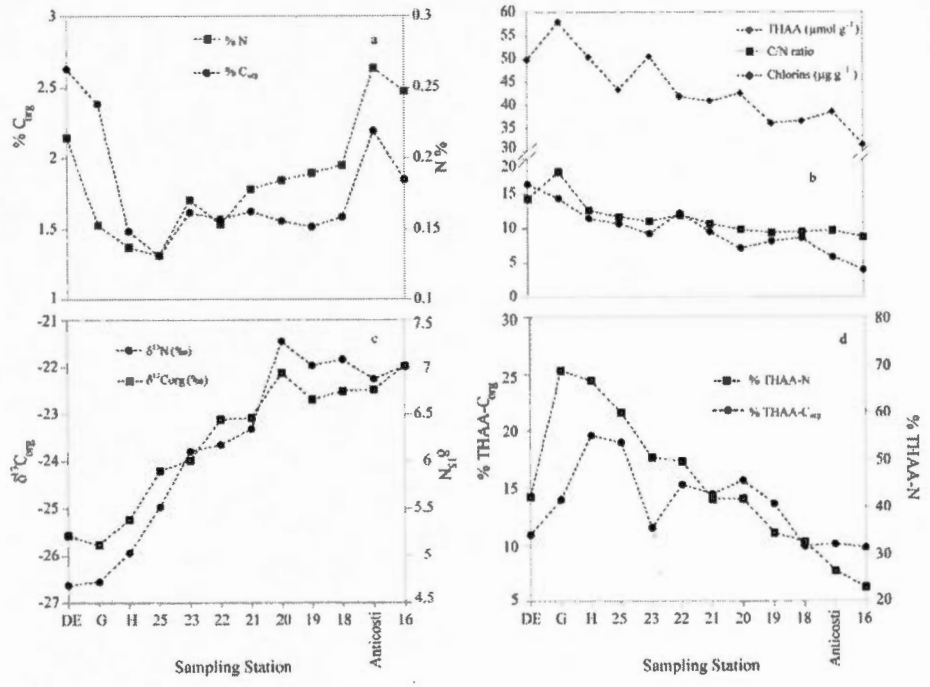

Fig. 1.2. (a) $\%C_{org}$ and %N, (b) C/N ratios, THAA (μmol g^{-1}), and chlorin concentrations (μg g^{-1}), (c) $\delta^{13}C_{org}$ and $\delta^{15}N$ (‰) and (d) contribution of THAA to C_{org} (%THAA-C_{org}) and N (%THAA-N) in surface sediments in the St. Lawrence Estuary and the Gulf of St. Lawrence.

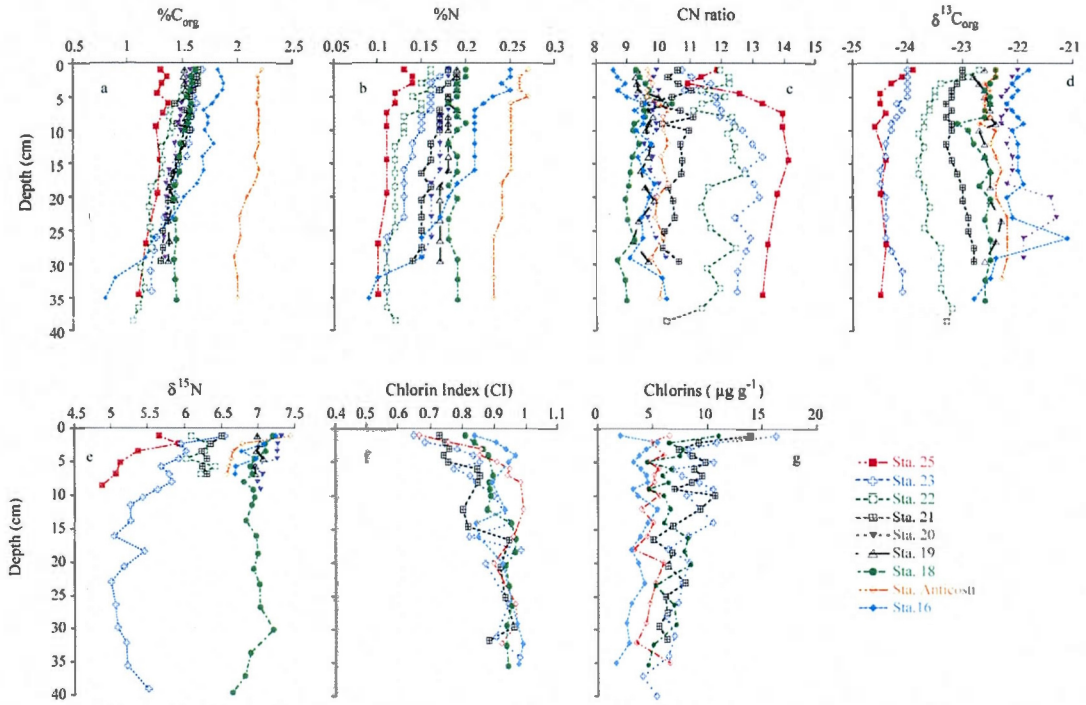

Fig. 1.3. Down-core sediment records in the Lower St. Lawrence Estuary and the Gulf of St. Lawrence: (a) $\%C_{org}$, (b) %N, (c) C/N ratios, (d) $\delta^{13}C_{org}$ (‰), (e) $\delta^{15}N$ (‰), (f) chlorin index (CI) (‰), and (g) chlorin concentrations ($\mu g\ g^{-1}$).

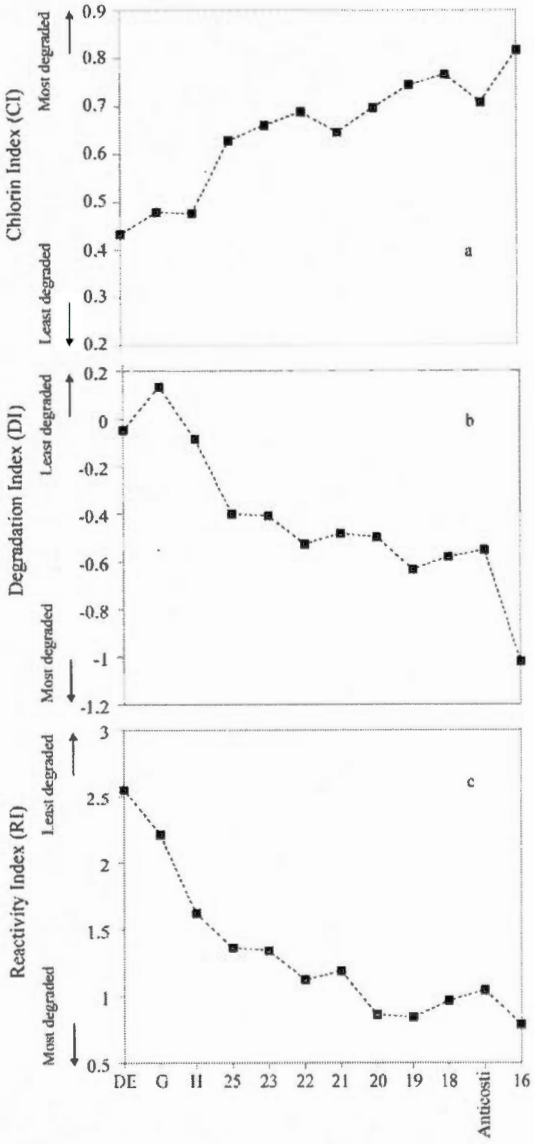

Fig. 1.4. OM reactivity indices in surface sediments in the St. Lawrence Estuary and the Gulf of St. Lawrence

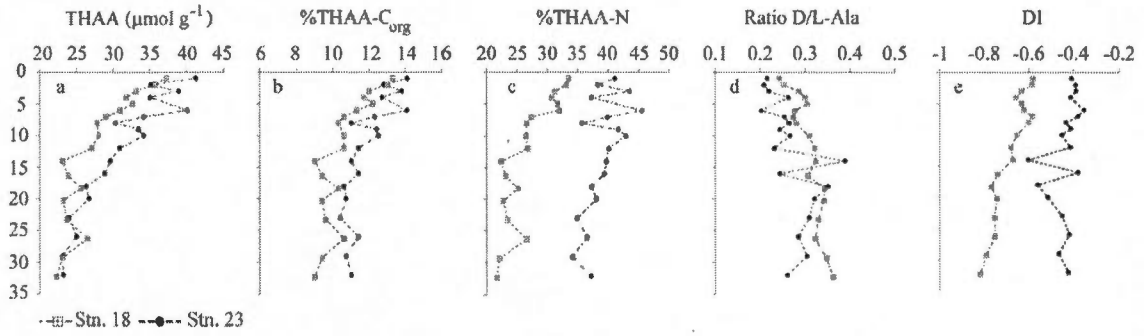

Fig. 1.5. Down-core profiles of (a) THAA (μmol g^{-1}), (b) %THAA-C_{org}, (c) %THAA-N, (d) D/L Ala, and (e) DI scores at Sta. 23 in the Lower Estuary and Sta. 18 in the Gulf.

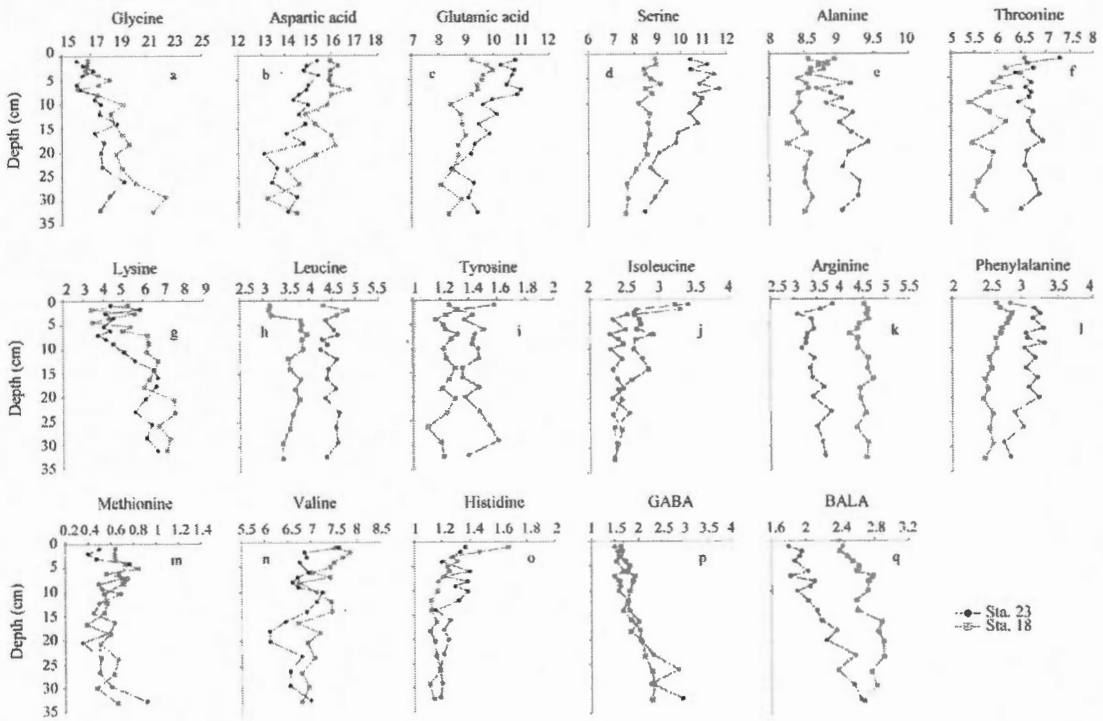

Fig. 6

Fig. 1.6. Amino acid distribution (mole% of THAA) in sediments down-core at Sta. 23 in the Lower Estuary and Sta. 18 in the Gulf.

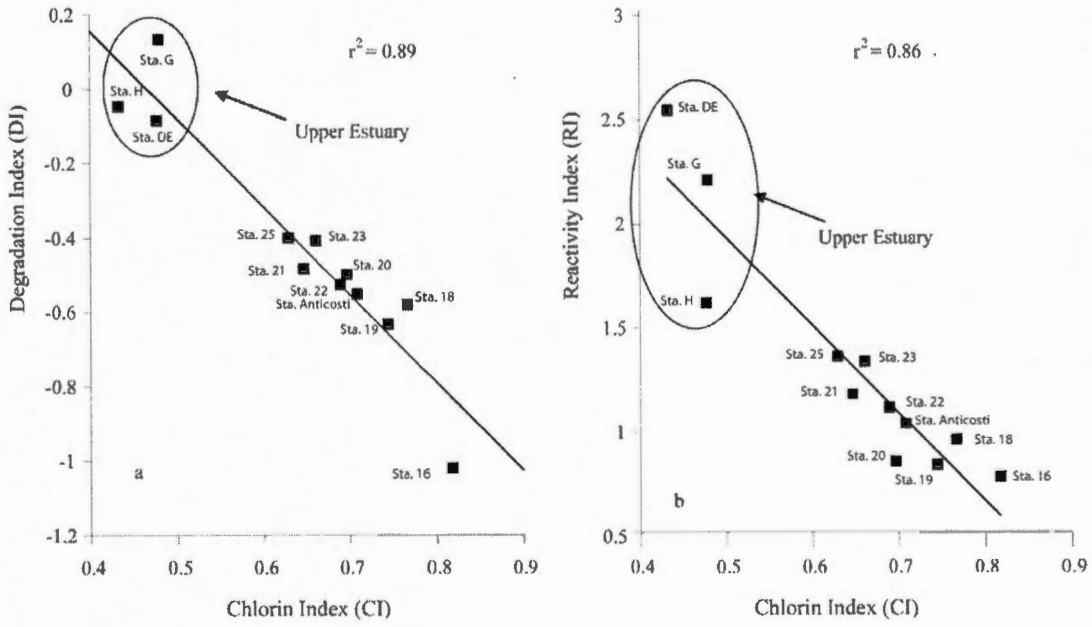

Fig. 1.7. Correlation between the chlorin index (CI) and (a) the degradation index (DI) and (b) the reactivity index (RI) in surface sediments along the St. Lawrence Estuary and the Gulf of St. Lawrence. The labels next to data points refer to the station names. The circle denotes the shallow locations in the Upper Estuary.

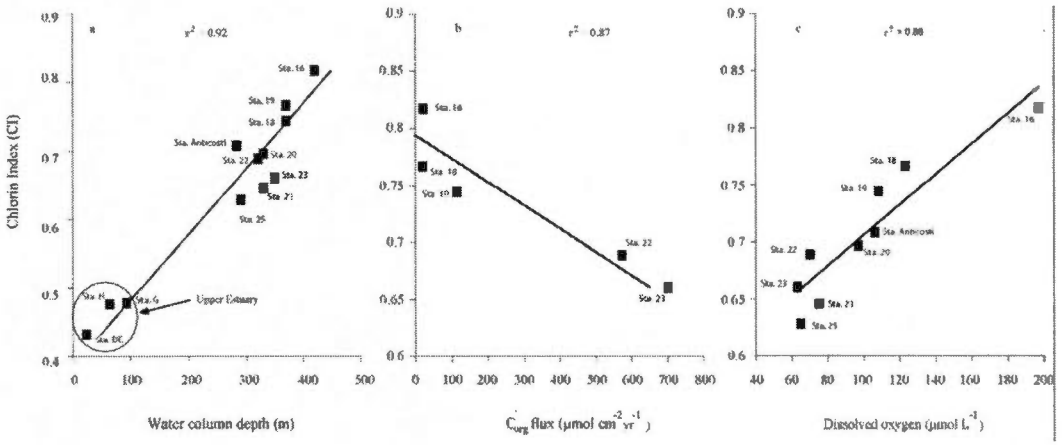

Fig. 1.8. Correlation between CI in surface sediments and (a) water column depth, (b) C_{org} vertical flux, and (c) dissolved oxygen in bottom water (DO; μmol L^{-1}). The labels next to data points refer to the station names. The circle denotes the shallow locations in the Upper Estuary.

CHAPTER II

Distribution of dissolved organic nitrogen in sediments of the Lower St. Lawrence Estuary: Links to benthic dissolved organic matter fluxes and selective organic matter degradation.

Distribution of dissolved organic nitrogen in sediments of the Lower St. Lawrence Estuary: Links to benthic dissolved organic matter fluxes and selective organic matter degradation

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ABSTRACT

The distribution of dissolved organic nitrogen (DON) and carbon (DOC) in sediment porewaters was determined at nine locations along in the St. Lawrence Estuary and in the Gulf of St. Lawrence. The study area is characterized by gradients in the sedimentary particulate organic matter (POM) reactivity, bottom water oxygen concentrations, as well as benthic respiration rates. Based on porewater profiles we estimated the benthic diffusive flux of DON and DOC. Our results show that DON fluxed out of the sediments at significant rates (110 to 430 μmol m^{-2} d^{-1}). DON fluxes were positively correlated with sedimentary POM reactivity and sediment oxygen exposure time (OET), suggesting direct links between POM quality, aerobic remineralization and the release of DON to the water column. DON fluxes were on the order of 30% to 64% of the total benthic inorganic fixed N loss due to denitrification, and often exceed the diffusive nitrate fluxes into the sediments. Hence they represented a large fraction of the total benthic N exchange, and this result is particularly important in light of the fact that DON fluxes are usually not accounted for in estuarine and coastal zone nutrient budgets. The ratio of the DON to nitrate flux increased from 60% in the Lower Estuary to 150% in the Gulf. In contrast to DON, DOC fluxes did not show any significant spatial variation along the Laurentian Channel (LC) between the estuary and the Gulf (2100 ± 100 μmol m^{-2} d^{-1}), suggesting that production and consumption of labile DOC components proceed at similar rates, irrespective of the overall benthic characteristics and the reactivity of POM. As a consequence, the molar C/N ratio of DOM in porewater and the overlying bottom water varied significantly along the transect, with lowest C/N fluxes in the Lower Estuary (5-6) and highest C/N fluxes (>10) in the Gulf. We observed large differences between the C/N of porewater DOM with respect to POM, and the degree of the C-versus-N element partitioning seems to be linked to POM reactivity and/or redox conditions in the sediment porewaters. Our

results thus highlight the variable effects selective OM degradation and preservation can have on bulk sedimentary C/N ratios, decoupling the primary source C/N signatures from those in sedimentary archives. Our study further underscores that the role of estuarine sediments as efficient sinks of bioavailable nitrogen is strongly influenced by the release of DON during early diagenetic reactions, and that DON fluxes from continental margin sediment represent an important internal source of N to the ocean.

2.1. INTRODUCTION

Coastal marine systems are areas under particularly high environmental stress due to increased inputs of nutrients and organic matter (OM) from different land sources, which strongly influence both water quality and the biota (Thibodeau et al., 2006; Gilbert et al., 2009). Marine and estuarine systems are biogeochemically responsive to fixed nitrogen (N), exhibiting rapid uptake of dissolved inorganic N (DIN) and dissolved organic N (DON) (Seitzinger et al., 2002; Seitzinger and Sanders, 1999). However, in estuaries, DIN accounts for only a portion of the total N inputs, while DON is often a much more important (30-80%) component of the dissolved N pool (Berman and Bronk, 2003; Lønborg et al., 2009). In marine sediments, during early sedimentary diagenesis, a large portion of particulate organic matter (POM) is recycled by bacterial hydrolysis to DON and dissolved organic carbon (DOC) directly in the sediment porewater. Subsequently, the DOC and DON are partitioned between several processes, namely the bacterially-mediated remineralization of DOM to CO_2 and nutrients, diffusion to the overlying bottom water, where it can ultimately be assimilated by phytoplankton and bacteria in the water column, and/or stabilization and burial in sediments due to abiotic adsorption on mineral and metal oxide surfaces and geo-polymerization (Alperin et al., 1999; Burdige, 2001). DOC and DON dynamics in sediments and their release to the water column are important in the context the productivity of these aquatic environments, and the exchange of climate relevant gases with the atmosphere.

DON has been seldom considered in N budgets of estuaries and the ocean, in part, because it has assumed to be mostly recalcitrant (e.g., Berman and Bronk, 2003; Knapp et al., 2005; Bourbonnais et al., 2009). However, some algae and bacteria have the capacity to use DON as a source of N for biosynthesis (Seitzinger et al., 2002; Seitzinger and Sanders, 1999). Therefore, N budgets for aquatic ecosystems based only on DIN sources and sinks may underestimate the pool of bioavailable N (Seitzinger et

al., 2002). Likewise, most benthic studies have mainly focused on net DIN fluxes when assessing total denitrification rates and the N elimination capacity of the estuarine bottom sediments, neglecting the reflux of remineralized N as DON. For example, Thibodeau et al. (2010) have shown that sediments of the Laurentian Channel (LC) function as an efficient sink for nitrate, especially in the Lower St. Lawrence Estuary section, but the role of sediments as a potential source of DON and its biogeochemical role of benthic DON fluxes have not been investigated yet.

Another aspect that has not been well explored to date is the connection between DON and DOC dynamics in estuarine sediments, which could potentially provide insight into element fractionation during the benthic remineralization of sedimentary POM. For example, the bioavailability of the DOC from sediments with respect to DON, and its role with regards to ecosystem respiration and the generation of basin-scale oxygen deficits (e.g., hypoxia) has been a matter of recent debate (Faganeli et al., 1991; Lahajnar et al., 2005; Lønborg et al., 2009). Therefore, the identification of key reaction and transport processes that affect porewater DOC and DON distributions is critical to assessing the significance of benthic metabolism during early sedimentary diagenesis and the associated alteration of the bulk sedimentary organic matter pool.

It is generally accepted that refractory POM in sediments has a higher C/N ratio and a lower potential for further remineralization (Meyers and Eadie, 1993). Hence, it is reasonable to assume that general sediment characteristics (i.e., amount, composition and reactivity/age of the POM) and redox conditions in estuarine and coastal-area sediments have an important influence not only on the overall rates of benthic (inorganic and organic) solute exchange (Alkhatib et al., in press; Thibodeau et al., 2010), but also on the element partitioning during remineralization. However, the effect of OM reactivity on the fluxes and fate of remineralized DOM remains uncertain.

Here we provide a detailed assessment of the distribution of DON and DOC in sediments and in near-bottom waters along a transect from the St. Lawrence River estuary to the Gulf of St. Lawrence, which is characterized by systematic changes in

sediment OM reactivity and bottom water oxygenation (Lehmann et al. 2009; Alkhatib et al. in press). We first tested the suitability of different pore-water extraction techniques for DOM. We then used the pore-water DON and DOC profiles to estimate benthic diffusive fluxes of DON and DOC in the estuary, and in the Gulf. The main goals of this study were 1) to quantify the spatial heterogeneity in DON and DOC fluxes in the estuary and the St. Lawrence proper 2) to establish the connection between these fluxes, 3) to place sediment DOC and DON fluxes in the context of the reactivity of the sedimentary POM, particularly in terms of potential elemental discrimination during hydrolysis and bacterial degradation, and 4) to assess the potential contribution of benthic DON fluxes to the net dissolved N exchange at the SWI along the estuary.

2.2. Materials and Methods

2.2.1. Study Area

The bathymetry of the St. Lawrence River Estuary is dominated by the Laurentian Channel (LC), a 250-500 m deep submarine valley that extends landward 1,240 km from the edge of the Atlantic continental shelf (Fig. 2.1). The Gulf of St. Lawrence, a large (250 000 km^2), roughly triangular inland sea, is connected to the Atlantic by the Strait of Belle Isle and Cabot Strait at the northeast and southeast corners, respectively. Water mass movement in the St. Lawrence system is characterized by an estuarine circulation: less saline water flows seaward in the surface mixed layer, overlying more saline water mass that formed in the northwest Atlantic and flows landward. The water in the deeper layer is isolated from the atmosphere by a permanent pycnocline at 100–150-m depth and the water residence time is approximately 3-4 yrs. (Gilbert et al., 2005). As the deep-water mass flows landward, it gradually loses oxygen through aerobic microbial respiration. Sedimentary respiration was plausibly pointed out as the most important sink of oxygen in the St. Lawrence estuarine system (Lehmann et al., 2009). In previous work, we have shown that POM source, oxygen concentration,

benthic nutrient and DO fluxes, as well as POM reactivity vary along the LC (Alkhatib et al. in press; Thibodeau et al., 2010). Chlorin- and amino acid-based degradation indices show that sedimentary OM in the Lower Estuary is more reactive than in the Gulf (see Table 2.1). C-isotope measurements and C/N ratios indicate that sedimentary POM along the Lower Estuary and the Gulf is mainly of marine origin. The terrestrial content of the sedimentary OM pool decreases with distance from the head of the Lower Estuary, as does the sediment reactivity (Alkhatib et al., in press; Table 2.1).

2.2.2. Sampling

Sediment multicores and box-cores were recovered at multiple stations along the LC (Sta. 16-25) and at one station at the head of the Anticosti Channel in the Gulf during two cruises in June and August 2006 (Fig. 2.1). Stations were chosen to represent the spatial heterogeneity in sedimentary OM characteristics (see above), and hydro-chemical parameters (e.g., DO) in the overlying water. Sediment porewaters were extracted immediately after core recovery using the whole core squeezing (WCS) method (Jahnke, 1988; Bender et al., 1987). During porewater extraction, the multicores were kept at in situ temperatures by wrapping the core tubes with ice-bags. Extracting porewater from the upper 1.5 to 2 cm generally took about 20-30 minutes. The WCS is a porewater sampling technique that yields millimeter depth resolution near the sediment-water interface. Water samples were collected with syringes equipped with Teflon coated pistons. For collecting porewater from greater depths than allowed by WCS, but also as a quality control of WCS data and for sampling method comparison, porewater was extracted using Rhizon membrane samplers (Seeberg-Elverfeldt et al., 2005). The sediments processed with the Rhizon membrane samplers were obtained from box cores using push-core liners with holes (0.5 mm diameter) at 1-cm intervals. DOM blank contribution of the Rhizon sampler was negligible. Using the Rhizon sampling technique, porewater can be sampled with minimum disturbance to the sediment

structure, while the WCS method applies high pressures to the sediments, which can lead to rupturing of benthic organisms and the lysis of bacterial or algal cells.

Water samples collected for nutrient and total dissolved N (TDN, the sum of NO_3^-, NO_2^-, NH_4^+ and DON) analyses were filtered through 0.45µm syringe filters. For DOC water samples, PTFE syringe filters were used. Nutrient samples were then stored frozen in pre-washed plastic vials until analysis in the lab. Porewater samples for DOC analyses were kept acidified (pH=2) in glass vials (combusted, 500°C, 3 hours) and stored in the dark prior to analysis.

2.2.3. Laboratory analyses

The concentration of TDN for water samples was measured using the perusulfate oxidation method following Solorzano and Sharp (1980). Persulfate was re-crystallized three times prior to oxidation to reduce N blanks (Bronk et al., 2000). Briefly, for [TDN] determination, 0.5 ml of alkaline perusulfate oxidizing reagent was added to 3 ml of sample in a borosilicate glass test tubes. Samples and three test tubes with POR only (blank) were then autoclaved for 45 min to quantitatively convert TDN to NO_3^-. TDN-derived nitrate was then quantified using an Antek 7020 Nitric Oxide Analyzer. Here, nitrate is reduced to nitric oxide (NO) in an acidic heated (90°C) vanadium (III) solution (Braman and Hendrix, 1989), and the NO is measured by chemiluminescence detection. Reproducibility for replicate analyses was better than 2% or ±0.2µmol L^1. The total procedural blank never exceeded 3µmol L^{-1} and was on average 2µmol L^{-1}. TDN concentrations were corrected for blank contribution. Sample nitrate+nitrite were also measured using the vanadium method. DON was determined by subtracting total dissolved inorganic nitrogen (DIN; nitrate, nitrite, and ammonia) from TDN, where NH_4^+ concentrations were measured using standard colorimetric autoanalysis.

DOC concentrations were measured by high-temperature catalytic oxidation (HTCO) using a Shimadzu TOC-5000 analyzer. Briefly, filtered samples were acidified and purged with oxygen to remove inorganic carbon. A sample aliquot of 100 µL was then

injected into the combustion column packed with platinum-coated alumina beads held at 680°C. Organic carbon compounds were combusted and converted to CO_2, which is then detected by a non-dispersive infrared detector.

2.2.4. DOC and DON benthic fluxes

DOM fluxes (F) across the SWI were estimated based on pore-water concentration gradients (WCS or Rhizon) using Fick's first law of diffusion (Berner, 1980; Boudreau, 1996):

$$F = D_{sed} \Delta C/\Delta z$$

where D_{sed} is the free diffusion coefficient (in $cm^2\ s^{-1}$) for DOM, D°, corrected for sediment porosity (Boudreau, 1996), and $\Delta C/\Delta z$ is the solute (i.e., DON or DOC) concentration gradient with depth z in the sediment. $\Delta C/\Delta z$ was calculated from the first derivative of best-fit curves of the WCS-derived DOC or DON concentration profiles just below the sediment-water interface. To calculate F from Rhizon membrane profiles, $\Delta C/\Delta z$ was calculated as the concentration difference between the overlying water and the first sample below the SWI, in all cases at depths less than 2 cm. A similar approach was used in previous work (Martin and McCorkle, 1993; Lahajnar et al., 2005; Hall et al., 2007). Bottom water temperatures ranged from 3 to 5°C, and the porosity of the surface sediments generally decreased from 95% to 75% within the top 5 cm. To determine D° for DOC and DON in seawater, we used the empirical relationship between molecular diffusion and molecular weight (MW; at 25°C in distilled water) reported by Burdige et al.'s (1992), where we assumed a fixed average MW of 2500 daltons for both DON and DOC. This MW represents an intermediate value of previous estimates for porewater DOM. Alperin et al. (1999) proposed an average MW of 5000 daltons, while other studies show that the vast majority (> 80%) of the DOC and DON in sediment porewaters has a molecular weight of less than 3000 daltons. Uncertainty in

the molecular weight has relatively little impact on the calculated fluxes because of the inverse cube-root relationship between D^o and MW (Burdige et al., 1992). *In situ* salinity and temperature were taken into consideration using the Stokes-Einstein formula (Boudreau, 1996) and the corrected value for D^o was 1.56×10^{-6} cm^2 s^{-1}, translating into a D_{sed} value of 1.5×10^{-6} cm^2 s^{-1} after correction for sediment porosity.

2.3. RESULTS

2.3.1. DON and DOC in sediment porewaters

Sediment profiles of DON and DOC are depicted in Fig. 2.2 and Fig. 2.3. Highest porewater [DON] was always observed at the bottom of the sediment cores (20-30cm; 40-200 μM DON), with a decreasing trend towards the SWI. In most cores, DON concentrations stabilize with depth, but at some locations it decreased again in the deepest portion of the sampled depth interval (we have not analyzed Rhizon samples for DOC for these depths). At all sites, average porewater [DON] in the uppermost cm of the sediment column were at least two-fold higher than in the overlying water column, suggesting a substantial flux of DON out of the sediments (see below). The agreement between WCS and Rhizon-sampler-derived DON profiles was good in the upper 1-3 cm, but (at least at several stations) poor at sediment depths below. In these deeper samples, WCS-derived DON concentrations were often several fold higher (Fig. 2.2), suggesting artefacts that probably result from the harsh sediment treatment during the core squeezing (see discussion) which may lead to the release of intracellular DON during the WCS. In general, average porewater [DON] of the uppermost centimeter in the sediment column decreased eastward along the LC, with the highest subsurface [DON] in sediment porewaters at the Lower Estuary stations (~21 μM), and lowest concentrations in the Gulf (8-18 μM). [DOC] in the uppermost centimeter of the sediment column did not have a clear spatial trend along the Lower Estuary (Fig. 2.4a). In general, it was higher in the Lower Estuary sediments (108-141 μM) than in the Gulf

sediments (97-127 μM), but highest [DOC] occurred in porewater at Sta. Anticosti Channel (~274 μM). The porewater molar ratio of [DOC] to [DON] (C/N_{DOM}) in the uppermost centimeter in the sediment column varied significantly between 5.2 at Sta. 25 in the Lower Estuary and 11 at Sta. 16. C/N_{DOM} was 13.3 at the Anticosti Channel station (Fig. 2.5).

2.3.2. Benthic DON and DOC fluxes

Benthic diffusive DON fluxes at the SWI based on Rhizon sample measurements were in general agreement with those determined using the WCS concentration gradients. DON Rhizon sample-based fluxes were slightly lower than the WCS ones only at Sta.18 and Sta. 20 in the Gulf (Table 2.2). Highest DON fluxes (average of both WCS- and Rhizon-based calculations) were observed at the head of the LC (~440 μmol m^{-2} d^{-1} at Sta. 25) and fluxes decreased downstream, with the lowest flux (~110 μmol m^{-2} d^{-1}) at Sta. 16. Diffusive DOC fluxes (0 to ~2cm depth profiles; WCS-based only) averaged ~2100 μmol m^{-2} d^{-1} and did not display any consistent trend along the Lower Estuary eastward up to Sta. 19 in the Gulf, yet significantly lower DOC fluxes (~1400 and ~1300 μmol m^{-2} d^{-1}) were observed at Sta.18 and at Sta. 16, respectively (see Table 2.2). The highest DOC flux (~3850 μmol m^{-2} d^{-1}) was observed at Sta. Anticosti (Table 2.2), consistent with the high porewater DOC and DON concentrations. The C/N ratio of the DOM efflux from the sediments was lowest in the Lower Estuary (average C/N of ~ 5 at Sta. 25), intermediate between Sta. 25and Sta. 20 (average C/N of ~ 7), and it was highest at Sta. 16 and Anticosti in the Gulf (average C/N of ~ 13) (Fig. 2.5).

2.3.3. DON and DOC in the bottom water

The DON concentrations in the bottom waters overlying the sediments were highest at the head of the LC at Sta. 25 and 23 (~10 μM) and were comparatively low in the Gulf of St Lawrence, with lowest concentrations at Sta. 20 (6.3 μM) (Fig. 2.4b). This pattern agrees well with the spatial trends in DON fluxes that we described above. In contrast, DOC concentrations were higher in the Gulf (between 60 and 70 μM) than in the Lower Estuary (between 40 and 60 μM. Highest DOC concentrations were observed at the Anticosti station (82 μM). The observed patterns in bottom water DOC and DON concentrations resulted in a clear increasing trend in the bottom water DOM C/N ratios, from 6 in the Lower Estuary to ~9 in the Gulf at Sta. 16 (Fig. 2.5).

2.4. DISCUSSION

2.4.1. Suitability of whole core squeezing for DOM extraction

Previous studies on the applicability of WCS in determining DOC in sediment porewaters have shown that this porewater extraction technique tend to overestimate [DOC] in deeper layers of the sediments, most likely due to the lysis of bacterial cells (Bolliger et al., 1992; Martin and McCorkle, 1993). This bias is likely to increase at greater depths, where the pressure is greatest (Martin and McCorcle, 1993). Indeed, at several stations and at depths greater than 2 - 3 cm, the average [DON] from WCS was several-fold higher than for that from Rhizon samples (Fig. 2.2), suggesting that intracellular metabolites from only partially lysed or living sediment microorganisms may have been released into the aqueous extract under the influence of high pressures (Bolliger et al., 1992; Martin and McCorkle, 1993). Thus, the observed differences were most probably due to the liberation of benthic N immobilized in bacteria and other benthic organisms (Benner, 2002; Tremblay and Benner, 2006) during the WCS processing. In the first two cm of the sediment column, however, the agreement between

WCS- and Rhizon sampler derived DON concentrations was good, demonstrating that possible pressure effects are negligible in the upper sediment layer below the SWI. Hence, at least in the St. Lawrence sediments, WCS can be used to sample porewater for benthic flux determination, as the gradient down to ~2 cm below SWI does not seem to be affected by WCS artefacts.

A large fraction of the total sedimentary organic matter is associated with mineral particles. Metal oxides that accumulate near the SWI at the oxic-anoxic interface can possibly act as DOM filters (Skoog et al., 1996; Arnarson and Keil 2000; Skoog and Arias-Esquivel 2009). Hence redox conditions, i.e. the distribution of redox-sensitive metals within the sediments, for example, may exert a strong control on the concentration of DOM within sediment porewaters (Skoog et al., 1996). Mineral matrix - DOM interactions can potentially have a biasing effect on the porewater DOM measurements. As porewater is slowly forced through the interstices of the sediment during core squeezing, porewaters from deeper sediment layers come into contact with surface-reactive oxic sediments that were in equilibrium with waters with different DOM concentrations. DOM may equilibrate with the ambient mineral phase through adsorption/desorption reactions that occur on a sub-minute time scale (Alperin et al., 1999; Arnarson and Keil, 2000), therefore, it is possible that DOC and DON concentration of WCS samples and the DOM composition is modified by solid-solution partitioning. It is difficult to predict where in a porewater DOM profile such an effect would be most expressed. Yet the agreement between WCS and Rhizon sampler derived DON concentrations suggest that DOM samples from near the SWI most probably were not affected by the adsorption/desorption reactions, probably because they are less likely to pass by different redox interfaces. We thus recommend the combination of the two porewater extraction methods. Porewater samples collected by WCS provides better resolution near the SWI, while Rhizon-membrane samples yield reliable data (although at lower depth resolution) at greater sediment depths.

DOC was not determined for the Rhizon membrane samples, but we can assume that the physico-chemical aspects are similar for DOC and DON (Burdige et al., 1992; Burdige and Zheng, 1998; Burdige and Gardner 1998), so that the insights gained from the DON data comparison also applies to porewater DOC.

2.4.2. DOC and DON Fluxes

Porewater [DON] and [DOC] were systematically elevated over those in the bottom water, across all sites (Fig. 2.4). The higher DOM concentration in porewaters implies that there was a net production of DOM inside the sediments, and a net flux across the SWI towards the overlying waters. Numerous studies from a wide variety of marine environments have shown that DOC and DON concentrations in sediment porewater may be several fold higher than in the overlying water (e.g., Martin and McCorkle, 1993; Blackburn et al., 1996; Burdige and Zheng, 1998; Alperin et al., 1999; Holcombe et al., 2001), and in Table 2.3 we have summarized published sedimentary fluxes of DON and DOC from different marine systems. Our calculated sediment DON fluxes were much higher than reported fluxes from deep-sea sediments (Brunnegård et al., 2004), within the lower limits of fluxes calculated for the Chesapeake Estuary (Burdige and Zheng, 1998) and in the Svalbard, Norway (Blackburn et al., 1996), much lower than DON fluxes calculated from the from a shallow coastal area (Knebel Vig) in Denmark (Lomstein et al., 1998), but generally within the range reported for other estuarine and continental shelves sites (reviewed by Bronk and Steinberg, 2008). DOC fluxes (1300 to 3900 μmol m^{-2} d^{-1}) are within the range of values reported previously by Colombo et al. (1996) at two locations in the LC (~1700 μmol m^{-2} d^{-1}), and similar to estimates reported by Burdige and Homstead (1994) from Chesapeake Bay sediments (1400 to 2900 μmol m^{-2} d^{-1}), but significantly higher, for example, than fluxes determined by Holcombe et al. (2001) for Mexican margin sediments (400 μmol m^{-2} d^{-1}).

We found that the highest DON fluxes occurred in sediments of the Lower Estuary, while lowest fluxes were observed in the Gulf sediments (Table 2.2). We attribute these variations in DON fluxes to patterns in POM reactivity across the same sites, as the average flux of DON from both WCS and Rhizon membrane samples correlated significantly with OM reactivity (CI, $r^2 = 0.83$, n = 9; DI, $r^2 = 0.74$, n = 9; Fig. 2.6a,b). In addition, there was an inverse exponential relationship between DON fluxes with [DO] in the bottom water ($r^2 = 0.83$, n = 9; Fig. 2.6c). Moreover, DON fluxes correlated linearly with the oxygen exposure time (OET), which defines the average time that sediment OM is exposed to "oxic" conditions during burial in the sediments (Hartnett et al., 1998; Hedges et al., 1999; $r^2 = 0.9$, n = 9; Fig. 2.6d). It would thus appear that higher DON fluxes were related to increased supply of more reactive POM, higher overall sediment POM reactivity, and probably to the lower [DO] in the overlying bottom water in the Lower Estuary than stations in the Gulf (See table 2.1; Alkhatib et al., in press).

A number of studies have shown a positive correlation between POM delivery to the sediment and [DON] in porewaters (e.g., Hansen and Blackburn, 1991; Sloth et al., 1995). The rate-determining step for both aerobic and anaerobic microbial degradation of POM and production of DOM is the hydrolysis by extracellular enzymes (Wilczek et al., 2005). Higher POM reactivity induces the production and activity of bacterial hydrolytic enzymes (Boetius and Lochte, 1994; Wilczek et al., 2005), and hydrolysis proceeds under both oxic and anoxic conditions, although not necessarily always at the same rates (Hansen and Blackburn 1991; Kristensen and Holmer 2001). In this regard, Thibodeau et al. (2010) recently reported that OM remineralization rates along the LC are highest in the Lower Estuary and decrease eastwards. Therefore, it would appear that a high POM flux of relatively reactive POM is particularly conducive to high rates of OM hydrolysis in the Lower Estuary, resulting in both high porewater [DON] and high DON fluxes.

The inverse relationship between DON fluxes and $[O_2]$ in the bottom waters, as well as OET, suggests that the redox conditions, in addition to the sediment POM reactivity, may play a role for DON production in the sediments and for the subsequent DON flux. In a previous paper (Alkhatib et al., in press), we argued that the sedimentary diagenesis and the degradation rate and extent are largely controlled by the reactivity of available organic substrate(s), as opposed to the relative supply of different electron acceptors. In vitro laboratory experiments have shown much slower and less efficient anoxic degradation of refractory POM and carbon rich substrates such as lipids (Kristensen and Holmer, 2001; Emerson and Hedges, 2003). In contrast, rates of OM remineralization for relatively reactive OM (and hence their potential to produce DOM) are largely controlled by the quality and the quantity of available organic matter and are also largely independent of sediment redox conditions (Burdige, 2007 and references therin). The correlations between $[O_2]$ and OET with DON fluxes suggest that oxygen-sensitive POM (i.e., POM requiring O_2, in some way, for degradation) is selectively concentrated with increasing POM diagenetic maturity eastwards along the LC. Therefore, our results suggest that redox conditions are important in controlling [DON] in the porewater and as a result DON fluxes increases as sediment POM reactivity declines.While the reduced bottom water $[O_2]$ and, as a result the surface sediment OET, is the result of a more reactive sediment regime in the Lower Estuary, which is at the same time also directly responsible for enhanced DON fluxes, the lower DON fluxes in the Gulf are most probably a result of low POM reactivity.

The minor variations in DOC fluxes that we observed under such a wide range of environmental conditions (vertical OM flux, [DO], sediment POM reactivity) suggest that production and consumption of labile DOC components proceed at roughly similar rates irrespective of the overall benthic activity (Otto and Balzer, 1998). The bottom water current moves westward along the LC (see section `study site`), and therefore we would expect the continuous increase in DOM (i.e. in both DOC and DON) due to

cumulative remineralization during transit. Fig. 2.4b shows that, as the bottom water currents move westwards along the LC, [DOC] in the bottom water decreased from 68 μmol L^{-1} at Sta. 20 to 40 μmol L^{-1} at Sta. 25, while DON increased from ~6 μmol L^{-1} to ~10 μmol L^{-1} at the head of the LC (Fig. 2.5). While the concentration gradient between the sediment and the bottom water DOC pools predicts a flux of DOC out of the sediments at all stations (Table 2.2), the eastward decline in bottom water DOC indicates that the benthic DOC fluxes were offset by water column respiration of the DOC.

To some extent, adsorption processes right at the SWI (not necessarily detected with our sample resolution) can modulate benthic DOC exchange. More precisely, adsorption to mineral and metal oxides/hydroxides surfaces can potentially remove significant amounts of DOC from the pore water before it actually fluxes into the water column (Boto et al., 1989; Skoog et al., 1996; Arnarson and Keil, 2000; Skoog and Arias-Esquivel, 2009). However, if adsorption were a significant process, it should be more effective in the more oxygenated locations in Gulf, with deeper oxygen penetration depths (Skoog et al., 1996; Arnarson and Keil, 2000; Skoog and Arias-Esquivel, 2009), but lower DOC fluxes in the East were not observed, suggesting that DOC levels are regulated by remineralization and the subsequent heterotrophic pathways requiring DOC as a C and energy source respiration. In addition, there is a priori no reason why DOC would adsorb to particles at a greater extent than DON.

2.4.4. C versus N partitioning during DOM production and remineralization

The discrepancy between the porewater DOM C/N ratios and the C/N of sedimentary POM along the estuarine transect suggests preferential hydrolysis of N-rich DOM during initial break-down of sediment POM (except at Sta. 16 and Anticosti Channel station; see Fig. 2.5). It has been shown previously that initial hydrolysis of freshly deposited POM in surface sediments produces DOM with C/N ratios that are lower than those of the deposited POM itself (Blackburn et al., 1996; Burdige and

Zheng, 1998; Weston et al., 2006). We observed that the difference between C/N ratios of sediment POM and porewater DOM ($\Delta C/N = C/N_{POM}-C/N_{DOM}$) decreases along the LC from ~ 6 at the head of the LC to ~1 at Sta. 18, at Sta. 16, and in the Anticosti Channel sediments the difference was in fact negative (~-3) (See Fig. 2.5). There is a significant correlation between sediment POM reactivity (CI and DI for definition see caption of Fig. 2.7) and the $\Delta C/N$, (r^2= 0.85, n=8; we excluded Channel Anticosti from the correlation as its point on the graph was located far from the trend line; Fig. 2.7a). The $\Delta C/N$ also correlates with the oxygen exposure time (OET) (r^2= 0.88, n=8; Fig. 2.7b). These patterns most likely are related to the nature of hydrolysed sediment POM (i.e. hydrolysable (reactive) vs. hydrolysis-resistant or recalcitrant OM) and to the mode of hydrolysis, i.e. the initial depolymerisation step (or oxidative cleavage) of sedimentary POM to form dissolved intermediates, which is likely to be a function of the redox conditions (Emerson and Hedges, 2003; Burdige, 2007). While the hydrolysis of more labile or fresh POM does not require oxygen and takes place at similar rates under both oxic and anoxic redox conditions (Hansen and Blackburn, 1991; Emerson and Hedges, 1988, 2003), effective degradation of hydrolysis-resistant refractory POM requires molecular oxygen (Emerson and Hedges, 1988; Fenchel et al., 1998). Most of the strong oxidants such as peroxide (H_2O_2) and other reactive oxygen-containing enzymes are nonspecific, that is, they do not target specific compounds or types of bonds during hydrolysis of more refractory POM (Fenchel et al., 1998; Emerson and Hedges, 1988), minimizing element partitioning during the initial breakdown to DOM. Given that the sedimentary POM reactivity is highest in the Lower Estuary (Sta. 25, 23, 22, and 21) and that POM becomes more refractory eastwards along the LC, we can assume that the fraction of hydrolysable POM decreases while that of hydrolysis-resistant POM increase as POM reactivity decreases eastwards. Low C/N DOM in the Lower Estuary, and a high $\Delta C/N$, suggest that mostly labile, hydrolysable POM gets depolymerized under the low-oxygen conditions in the Lower Estuary, inducing a relatively high C-N elemental partitioning. As POM reactivity decreases eastwards

along the LC, and with increased O_2 concentrations in near-bottom waters, the hydrolytic attack of POM under increasingly aerobic conditions becomes increasingly non-specific, and the C/N of hydrolysed DOM gradually approaches that of the POM. Consistent with the observed increase in DOM C/N ratios (both in the porewater and in the bottom water) eastward towards the Gulf, we see a complementary decline in the C/N ratio of the sedimentary POM pool (Fig. 2.5). It remains uncertain, to what extent immobilized DOM in bacterial biomass contributes to the C/N signatures of the POM (Alkhatib et al. in press.), yet, it is clear that early diagenetic degradation of the bulk OM, and the partitioning between the particulate and dissolved OM pools is associated with an elemental fractionation of C versus N, which appears to be sensitive to the environmental conditions (i.e., bottom water O_2) and sediment characteristics (i.e., OM reactivity).

The comparatively low difference in C/N between porewater DOM, the DOM flux, and bottom water DOM in the St. Lawrence Estuary indicates that post-hydrolytic DOM remineralization during bacterial degradation proceeds with little, if any, C versus N elemental fractionation, and suggests that porewater DOM diffusing out of the sediments is the dominant source of DOM to the overlying bottom waters. In this context, however, the large discrepancy between porewater-flux-bottom water DOM C/N ratios at some stations (e.g., Sta. 16 and Anticosti) is difficult to explain. At these easternmost Gulf stations, the lower C/N of DOM in the overlying bottom water with respect to both porewater DOM and DOM fluxes suggests DOM sources other than benthic. Our data indicate bottom water DOM with a very low C/N compared to the diffusive DOM flux (see Fig. 2.5). Burdige (2001) proposed that low C/N DOM, such as urea, can be added to bottom water directly through macrofaunal excretion at the surface sediments or as a result of bioirrigation, helping to bypass the porewater DOM pool. However, the reasons as to why such a mechanism would be more important in the Gulf than in the St. Lawrence Estuary remain unclear.

2.4.5. Significance of benthic DON fluxes for regional and global budgets

In the Lower Estuary and the Gulf, DON was the most important dissolved nitrogen component in pore water, comprising up to 85% of the TDN at the sedimentary redox transition zone. In this regard, Thibodeau et al. (2010) reported that nitrate and ammonium fluxes out of the sediments are zero or very low. To place the DON fluxes in the context of the total benthic nitrogen budget of the St. Lawrence system, we compared the N loss rates by sediment denitrification (Thibodeau et al., 2010) to the benthic DON fluxes estimated in this study. Observed DON fluxes would account for 30% to 64% of the total benthic denitrification (including nitrification-denitrification coupling), as estimated by Thibodeau et al. (2010) (see Table 2.1). Along the study transect, the ratio of DON efflux from the sediments to the nitrate flux into the sediments (diffusive+advective) increased from 0.6 and 0.96 in the Lower Estuary to 1.5 in the Gulf. The extrapolation of the calculated DON fluxes (on average 310 μmol m^{-2} d^{-1}) from the LC sediments to the sediment surface area of the Laurentian Channel between Sta. 23 and 16 (~ 35,700 km^2) suggest that between 0.04 and 0.06 Tg N yr^{-1} of DON are lost annually to the water column of the St. Lawrence Estuary and Gulf.

The DON fluxes that we present here are within the range of DON fluxes reported from various coastal marine environments (Review by Bronk and Steinberg, 2008), and we assume that the DON fluxes from the St. Lawrence Estuary and Gulf sediments can be considered representative for other continental shelf regions. Given a global shelf area of ~30 × 10^6 km^2, (Milliman, 1993) and an average DON flux of 1.58 × 10^6 g N km^{-2} yr^{-1} (equivalent to 310 μmol m^{-2} d^{-1}), we calculate that the global benthic DON flux from shelf sediments may result in a flux of approximately 48 Tg N yr^{-1}. This rate is ~1.5 times higher than the estimated riverine DON input to the ocean (~35 Tg N yr^{-1}), and about half to one third of the total oceanic biological N_2 fixation

(100-150 Tg N yr^{-1}; Codispoti et al., 2001). In agreement with previous work (e.g. Middelburg and Nieuwenhuize, 2000; Berman and Bronk, 2003), our data confirm that benthic DON fluxes represent a key source of reduced N to the marine environment, representing an important component of the internal marine N cycle, at the ecosystem scale (e.g., the St. Lawrence system) and globally.

The fate of the DON from the sediments and its bioavailability for microorganism in the oceanic water column is not fully understood, however it is reasonable to assume the benthic DON plays an active part in the water column N cycle. Several studies have reported on the dynamic nature of DON in the ocean water column and the susceptibility of DON to bacterial enzymatic remineralization (Bastviken et al., 2004). Dominant fractions of the DON that originates from diagenetic processes within marine sediments are likely to undergo rapid oxidation and to add to the oceanic DIN pool (e.g. Burdige, 2007). DON is a significant source of N that supports both auto- and heterotrophic production in marine environments, and there is evidence that DON concentrations fluctuate significantly on seasonal time scales in near shore waters and on annual time scale in open ocean (Engeland et al., 2010). In addition, the C/N ratio of DOM flux from the St. Lawrence sediments is very low (4-10), which is indicative of a relatively high DOM reactivity.

2.5. SUMMARY AND CONCLUDING REMARKS

In this study, we measured the DON and DOC concentration of sediment porewater along the Laurentian Channel, and determined benthic DOC and DON fluxes in the Lower Estuary and the Gulf of St. Lawrence. We observed large discrepancies with regards to DON concentrations between the two different porewater extraction methods applied (WCS versus Rhizon samplers), at sediment depths below 2 cm. Bacteria are able to concentrate bioavailable forms of DOM (and DIN) inside their cells and transfer it to deeper sediments where most of the DOM is bio-refractory (e.g.,

Prokopenko et al., 2011). We speculate that the observed differences were most probably due to the release of benthic N immobilized in bacteria and other benthic organisms (Benner, 2002; Tremblay and Benner, 2006) during the sediment squeezing process. The WCS porewater extraction method, while compromising the assessment of free DOM in the deeper sediment porewaters, may thus provide insight into this "hidden" N pool and hence into benthic C and N related to the intracellular storage, biological transport, and release of DOM. This aspect should be further exploited in future investigations.

The modest spatial variations in DOC fluxes despite pronounced changes in environmental conditions (vertical OM flux, [DO], sediment POM reactivity) suggest that production and consumption of DOC by microbial hydrolysis and degradation result in a relatively constant net DOC flux out of the sediments, irrespective of the overall OM reactivity. Our results suggest that both the sediment POM reactivity and the oxygen exposure time of organic particles after sedimentation influence the C/N ratio of hydrolysed DOM. The degree of C versus N element partitioning between the particulate and dissolved OM pools was greatest when fresh, reactive OM is hydrolyzed under less oxygenated conditions in the Lower St. Lawrence Estuary. Hydrolysis of less reactive OM under aerobic conditions in the Gulf of St. Lawrence appears to be less specific with regards to C and N containing components. The general validity of these results need to be further tested in other environments, and we cannot be certain whether the C/N fractionation between POM and DOM is mostly due to the hydrolysis step of the OM degradation or whether it is the result of the elemental fractionation during subsequent DOM mineralization. Nonetheless, our results have implications for the interpretation of bulk OM C/N ratios in sedimentary archives, as they indicate that variable preservation conditions and sedimentation regimes can result in variable early diagenetic C/N shifts that can compromise the use of C/N ratios of sedimentary OM as basic OM source indicator.

Both in the Lower Estuary and in the Gulf, DON contributes significantly to the overall dissolved N exchange between the sediments and the water column. DON fluxes out of the sediments were equivalent to 30-64% of the total sediment denitrification rates and 60-150% of the average nitrate flux into the sediments. Assuming that these DOM fluxes are representative of shelf environments in general, extrapolation of our results reveals that- DON fluxes from continental shelf sediments (depth $\leq$500 m) may add 48 Tg N yr^{-1} to the ocean water column globally. This flux is approximately 1.5 times higher than the estimated riverine DON input to the ocean (~35 Tg N yr^{-1}), and about half to one third of the total oceanic biological N_2 fixation (100-150 Tg N yr^{-1}; Codispoti et al., 2001). The fate and reactivity of the DON escaping the sediments is uncertain. Given the discrepancy between relatively large marine benthic DON fluxes (this study) and the low DON concentrations in the ocean water column (e.g. Knapp et al., 2005; Bourbonnais et al., 2009), however, it is likely that DON remineralization ultimately adds substantially to the reactive water column N pool. Benthic DON fluxes therefore represent an important component of the internal N cycle, eventually supporting a significant fraction of ecosystem productivity.

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Table 2.1. Biogeochemical parameters of sediments in the St. Lawrence Estuary and the Gulf of St. Lawrence.

Station I.D.	OET[(B)] †	OPD[(B)] ‡	O_2 flux[(A)]	%N[(B)]	$\%C_{org}$[(B)]	C_{org} flux	Nitrate flux from incubation[(A)]	Total denitrification[(A)]	CI[(B)] ♦	DI[(B)] ♦
	(yr)	(cm)	(μmol m^{-2} d^{-1})	(dry weight sediment)		(mg C_{org} m^{-2} d^{-1})	(μmol m^{-2} d^{-1})			
25	1.2	0.64	-6410	0.13	1.31	N.D.	-450	-870	0.63	-0.40
23	1.7	0.92	-4300	0.17	1.62	471 to 932[(C)]	-580	-640	0.66	-0.41
22	N.D.	N.D.	N.D.	0.15	1.57	573[(D)]	N.D.	N.D.	0.69	-0.53
21	6.6	1.46	-3220	0.18	1.63	N.D.	-250	-510	0.65	-0.48
20	N.D.	N.D.	N.D.	0.18	1.56	~22[(E)]	N.D.	N.D.	0.7	-0.50
19	7.7	1.82	-3750	0.19	1.52	66 to 160[(F)]	N.D.	-540	0.74	-0.63
18	13.6	1.56	-4950	0.2	1.59	~19[(E)]	-130	-660	0.77	-0.68
Anticosti	N.D.	1.53	-4360	0.26	2.19	N.D.	N.D.	-630	0.71	-0.55
16	N.D.	N.D.	N.D.	0.25	1.85	~22[(E)]	N.D.	N.D.	0.82	-1.02

Data from: [(A)] Thibodeau et al. (2010), [(B)] Alkhatib et al. (in press), [(C)] Silverberg et al. (1987), [(D)] Colombo et al. (1996), [(E)] Muzuka and Hillaire-Marcel (1999), [(F)] Silverberg et al. (2000)).

†OET: Oxygen exposure time

‡OPD: Oxygen penetration depth

♦CI: Chlorin index indicates OM reactivity (Schubert et al., 2005). The CI scale ranges from 0.2 for pure chlorophyll to approximately 1 for highly degraded OM.

♦DI: Degradation index indicates OM reactivity (Dauwe et al., 1999). DI scores for phytoplankton and sediment trap material vary between 1 and 1.5, while coastal and ocean margin sediments have scores between -1 for extensively degraded materials and 1 for relatively reactive OM.

N.D.: not determined

Table 2.2. DOC and DON fluxes at several locations along the Laurentian Channel and Anticosti Channel. DON flux was calculated from both WCS and Rhizon membrane porewater samples. The average DON flux is used for the calculation of flux ratios and % DON flux relative to nitrate fluxes and the total denitrification rates (from Thibodeau et al., 2010)

Sta. ID	DOC flux (WCS)	DON flux (Rhizon)	DON flux (WCS)	Av. DON flux	DON efflux /total NO_3^- flux	% of DON efflux/total denitrification
	(μmol m^{-2} d^{-1})					
Sta. 25	2150	440	420	430	0.95	49.4
Sta. 23	2120	N.D.	350	350	0.60	54.7
Sta. 22	2230	260	340	300	N.D.	N.D.
Sta. 21	2270	320	330	330	1.30	63.8
Sta. 20	1950	160	260	210	N.D.	N.D.
Sta. 19	2080	300	260	280	N.D.	51.8
Sta. 18	1430	170	230	200	1.54	30.5
Sta. 16	1310	110	N.A.	110	N.D.	N.D.
Anticosti	3850	260	290	270	N.D.	43.5

Table 2.3. Comparison of calculated benthic DON and DOC fluxes along the Laurentian Channel and Anticosti Channel with benthic DON and DOC fluxes from other estuarine and coastal environments. All fluxes are expressed in μmol m^{-2} d^{-1}, and all fluxes direction is out of the sediments.

Site	DON flux	DOC flux	References
Laurentian Channel, Canada	110-430	1300-2150	This study
Anticosti Channel, Canada	270	3850	This study
Chesapeake Bay, USA, Site M3	60-320	670-1650	Burdige and Zheng (1998)
Chesapeake Bay, USA, Site S3	40-550	200-850	Burdige and Zheng (1998)
Laholmm Bay, Sweden	100-400	-	Enoksson (1993)
Porcupine Abyssal Plain, NE Atlantic	100	-	Brunnegård et al. (2004)
Svalbard, Norway	950	-	Blackburn et al. (1996)
Temperate Australian Estuaries	-	up to ~50000	Maher and Eyre (2010)
Knebel Vig, Denmark	3900	-	Lomstein et al. (1998)
Mexican Margin	-	250-400	Holcombe et al. (2001)
California continental margin	-	100 - 3100±2700	Burdige et al. (1999)_

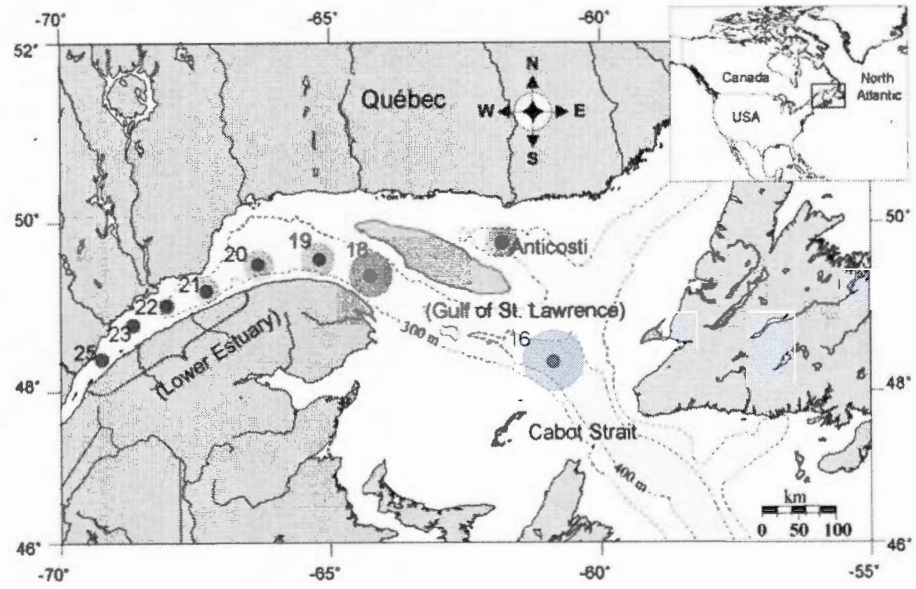

Fig. 2.1. Map showing the sampling locations in the St. Lawrence Estuary and the Gulf of St. Lawrence. Bathymetric contours outline the Laurentian Channel along the 300 and 400 m isobaths. The size of shadowed circles around study sites denotes the relative DO concentrations. For absolute values of bottom water DO see Table 2.1.

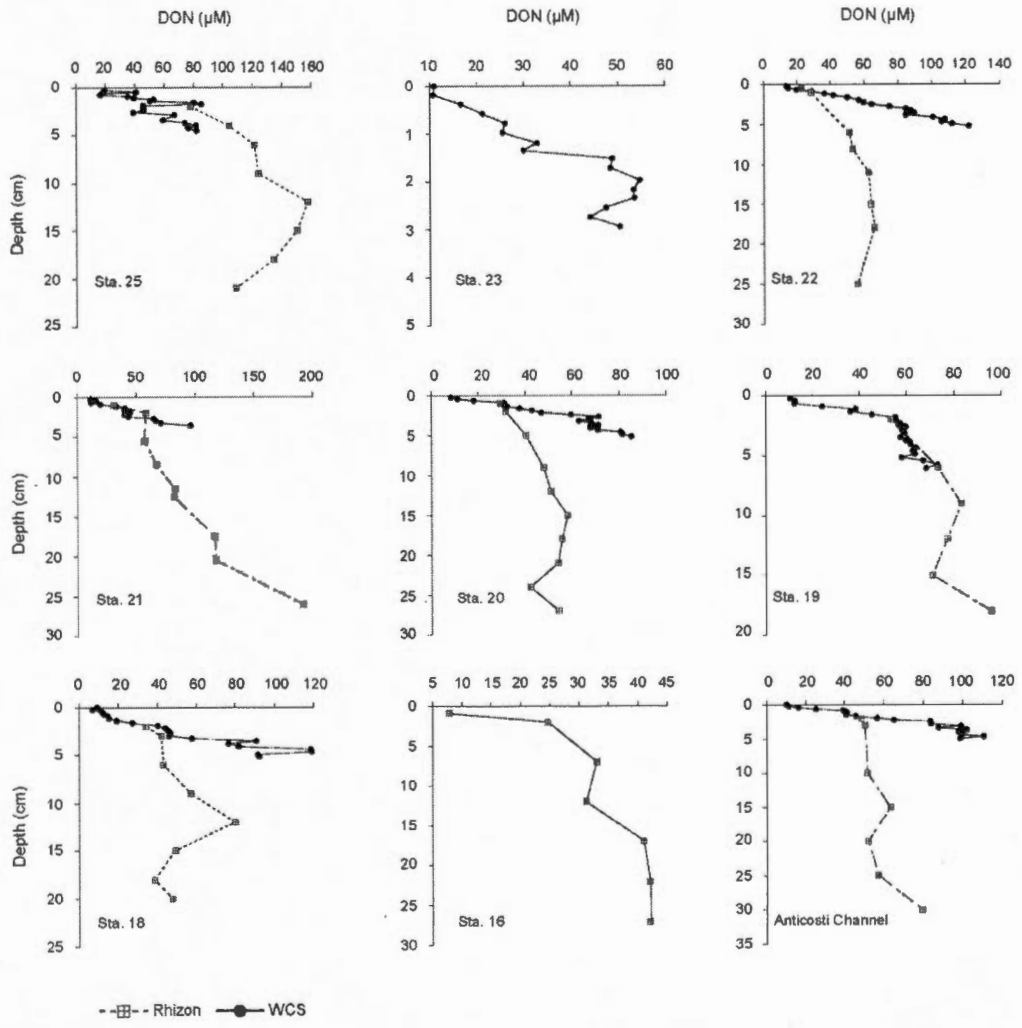

Fig. 2.2. Porewater profiles of dissolved organic nitrogen (DON) in the Lower Estuary and the Gulf of St. Lawrence. Data for both whole core squeezing (WCS) and the Rhizon membrane samples are shown.

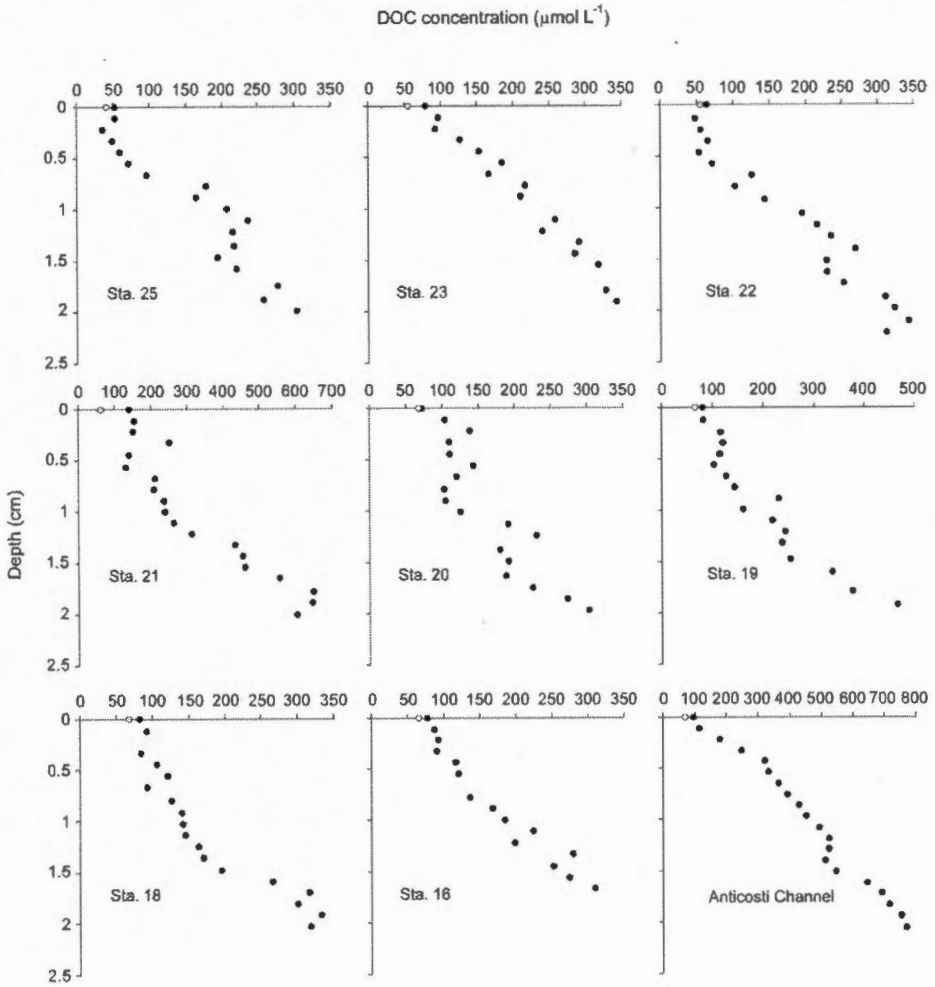

Fig. 2.3. Porewater profiles of dissolved organic carbon (DOC) in the Lower Estuary and the Gulf of St. Lawrence. Open circles on the upper x-axes represent bottom-water [DOC].

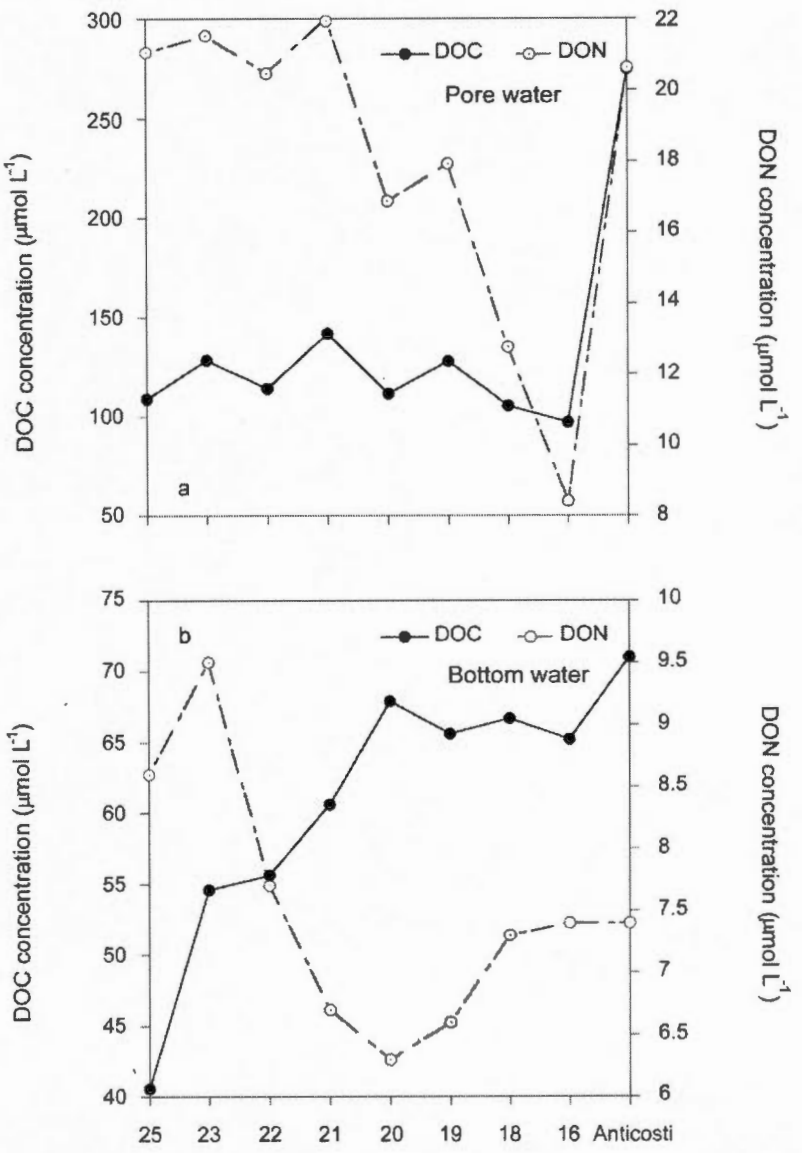

Fig. 2.4. Dissolved organic carbon (DOC) on the left Y-axis and dissolved organic nitrogen (DON) on the right Y-axis concentrations (µmol L^{-1}) in : a) porewaters (average of the first cm), b) overlying bottom water along the lower Estuary and Gulf of St. Lawrence.

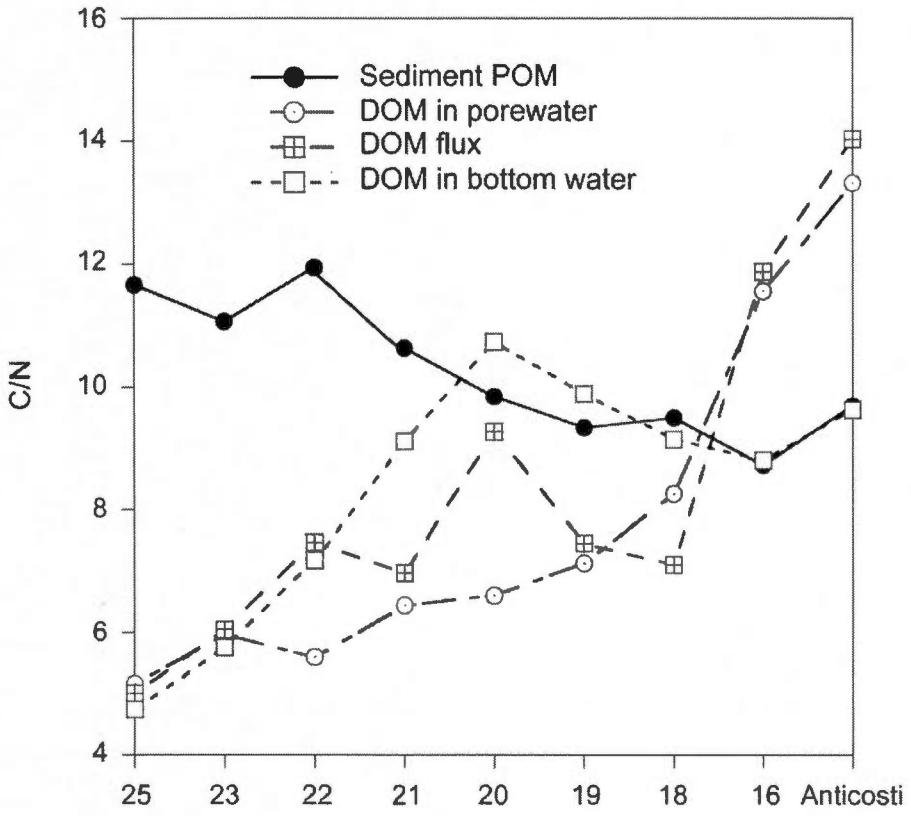

Fig. 2.5. C/N ratios (mole/mole) in the sedimentary particulate organic matter (POM), in porewater dissolved organic matter, and in sediments overlying bottom water along the Lower Estuary and Gulf of St. Lawrence.

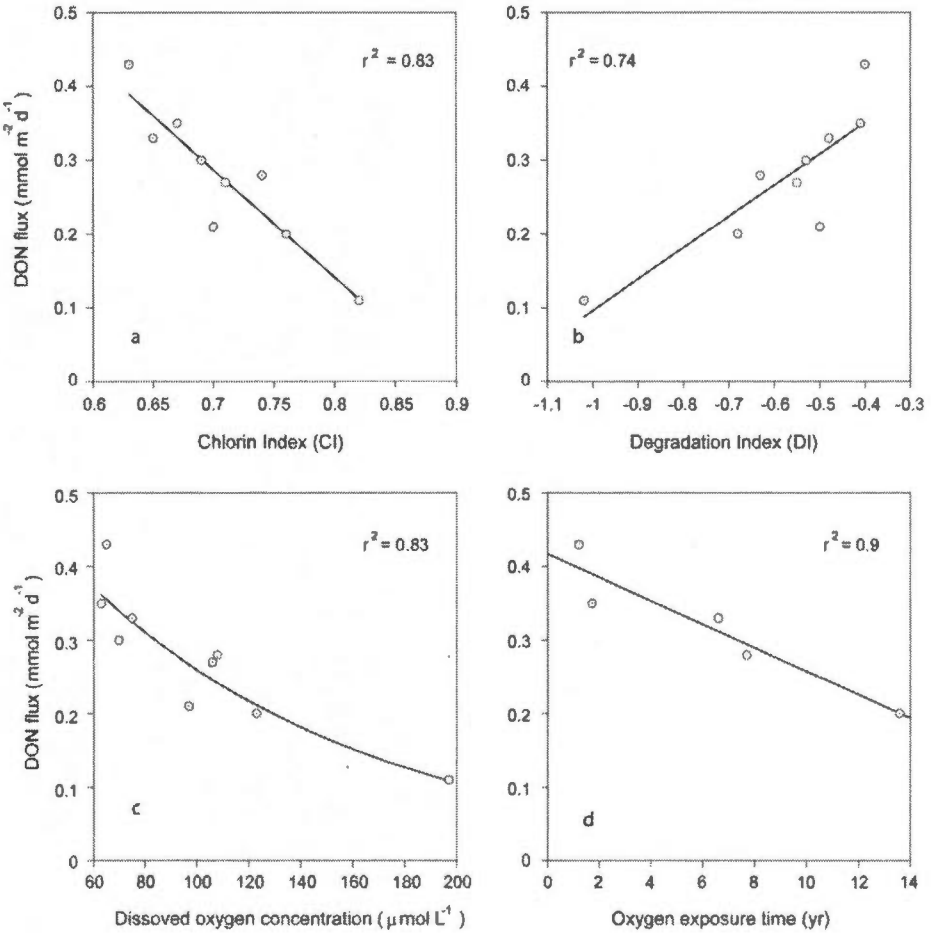

Fig. 2.6. Correlation between the dissolved organic nitrogen (DON) fluxes and reactivity indices: (a) the chlorin index (CI), (b) the degradation index (DI), as well as (c) dissolved oxygen, and (d) oxygen exposure time in sediments along the St. Lawrence Estuary and the Gulf of St. Lawrence.

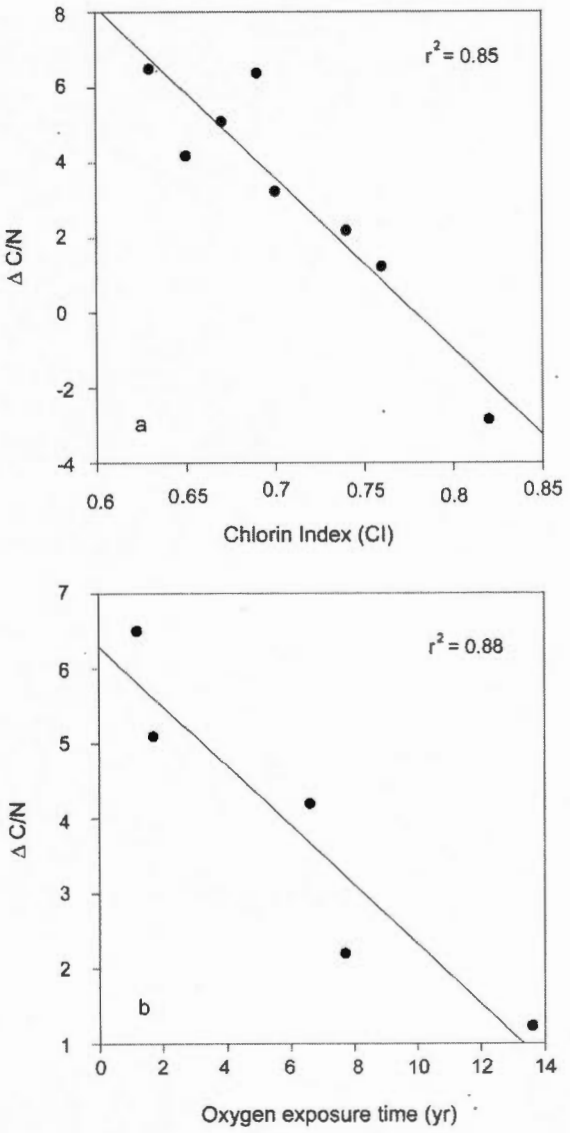

Fig. 2.7. Correlation between the differences between C/N (mole/mole) in the sedimentary particulate organic matter (POM) and in porewater dissolved organic matter (ΔC/N) and (a) the chlorin index (CI) and (b) oxygen exposure time in sediments along the St. Lawrence Estuary and the Gulf of St. Lawrence.

CHAPTER III

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The nitrogen isotope effect of benthic remineralization-nitrification-denitrification coupling in an estuarine environment

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ABSTRACT

The nitrogen (N) stable isotopic composition of pore water nitrate and total dissolved N (TDN) was measured in sediments of the St. Lawrence Estuary and the Gulf of St Lawrence. The study area is characterized by gradients in organic matter reactivity, bottom water oxygen concentrations, as well as benthic respiration rates. N isotope effects on the water column associated with the benthic exchange of nitrate (ε_{app}) and TDN (ε_{sed}) during benthic nitrification-denitrification coupling were investigated. The sediments were a major sink for nitrate and a source of reduced dissolved N (RDN = DON + NH_4^+). We observed that both the pore water nitrate and RDN pools were enriched in ^{15}N relative to the water column, with increasing $\delta^{15}N$ downcore in the sediments. As in other marine environments, the biological nitrate isotope fractionation of net fixed N loss was barely expressed at the scale of sediment-water-exchange, with ε_{app} values < 3‰. The strongest under-expression (i.e., lowest ε_{app}) of the biological N isotope fractionation was observed at the most oxygenated sites with the least reactive organic matter, indicating that, through their control on the depth of the denitrification zone, bottom water oxygen concentrations and the organic matter reactivity can modulate ε_{app}. For the first time, actual measurements of $\delta^{15}N$ of pore water RDN were included in the calculations of ε_{sed}. We argue that large fractions of the sea-floor-derived DON are reactive and, hence, involved in the development of the $\delta^{15}N$ of dissolved inorganic N (DIN) in the water column. In the St. Lawrence sediments, the combined benthic N transformations yield a flux of ^{15}N- enriched RDN that can significantly elevate ε_{sed} above ε_{app}. Calculated ε_{sed} values were within the range of 4.6 ± 2 ‰ and were related to organic matter reactivity and oxygen penetration depth in the sediments. ε_{sed} reflects the $\delta^{15}N$ of the N_2 lost from marine sediments and thus best describes the isotopic impact of fixed N loss from sediments on the oceanic fixed N pool. Our mean

value for ε_{sed} is larger than assumed by earlier work, questioning current ideas with regards to the state of balance of the modern N budget.

3.1. INTRODUCTION

Nitrogen (N) plays a critical role as a nutrient in marine systems (Rabalais, 2002). N is mainly removed from the ocean by conversion of fixed N to N_2 by denitrification and/or anammox (Strous et al., 1999; Zehr and Ward, 2002; Brandes and Devol, 2002; Hulth et al., 2005). Loss by suboxic N_2 production occurs in the absence of dissolved oxygen (DO), in the global ocean oxygen-deficient zones, and, more importantly, in marine sediments. Previous work has demonstrated that benthic denitrification in continental shelf sediments is by far the largest sink of oceanic N, accounting for up to ~70% of the total global denitrification (Middelburg et al., 1996; Codispoti et al., 2001; Brandes and Devol, 2002). However, benthic N elimination in pelagic sediments can also drive basin-wide nitrate deficits (Sigman et al., 2003; Lehmann et al., 2005). During early sedimentary diagenesis, particulate organic N (PON) is hydrolyzed to dissolved organic nitrogen (DON) by bacterial hydrolytic enzymes, and a large fraction of this DON is ultimately remineralized to ammonium (Fig. 3.1). Both the DON and NH_4^+ can diffuse to the overlying bottom water, but in the presence of O_2, a portion of the regenerated NH_4^+ is oxidized to NO_3^- (nitrification) before it can escape from the sediments. This NO_3^- may, in turn, be used as a terminal electron acceptor by denitrifying bacteria producing gaseous forms of N (coupled nitrification-denitrification). Rates and occurrence of diagenetic N-cycle reactions are influenced by substrate availability, organic matter reactivity, and redox conditions (Lehmann et al., 2007; Thibodeau et al., 2010).

Given that the N-isotope effects of specific N-loss terms are known, the isotopic composition of the marine fixed N pool can be used to constrain regional or global N fluxes (Sigman et al., 2003; Brandes and Devol, 2002; Lehmann et al., 2005). The preferential consumption of $^{14}NO_3^-$ by denitrifying bacteria causes the remaining NO_3^- pool to become progressively enriched in ^{15}N (Cline and Kaplan, 1975; Mariotti et al., 1981). Hence, around suboxic water masses, $\delta^{15}N\text{-}NO_3^-$ is significantly elevated with respect to the mean ocean value of 5‰ (Liu and Kaplan, 1989; Voss et al., 2001; Sigman et al., 2003). In agreement with higher estimates of the organism-level N isotope effect for denitrifiers in cultures (Granger et al., 2008), N isotope effects (ε_{cell}) observed in natural environments range between 20‰ and 30‰ (Fig. 3.1). In contrast, the nitrate N isotope effect of benthic denitrification at the scale of sediment–water exchange (ε_{app}) has been proposed to be significantly less than ε_{cell} if denitrification is limited by the rate of diffusive nitrate supply to the active denitrification sites within the sediments (Brandes and Devol, 1997; Lehmann et al., 2004, 2007; see illustration in Fig. 3.1). Moreover, it has been shown that such suppression of the biological N isotope effect of denitrification due to diffusion limitation applies to both reactive coastal sediments and less reactive pelagic sediments (Brandes and Devol, 1997; Lehmann et al., 2004; Lehmann et al., 2007), yet possibly at different degrees.

The use of marine nitrate $\delta^{15}N$ as tracer of sedimentary versus water column denitrification is dependent on the knowledge of the total, or net, sedimentary N isotope effect. Model simulations by Lehmann et al. (2007) suggest that differences in sediment reactivity and the oxygen concentration of the overlying water have a significant effect on the geometry of the oxic layer and the denitrification zone, and thus on the expression of the biological N-isotope effect of denitrification in the water column. An important point considered by previous work, but not directly addressed using measured data, is the potential contribution of dissolved N-forms other than nitrate (i.e., ammonium and DON) to the overall N isotope effect of benthic N-cycling. Thus far,

ammonium and DON fluxes out of the sediments have not been investigated in terms of their N-isotopic composition. Based on model simulations, Lehmann et al. (2007) argued that partial nitrification may, while generating ^{15}N-depleted nitrate within the pore water DIN pool, produce pore water ammonium that is strongly enriched in ^{15}N. If this ammonium escapes to the water column, it can shunt significant amounts of "heavy" fixed N to the water column, an aspect that has until today been neglected in global or regional N isotope balances. Moreover, DON effluxing from sediments has a high potential to be remineralized in the overlying water, and as a result may impact the isotopic composition of the DIN pool in the water column as well. It is important to understand that ultimately it is the combined net flux of N, including the sediment water exchange of reduced dissolved N species (i.e., RDN = NH_4^+ + DON, and TDN = NO_3^- + RDN) that is pertinent to the understanding of the isotopic impact of benthic N elimination on the water column of a marine environment. This combined effect (referred to as $\varepsilon_{sed,}$ as introduced by Lehmann et al., 2007; Fig. 3.1) has been posited to vary as a function of environmental conditions and sediment characteristics.

Here we present high-resolution profiles of the concentration and N isotopic composition of pore water nitrate and total dissolved nitrogen (TDN) from several locations along the Laurentian Channel. The objective of this study was to assess the N isotope effect of benthic N cycling on the water column fixed N pool in the estuary. Our study explores, for the first time based on observational data, the impact of benthic-pelagic exchange of dissolved reduced N compounds (i.e., RDN). The Laurentian Channel of the St. Lawrence Estuary displays pronounced spatial gradients with respect to benthic DIN fluxes, sediment organic matter (OM) reactivity, and degradation state, as well as bottom water oxygenation (Lehmann et al., 2009; Thibodeau et al., 2010; Alkhatib et al., 2012). Hence, it is an interesting environment to study possible links between ε_{app}, ε_{sed}, and these spatial geochemical changes, testing previous hypotheses on the environmental controls on benthic dissolved N isotope effects.

3.2. MATERIAL AND METHODS

3.2.1 Study site and sampling

The Laurentian Channel (Fig. 3.2) displays pronounced spatial variations in OM source, water column depth, surface water productivity, and water column DO concentration (Table 3.1; Alkhatib et al., 2012, and references therein). DO concentration measurements along the Laurentian Channel revealed the presence of year-round hypoxic bottom waters covering approximately 1300 km^2 of the Lower Estuary sea floor, with [DO] as low as ~50$\mu molL^{-1}$ (Gilbert et al., 2005), while the bottom water [DO] in the Gulf is > 150 $\mu mol\ L^{-1}$. The sediment reactivity in the Lower Estuary and the Gulf, which we determined previously using amino acid and chlorin-based preservation indicators (DI and CI, respectively), shows a clear trend along the Laurentian Channel, with the highest organic matter reactivity (i.e., low CI and high DI; for definition see Table 3.2 caption) at the head of the Lower Estuary (Sta. 25, 23 and 22 in Fig. 3.2) and significantly lower OM reactivities at the Gulf stations (Alkhatib et al., 2012).

Sediment multicores were recovered from nine stations along the Laurentian Channel during two summer 2006 cruises (June and August) aboard the *R/V Coriolis II* (Fig. 3.2) using a Bowers & Connelly multicorer (10 cm internal diameter). Immediately upon corer recovery, cores were capped without headspace, and pore water samples were collected from cores using shipboard whole core squeezing (WCS) (Bender et al., 1987). WCS is a pore water sampling technique designed for millimeter depth resolution near the sediment-water interface. Cores were kept at in situ temperatures by wrapping them with ice bags during WCS. Collected pore-waters were filtered through a 0.45 μm Nylon membrane filter (with polypropylene housing), and stored frozen in acid-washed

polyethylene bottles. Previous work has shown that the WCS method agrees well with other, lower-resolution, pore-water extraction techniques (i.e., centrifuging/sectioning, Rhizon-membrane sampling), for both inorganic and organic solutes in the uppermost two cm (Lehmann et al., 2005; Thibodeau et al., 2010; Alkhatib et al., in prep.). Nevertheless, WCS may have effects on the concentration of solutes that are affected by adsorption or, as is probably the case with DON, which are liberated due to cell fracturing at high pressures (Bender, 1987). Alkhatib et al. (in prep.) have shown that below two cm depth, WCS-derived DON concentrations are artificially elevated by the squeezing process and do not represent the natural condition. However, at and just below the sediment-water-interface (SWI), the agreement between the different sampling methods is excellent, so that we can assume that WCS data near the SWI provide reliable estimates on RDN concentrations and fluxes.

3.2.2. Total and dissolved inorganic nitrogen concentrations

NOx (NO_3^- and NO_2^-) concentrations from pore water samples were determined by reduction to nitric oxide (NO) in a solution of acidic vanadium (III), followed by chemiluminescent detection of the NO using an Antek Model 7020 Nitric Oxide Analyzer (ANTEK Instruments, Houston, TX), with a precision of ±0.1 μmol L^{-1} (Braman and Hendrix, 1989). [NH_4^+] from pore water samples was determined using standard colorimetric autoanalyzer techniques using a Braan and Luebbe autoanalyzer, with a precision and a detection limit of ~ 0.25 μmol L^{-1}. TDN concentrations were determined by transforming all N containing compounds to NO_3^- by persulfate oxidation (Knapp et al., 2005), followed by chemiluminescent detection of the NO_3^- (Braman and Hendrix, 1989). [TDN] was corrected for any blank contribution from the persulfate oxidation reagent (on average < 3 μmol L^{-1}), accounting for sample dilution. To test the oxidation efficiency of the persulfate reaction, we have measured a batch of urea and

ammonia standards (10 μmol L^{-1}, n =10), as well as mixed ammonia/urea standards (10 μmol L^{-1}, n =10). The total yield was always in the range of 99 to 103%. Ammonium and dissolved organic N are treated together as reduced dissolved nitrogen ([RDN]), which we calculate here as the difference between [TDN] and [NOx].

3.2.3. Nitrogen isotope ratios

N isotope measurements of nitrate were performed using the denitrifier method of Sigman et al. (2001). Briefly, 20 nmoles of sample nitrate are quantitatively converted to nitrous oxide (N_2O) by denitrifying bacteria that lack active N_2O reductase (Pseudomonas chlororaphis, ATCC 43928). N_2O is stripped from the sample vial using helium as carrier gas, purified, and analyzed for its N isotopic composition with a Micromass Isoprime™ isotope ratio mass spectrometer in continuous flow mode. Blank contribution was generally lower than 0.3 nmol (as compared to 20 nmol of sample N). Isotope values were calibrated using IAEA-N3 and USGS-34, international KNO_3 reference materials with assigned $\delta^{15}N$ values of +4.7‰ and -1.8‰, respectively (Gonfiantini et al., 1995). N isotope ratios are reported in the conventional δ-notation with respect to atmospheric di-nitrogen:

$$\delta^{15}N = [R_{sample} / R_{Standard} - 1]*1000 \quad (1)$$

where R represents the $^{15}N/^{14}N$ ratio. On the basis of replicate measurements of laboratory standards and samples, the analytical precision for $\delta^{15}N$ was generally

$< \pm 0.2\%$ (1 SD).

The $\delta^{15}N$ of TDN was determined by peroxidation of the TDN to NO_3^- (see above), followed by N isotope analysis with the denitrifier method (Knapp et al., 2005; Bourbonnais et al., 2009). TDN-$\delta^{15}N$ was corrected for the blank contribution. IAEA-N1 (0.4‰) and IAEA-N2 (20.3‰) ammonium standards were oxidized and analyzed

with each denitrifier run as a quality control. Replicate measurements of the standards yielded reproducible (±0.2‰) and accurate results (within 0.2‰ for IAEA-N1 and 0.7‰ for IAEA-N2). A secondary "ammonium standard" correction has been applied accounting for the slight N isotope scale compression. The $\delta^{15}N$ of RDN was then approximated according to the equation:

$$\delta^{15}N_{RDN} = (\delta^{15}N_{TDN} * [TDN] - \delta^{15}N_{NO3-} * [NO_3^-]) / [RDN]. \quad (2)$$

3.3. RESULTS

3.3.1. Dissolved nitrogen concentration profiles

Pore water nitrate and nitrite (referred to as nitrate in the discussion of this paper; nitrite in the upper portion of the profiles, near the SWI was always below detection levels) and ammonium concentration profiles in the sediments along the Laurentian Channel are presented in Fig. 3.3. Pore water DIN concentrations and fluxes are discussed in detail in Thibodeau et al. (2010). In brief, nitrate concentration profiles display sharp negative gradients from the SWI down to 2-3 cm depth at most stations, except at Sta. 16 where a subtle subsurface NO_3^- maximum can be discerned. In some cores, nitrate concentration increased slightly at depth, but it is likely that these concentration changes were due to WCS artefacts at high pressures in the lower segments of the WCS core (Lehmann et al., 2005). The negative nitrate concentration gradients indicate that sediments were, in general, a net nitrate sink. Only at Sta. 16 did nitrate production by nitrification exceed nitrate consumption. Diffusive fluxes of nitrate calculated by Thibodeau et al. (2010) (using the same concentration profiles) were highest in the Lower Estuary (130-190 μmol m^{-2} d^{-1}), while lowest fluxes were found in the Gulf (95-110 μmol m^{-2} d^{-1}). Based on Thibodeau et al. (2010) and Crowe et al.

(2011), 70-90% of the nitrate that was reduced (either by denitrification or by anammox) originated from organic N remineralization and nitrification. Ammonium in pore water generally displayed an increasing concentration trend with depth, yet, the concentrations remained relatively low within the first 6 cm (<25 μmol L^{-1}). Pore water [NH_4^+] dropped to zero-levels in the oxic-suboxic transition zone, due to oxidation of ammonium to nitrate (nitrification). However, at some sites, incomplete nitrification within the suboxic-oxic transition zone resulted in the efflux of ammonium from sediments (e.g., Sta. 20 and 21). Clear hydrochemical evidence for anaerobic ammonium oxidation (i.e., depletion of NH_4^+ below the redox transition) was not observed, yet a recent study by Crowe et al. (2011) suggests that potential anammox can contribute as much as one third to the total DIN elimination in the Lower Estuary. TDN concentrations in the water column were ~35 μmol L^{-1}, with the dominant TDN fraction being NOx (~25 μmol L^{-1}). Just below the SWI, the rapid decrease in [NO_3^-] resulted in a TDN minimum of ~25uM. With depth in the sediments, pore water [TDN] increased in a quasi-linear fashion to 80-160 μmol L^{-1} at 4-6 cm depth. Below 2-3 cm, RDN comprises essentially all TDN. RDN, in turn, consists to the larger part of DON (Figs. 3 and 5; DON concentrations are calculated as the difference between RDN and NH_4^+ (not shown here); Alkhatib et al. in prep). Comparison with pore water RDN data obtained using Rhizon sampling (Alkhatib et al., in prep.) show that, in spite of a very good agreement in the uppermost sediment column, below 2 cm sediment depth, WCS-derived DON concentrations were up to three-fold higher than concentrations derived using Rhizon membranes. This seems to be consistent with the liberation of DON during cell rupture at high WCS pressures, confirming previous studies that demonstrated that WCS has a biasing effect on DOM concentrations (and likely on the N isotope ratios) below a certain depth (Martin and McCorkle, 1993).

3.3.2. Nitrate $^{15}N/^{14}N$ in bottom water and pore water

The $\delta^{15}N$ of bottom water nitrate followed a decreasing trend from the Lower Estuary to the East. The $\delta^{15}N$ was 7.2 ‰ and 6.3 ‰ at the hypoxic stations 25 and 23, respectively, and decreased towards the more oxygenated locations (mean value of 5.9 ‰ at Sta. 22, 21 and 20), with lowest values (~5 ‰) in the Gulf (Fig. 3.4, Table 3.2). The observed trend seems to be representative not only for the near-bottom waters but for the complete water masses below the thermocline, as indicated by water column nitrate $\delta^{15}N$ profiles presented in Thibodeau et al. (2010).

The distribution of nitrate $^{15}N/^{14}N$ in pore water was similar for all stations: At some point below the SWI the $\delta^{15}N\text{-}NO_3^-$ increases as $[NO_3^-]$ decreases with depth (see Fig. 3.4), with maximum $\delta^{15}N\text{-}NO_3^-$ of ~11 to 25 ‰ in the deeper sediments (between 2 and 3cm). Nevertheless, the $\delta^{15}N\text{-}NO_3^-$ gradients across the SWI varied significantly. While in the Lower Estuary (Sta. 25 to 20), the pore water $\delta^{15}N\text{-}NO_3^-$ increased right below the SWI, sediment pore water $\delta^{15}N\text{-}NO_3^-$ profiles in the Gulf (Sta. 19, 18, 16) did not show clear changes with depth in the uppermost few mm below the interface, especially not at Sta. 16 (see Fig. 3.4), where nitrate production is evidenced by a $[NO_3^-]$ maximum at 0.7cm.

3.3.3. Sediment-water nitrate $^{15}N/^{14}N$ flux and the apparent N isotopic effect (ε_{app})

ε_{app} quantifies the apparent nitrate N isotope effect of benthic nitrate reduction to N_2, that is, the degree, to which the biological N isotope effects of denitrification, ε_{cell}, is expressed in the above-lying water column nitrate pool. ε_{app} was derived from the $[NO_3^-]$ and $\delta^{15}N\text{-}NO_3^-$ pore water profiles by calculating the net $^{14}NO_3^-$ and $^{15}NO_3^-$ fluxes across the SWI. The latter (i.e., F_{14NO3-} and F_{15NO3-}, respectively) were determined from the measured concentration gradients ($\Delta C/\Delta z$) across the SWI (i.e., the slope of the linear

fits to the upper portion of profiles). Assuming same diffusion coefficients for $^{15}NO_3^-$ and $^{14}NO_3^-$, the $\delta^{15}N\text{-}NO_3^-$ of the nitrate flux is independent of the molecular diffusivities and can be calculated as

$$\delta^{15}N_{Flux\_NO3-} = [(\Delta^{15}NO_3/\Delta^{14}NO_3)_{\Delta z}/ R_{AIR} - 1] * 1000, \qquad (3)$$

where Δ refers to the concentration gradients, and R_{AIR} is the $^{15}N/^{14}N$ ratio of atmospheric nitrogen ($R_{AIR} = 0.003677$). The $\delta^{15}N\text{-}NO_3^-$ of the nitrate flux relative to the diffusive-source $\delta^{15}N\text{-}NO_3^-$ then corresponds to ε_{app} (i.e., it is quantified as the difference between the bottom water nitrate $\delta^{15}N$ and the $\delta^{15}N$ of the benthic nitrate flux). At all stations, the nitrate removed from the bottom water was only slightly more depleted in ^{15}N (i.e., lower $\delta^{15}N\text{-}NO_3^-$) than the bottom water NO_3^-, and ε_{app} ranged from 1.3 to 2.9 (Table 3.2). ε_{app} was highest in the hypoxic portions of the Lower Estuary (Sta. 25 and 23) and at Sta. Anticosti in the Gulf, and decreased eastward along the Laurentian Channel (see Table 3.2).

3.3.4. $^{15}N/^{14}N$ of the total and reduced dissolved N fluxes (ε_{sed})

$\delta^{15}N$-TDN in the bottom waters was dominated by the nitrate $\delta^{15}N$, and ranged between +4 and +7 ‰, with the higher values at the hypoxic locations (~+7 ‰) and more ^{15}N-depleted values in the Gulf stations (4.3-5 ‰) (Fig. 3.5, Table 3.2). Below the SWI, as $[NO_3^-]$ decreased and [RDN] increased, the $\delta^{15}N$-TDN was dominated by the N isotopic composition of ammonium and DON (i.e., RDN). The $\delta^{15}N$-RDN ranged between 2‰ and 6‰ in the bottom waters and generally increased with depth and concentration to values between 5 and 10 ‰ at 2 cm depth (Fig. 3.5). At Sta. 25 and to a lesser extent at Sta. 23, Sta. 22, and Sta. 21, the change in $\delta^{15}N$-RDN with depth was relatively minor. The difference between the $\delta^{15}N$-TDN in the bottom water column and that in the pore water (which is the master factor in determining the overall N isotope

effect during the exchange of the different N species across the SWI) seems to increase eastwards along the Laurentian Channel. Using the RDN isotope data in the upper 2 cm of the sediments (i.e., down to a depth which is not affected by WCS artefacts), analogous to the use of nitrate N isotope data above to calculate ε_{app}, we calculated the RDN N isotope flux across the SWI. However, RDN profiles were clearly not linear in the vicinity of the SWI, and the first derivative at z = 0 of the exponential fits was used to calculate nitrate isotope concentration gradients. The RDN N isotope flux was then combined with the nitrate N isotope flux from above to yield estimates of ε_{sed}, the overall N isotope effect of benthic DIN removal. We found that the ε_{sed} values were in the same range, or slightly higher than, the ε_{app} values for all the St. Lawrence Estuary and Gulf stations, yet all of them were < 7‰ (see Table 3.2).

3.4. Discussion

3.4.1. Distribution of the nitrate and TDN ^{15}N in sediment pore waters

The inverse correlation between nitrate $\delta^{15}N$ and $[NO_3^-]$ suggests that nitrate consumption within the sediments occurs with a significant intrinsic (or biologic) N isotope effect, which results from the discrimination against ^{15}N by nitrate-reducing bacteria. Although not completely adequate to describe nitrate N isotope fractionation within sediment pore waters (Lehmann et al., 2007), the biological N isotope effect ε_{cell} of net nitrate consumption can be approximated assuming Rayleigh model dynamics: The slope of the linear regression between $\delta^{15}N\text{-}NO_3^-$ vs. $\ln[NO_3^-]$, yields estimates of the biological N-isotope fractionation during nitrate consumption in the sediments ($\varepsilon_{cell\_Rayleigh}$) (Mariotti et al., 1981). $\varepsilon_{cell\_Rayleigh}$ ranged between 3‰ and 7‰ in the St. Lawrence estuarine and Gulf sediments (Fig. 3.6), within the lower range of the values

reported for deep-sea sediments in the Bering Sea (between 4‰ and 17‰). N elimination in St. Lawrence Estuary sediments is dominated by nitrification-denitrification coupling (Thibodeau et al., 2010; Crowe et al., 2011), and diffusive supply of water-column nitrate for denitrification plays a lesser role. While sediments in general violate one of the basic assumptions of a closed-system model, leading to underexpression of the biological N isotope effect when calculating $\varepsilon_{cell\_Rayleigh}$ (Lehmann et al., 2007), the production of ^{15}N-depleted nitrate from nitrification may be particularly efficient in dampening the actual nitrate ^{15}N enrichment with respect to a given nitrate deficit. Furthermore, physiological effects may play a certain role in keeping ε_{cell} at low levels (e.g., low substrate availability at the active denitrification site; Lehmann et al. 2007).

At most stations, we observed a decrease of the $\delta^{15}N$-RDN from ~2cm sediment depth towards the SWI (see Fig. 3.5d). In accordance with observations by Prokopenko et al. (2006) in marine sediments of the Eastern Subtropical North Pacific and the Santa Barbara Basin, the $\delta^{15}N$-RDN at the deeper end of this depth segment matches the $\delta^{15}N$ of sediment POM (± 0.5‰) (except at Sta. 23 where the average pore water $\delta^{15}N$-RDN was significantly higher than $\delta^{15}N$-POM), suggesting the liberation of N during POM remineralization without significant N isotope fractionation. The cause for the ^{15}N depletion of RDN in the subsurface pore water (and ultimately in the RDN pool in the water column) is unclear. The observed decrease in $\delta^{15}N$-RDN implies a source of low-$\delta^{15}N$ DON just below the SWI. A low-$\delta^{15}N$ ammonium source is unlikely, as almost complete consumption of ammonium would produce RDN ^{15}N-enrichment rather than ^{15}N-depletion. Urea can be a major nitrogen excretion product from bacteria in marine sediments (Lomstein et al., 1989; Pedersen et al., 1993; Sloth et al., 1995), which could, if depleted in ^{15}N, contribute to the observed $\delta^{15}N$ decrease in the pore water RDN just below the SWI where bacterial respiration rates are high. Alternatively, bacterial uptake of DON could have caused the observed decrease in $\delta^{15}N$-RDN, if preferential

incorporation of high δ^{15}N-DON into bacterial biomass occurs. Given the work of Macko and Estep (1984), the partitioning of DON during bacterial DON assimilation into a "lighter" ammonium and a "heavier" PON pool is indeed plausible. While we can only speculate about the exact controls on the pore water δ^{15}N-RDN trend, it is evident that the bulk pore water RDN pool in the upper two cm of the sediment column has still a slightly higher δ^{15}N than the RDN in the water column, implying that the sediments are a source of ^{15}N-enriched RDN.

3.4.2. Controls on the nitrate N isotope ε_{app} effect during nitrate removal

As discussed in the previous section, net denitrification in the St. Lawrence estuarine sediments was associated with a substantial biological N isotopic fractionation at the cell-level, leaving the residual pore water nitrate pool enriched in ^{15}N. A significant water column nitrate deficit ($N^* \sim -10$ µmol L^{-1}) has been identified by Thibodeau et al. (2010) in the hypoxic bottom water column in the Lower Estuary, yet this nitrate deficit, which can exclusively be attributed to benthic denitrification (see Table 3.1 for rates), is not associated to any significant nitrate N isotope effect in the water column. Hence, as has been described for other marine environments (Brandes and Devol, 1997; Lehmann et al., 2004, 2007), the biological N isotope fractionation, as evidenced by strong nitrate δ^{15}N gradients within the sediment pore water, appears to be suppressed at the scale of sediment water nitrate exchange also in these estuarine sediments of the Laurentian Channel. In agreement with reports from other marine benthic environments (Brandes and Devol, 1997; Sigman et al., 2003; Lehmann et al., 2004), the calculated ε_{app} values between 1 and 3 ‰. confirmed a large degree of under-expression of the biological N isotope fractionation during nitrate consumption from the bottom waters. Despite extensive NO_3^- removal within the sediment pore water pool (Thibodeau et al., 2010; Table 3.1), and in spite of the strong nitrate ^{15}N enrichment associated with the nitrate removal, the δ^{15}N-NO_3^- in the bottom water barely increased. Based on previous work by Brandes and Devol (1997) and Lehmann et al. (2004, 2007),

we attribute the apparent suppression of the biological N isotope effect to the limited diffusive NO_3^- supply to the site of denitrification in the sediment, and/or the gross production of ^{15}N depleted nitrate through incomplete nitrification of pore water ammonium.

Although the spatial changes in ε_{app} are subtle, it appears that higher ε_{app} values were generally found in the Lower Estuary at stations that exhibit the highest sedimentary OM reactivity and that are overlain by the least oxygenated (hypoxic) bottom water along the Laurentian Channel (see Table 3.2; Alkhatib et al., 2012). Relatively low ε_{app} values were determined for the Gulf sediments (e.g., Sta. 16), which are characterized by the lowest OM reactivity and high bottom water $[O_2]$. The general under-expression of ε_{cell} along the Laurentian Channel ($\varepsilon_{app} \leq 3$ ‰) appears to be related to the supply and consumption of nitrate within the denitrification zone under diffusion limitation. In particular, the reactivity of the OM and the bottom water oxygenation may both affect the oxygen penetration depth (OPD) and, hence, the nitrate gradient across the SWI, as well as the corresponding diffusive nitrate flux.

We found a good correlation between ε_{app} and OPD (r^2=0.66, n=6; Fig. 3.7a), and a significant inverse correlation between the overlying bottom water $\delta^{15}N\text{-}NO_3^-$ and OPD (r^2=0.82, n=6; Tables 3.1 and 3.2). Hence our observational data seem to confirm previous model-based results by Lehmann et al. (2007), which indicate close links between ε_{app}, OPD, and the depth of denitrification. There is also a significant correlation between OM reactivity (quantified as Chlorin Index; data from Alkhatib et al., 2012) and ε_{app} along the Laurentian Channel (r^2=0.52, n=9; Fig. 3.7b), which further suggests that OM reactivity has a significant influence on ε_{app} at low levels by modulating OPD, ammonium production, and thus nitrate availability. As the denitrification zone is forced deeper into sediments, the diffusive transport of nitrate to the denitrification zone is decreased, and it is less likely that ε_{cell} is expressed in the water column. As the denitrification zone shifts closer to the sediment surface, due to a

lowered OPD (e.g., in a more reactive benthic environment), denitrifiers will consume nitrate from a larger nitrate pool, enhancing the expression of ε_{cell} at the scale of sediment-water solute exchange (Lehmann et al., 2007). OPD, and hence the depth of the denitrification zone, seems to be the main constraint on ε_{app}, through its direct control on the nitrate pool size at the depth where denitrification occurs. At the same time, in particular in low-reactivity environments, it is also possible that nitrification rates can exceed net denitrification (Berelson et al., 1990). For example, at Sta. 16 the sediments represent a source of nitrate (Table 3.1). Partial nitrification of upward diffusing and regenerated ammonium produces ^{15}N-depleted nitrate (e.g., Sigman et al. 2001; Wankel et al. 2009), which can compensate any nitrate ^{15}N enrichment to the point that the nitrate $\delta^{15}N$ gradient across the SWI is significantly lowered or even reversed.

3.4.3. The total sedimentary N isotopic effect ε_{sed}

As mentioned in the last section, nitrate produced from incomplete remineralization/nitrification can partially offset the ^{15}N enrichment by denitrification. Another more direct effect of remineralization on the N isotope composition of the water column TDN can result from the actual NH_4^+ and DON efflux from the sediments into the bottom water. For example, as low $\delta^{15}N\text{-}NO_3^-$ is produced from partial nitrification, ^{15}N-enriched ammonium is left behind and may escape to the water column, increasing the overall N isotope effect of benthic N cycling (Lehmann et al., 2007). As a consequence, while the N-isotopic fractionation during nitrification may reduce ε_{app} it can increase ε_{sed}, and we suggest this is the case at least at some of our sites. At stations where $[NH_4^+]$ was not zero at the SWI, and NH_4^+ fluxes out of the sediments, N isotope signatures that result from partial nitrification are propagated into the water column. In the same vein, under suboxic conditions, nitrate can be converted into ammonium via DNRA. Although the isotopic effect of DNRA has not yet been

investigated, it is likely that partial ammonification of nitrate most probably produces ^{15}N depleted ammonium (Lehmann et al., 2007). Through $^{15}NO_3^-$ label ex-situ core incubations, Thibodeau et al. (2010) demonstrated that DNRA contributes to total nitrate reduction at Sta. 18. However, the relatively high $\delta^{15}N$ values for porewater RDN at essentially all stations argue against an important effect of DNRA on the reduced nitrogen pool, in agreement with Crowe et al. (2011), who found that the rate of DNRA is three orders of magnitude lower than denitrification and anammox and is therefore insignificant to N-cycling in the St. Lawrence Estuary.

Calculated ε_{sed} values from sediments along the Laurentian Channel varied between 1.5‰ and 6.7‰, and ε_{sed} seemed to increase eastward along the Laurentian Channel (i.e., lower ε_{sed} values were observed for the Lower Estuary, while rather high values were found in the Gulf; see Table 3.2). The ε_{sed} values are, on average, higher than the ε_{app} values. The elevation of ε_{sed} over ε_{app} at most Laurentian Channel stations (particularly to the east) can be explained by the efflux of RDN into the water column, which has, at most stations, a higher $\delta^{15}N$ than the water column RDN pool. One would expect that OM remineralization (and associated N-isotope fractionation) and the $\delta^{15}N$ of the source OM, as well as the degree to which nitrification of mineralized NH_4^+ by bacteria is complete is a major determinant of ε_{sed}. Indeed, ε_{sed} correlates significantly with both CI (r^2=0.65, n=8; Fig. 3.7c) and DO in the bottom water (r^2=0.76, n=8; Tables 3.1 and 3.2). ε_{Sed} also correlates with the oxygen penetration depth in sediments (r^2=0.76, n=6; Fig. 3.7d), as well as with the oxygen exposure time (r^2=0.57, n=5; Tables 3.1 and 3.2), suggesting that, as with ε_{app}, OM reactivity and oxygen concentration play a significant role in controlling the combined isotope effects of benthic N-cycle processes on the water column TDN pool. As mentioned earlier, the efflux of high $\delta^{15}N$-NH_4^+, can by itself raise ε_{sed} values. However, it is important to note that, as the NH_4^+ ^{15}N-enrichment in the pore water NH_4^+ pool must be paralleled by the production of low- $\delta^{15}N$ nitrate, isotope fractionation during nitrification only increases

ε_{sed} if new, low $\delta^{15}N\text{-}NO_3^-$, is directly denitrified (coupled nitrification-denitrification), and the NH_4^+ is not completely oxidized within the sediments. Granger et al. (2011) found a significant direct relationship between the efflux of ammonium from Bering Sea Shelf sediments and the water column $\delta^{15}N\text{-}NO_3^-$. Similarly, Brandes and Devol (1997) found that the $\delta^{15}N\text{-}NH_4^+$ that diffused out of the sediments of Puget Sound was on average 4.5 ‰ heavier than both that of source organic matter within the sediments and the overlying water NO_3^-. It could be hypothesized that ε_{sed} is a function of the ammonium efflux ratio (i.e., the ratio between NH_4^+ remineralization and NH_4^+ efflux; Lehmann et al. 2004) in the Laurentian Channel, as was proposed for the Bering Sea shelf (Granger et al., 2011). However, at most of our sites, NH_4^+ appeared to be quantitatively oxidized within the sediments, and where NH_4^+ flux was observed, ε_{sed} was not particularly high (e.g., Sta.21). Hence, we argue that it is the amount and N isotopic composition of the effluxing DON that represent the most important constraints on ε_{sed}. Interestingly, ε_{sed} was highest where surface sediment OM $\delta^{15}N$ values were also highest (see Table 3.2).

Finally, bioturbation/bioirrigation is yet another aspect that may have contributed to the observed trends in ε_{sed} (and ε_{app}) along the Lower Estuary and the Gulf. Belley et al. (2010) found that the macrobenthos surface-trace density was highest at the head of the channel (Sta. 25 and Sta. 23) and that it decreased noticeably eastward along the LC. This observation was explained by the fact that the hypoxic area of the LC is now in a transition phase, where suspension feeders, non-tolerant to hypoxia, are progressively replaced by low-oxygen tolerant deposit feeders that are mainly responsible for surficial benthos traces (e.g., Ophiura sp.). The possible effect of bioturbation/bioirrigation on ε_{sed} remains uncertain. Bioirrigation in general facilitates the DIN and RDN exchange between the bottom and porewaters, and thus may allow "deeper" RDN with higher $\delta^{15}N$ to escape the sediments, by-passing the diagenetically active reaction zone right below SWI, and increasing the ε_{sed}. At the same time, oxygen is supplied to anoxic

regions within the sediments, pushing down the active denitrification zone, and reducing the porewater nitrate concentration gradient. This deepening of the oxic layer can, on the one hand, enhance nitrate diffusion limitation and thus reduce ε_{sed} (see above), it may also stimulate nitrification at greater sediment depths and may thus act to remedy nitrate diffusion limitation on the other hand. Lehmann et al. (2004) have argued that even in strongly bioirrigated environments the N isotope effect of benthic microbial denitrification is essentially not expressed. Our study attests to this notion, as none of the observed ε_{app} is greater than 3‰, and lowest ε_{Sed} co-occur with the highest density of surface traces.

3.4.4. Implications for the marine N-isotope budget

ε_{sed} approximates the difference between the $\delta^{15}N$ of all reactive N in the water column and the $\delta^{15}N$ of N lost by suboxic N_2 production, and thus, understanding ε_{sed}, more than ε_{app}, is critical for N isotope budgets (Lehmann et al., 2007; Granger et al., 2011). Lehmann et al. (2007) have simulated environmental scenarios where ε_{sed} can be up to 5‰ (when coupled nitrification-denitrification is associated with a flux of NH_4^+ out of the sediments). While they did not include DON in their N isotope flux calculations, their scenario of intermediate OM reactivity and intermediate oxygenation of bottom water reflects well the environmental conditions along the Laurentian Channel, and the agreement between modelled and observed values of ε_{sed} and ε_{app} is remarkably good (see Tables 3.1 and 3.2). Moreover, using a different approach, Granger et al. (2011) estimated that the N isotopic effects of sedimentary nitrification-denitrification coupling on the Bering Sea Shelf water column (derived from the $\delta^{15}N$ of surface sediments with respect to the reactive N deficit in the water column) ranged between 6 ‰ and 8 ‰. The weak coupling between nitrification and denitrification in the Bering Sea shelf allowed for large amounts of ammonium-N to escape consumption

inside the sediments, which resulted in such high ε_{sed} values. From the global N-isotope balance perspective, the degree to which N isotope fractionation due to benthic N elimination, the main N sink in the oceanic N cycle, finds its expression in the water column is of fundamental importance. Our data reveal that the biological N isotope effect of estuarine benthic N elimination is highly suppressed, confirming observations from other benthic environments (Brandes and Devol, 2007; Lehmann et al., 2004, 2007; Granger et al., 2011). However, both ε_{sed} and ε_{app} can be significantly different from zero, and can vary as a function of the sediment reactivity and bottom water oxygenation. The conditions along the Laurentian Channel, especially in the Gulf of St. Lawrence can be considered representative of coastal and continental shelf environments where most of the global sedimentary denitrification occurs. Given the mean ocean DIN $\delta^{15}N$ of approximately 5.5‰ and an input of N through N_2 fixation with a $\delta^{15}N$ of -2-0‰, a globally applicable ε_{sed} value of 5‰ (as calculated using our field data) or even higher (Granger et al., 2011) would indicate that essentially all denitrification occurs within the benthic environment, leaving little space for water column denitrification with a significantly higher N isotope effect (according to the approach by Brandes and Devol, 2002). Such a large attribution of benthic denitrification to the global N loss is not considered in current observations (Brandes and Devol, 1997; Fennel et al. 2006), and would imply that global N loss by far exceeds global N inputs, yielding a completely imbalanced N budget. While our observational data now confirm that ε_{sed} values of 5‰ and greater are possible, benthic denitrification must occur to large parts with a lower ε_{sed}, for example in deep sea sediments, where the average ε_{sed} is assumed to be ~2‰ (Lehmann et al., 2007).

3.5. CONCLUSIONS

Independent of the sedimentary OM reactivity and bottom water [O_2] regimes, the biological nitrate N isotope effect is significantly under-expressed at the scale of sediment-water nitrate exchange. This observation, for the first time made in an estuary, is in agreement with findings from other marine environments (Brandes and Devol, 1997, Lehmann et al., 2004, 2007), underscoring that a low ε_{app} applies to a wide spectrum of environmental conditions. We conclude that sediment OM reactivity and [DO] in bottom water interact to yield a low nitrate supply to the denitrification zone inside the sediment, so that diffusion limitation will keep the ε_{app} <3‰. Nevertheless, subtle but consistent changes in ε_{app} were discerned along the Laurentian Channel, with putative links to bottom water oxygenation and sediment OM reactivity. Hence, our observational data are consistent with recent model results that point to OM reactivity and the O_2 penetration depth as important controls of the N isotope effect during nitrate exchange between the sediments and the overlying water column (Lehmann et al., 2007).

We have analyzed the patterns in ε_{sed} for the first time in the context of N isotopic measurements of reduced dissolved N species (DON + NH_4^+). The values of ε_{sed} approximate the difference between the $\delta^{15}N$ of reactive N in the water column that is ultimately lost in sediments through suboxic N_2 production and the $\delta^{15}N$ of the lost N_2. Thereby, they consider the coupling between remineralization, nitrification, and denitrification (and other N transformations not explicitly addressed here; e.g., anammox). Previous work has not included the DON flux from the benthic environment in ε_{sed} calculations, on the basis that DON remineralization does not add substantially to the reactive water column N pool. While we agree that the mean oceanic N pool consists of DON that is of rather refractory nature (Knapp et al., 2005; Bourbonnais et al., 2009), we argue that the inert character of the water column DON pool does not preclude that a

dominant fraction of DON that fluxes out of marine sediments undergoes rapid oxidation and adds to the oceanic DIN pool. Otherwise, it would be difficult to explain the discrepancy between the relatively large estuarine and coastal benthic DON fluxes (Alkhatib et al., in prep.) and the low DON concentrations in the ocean water column (e.g. Knapp et al., 2005; Bourbonnais et al., 2009). As a consequence, we emphasize here that DON fluxes need to be included in ε_{sed} calculations and conclude that OM remineralization can modulate the overall N isotope effect of benthic N elimination. As we did not distinguish between NH_4^+ and DON, we can only speculate on the reason for a generally low $\delta^{15}N$ of the RDN in both the water column and the sediment pore waters, as well as processes that determine the RDN efflux. Future work should directly address the N isotopic impact of sediment/water ammonium versus DON fluxes. Our data clearly show that the flux of RDN out of the sediments is consistently enriched in ^{15}N with respect to the water column RDN pool, most often elevating ε_{sed} over ε_{app}. In agreement with previous model simulations (Lehmann et al. 2007), our observational data indicate that the degree of under-expression of the biological N isotope effect of sedimentary N-elimination seems to be a function of the environmental conditions (bottom water oxygenation and oxygen penetration depth in particular), and also of the reactivity and $\delta^{15}N$ of the sedimenting OM. These parameters all modulate the amount and the $\delta^{15}N$ of the regenerated N that is ultimately denitrified. The variability in ε_{sed} within a range of 4.6 ± 2.5‰ observed for the St. Lawrence system is consistent with previous projections of a mean global ε_{sed} value on the order of 4‰ (Lehmann et al., 2007). Nevertheless, these values of ε_{sed} are larger than previously assumed, and result in inconsistencies with current N isotope budgets, because they imply a greater sediment-to-water column denitrification ratio and, thus a greater overall imbalance in the global N budget than currently considered.

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Table 3.1. Sampling site characteristics along the Laurentian Channel: Depth, oxygen concentration in bottom water, oxygen penetration depth (OPD), and benthic fluxes of O_2 and nitrate.

Sta. I.D.	Depth (m)	Bottom water $[O_2]$ [a] ($\mu mol\ L^{-1}$)	OPD[b] (cm)	O_2 flux [a] ($\mu mol\ m^{-2}\ d^{-1}$)	NO_3^- benthic flux [a] ($\mu mol\ m^{-2}\ d^{-1}$)*	NO_3^- diff. flux [a] ($\mu mol\ m^{-2}\ d^{-1}$)†
25	290	65	0.64	-6410	-450	-130
23	350	63	0.92	-4300	-580	-140
22	321	70	N.D.	N.D.	N.D.	-140
21	330	75	1.64	-3220	-250	-140
20	330	97	N.D.	N.D.	N.D.	-130
19	370	108	1.82	-3750	N.D.	-110
18	370	123	1.56	-4950	-130	-100
Anticosti	283	106	1.53	-4360	N.D.	-130
16	420	197	N.D.	N.D.	N.D.	25

*from ex situ incubations

†based on concentration gradients

[a] from Thibodeau et al. (2010)

[b] from Alkhatib et al., (2012)

Table 3.2. Sedimentary bulk particulate OM characteristics, bottom water TDN and NO_3^- $\delta^{15}N$, benthic N isotope effects (ε_{app} and ε_{sed}), and the $\delta^{15}N$ of the benthic nitrate flux along the Laurentian Channel.

	Sediment POM[a]					Bottom water			
Sta. I.D.	$\delta^{15}N$	%N	$\%C_{org}$	CN	Chlorin Index (CI)*	$\delta^{15}N$-TDN (‰)	$\delta^{15}N$-NO_3^- (‰)	ε_{app} (‰)	ε_{sed} (‰)
25	5.52	0.13	1.31	11.66	0.63	6.99	7.25	2.7	1.5
23	6.11	0.17	1.62	11.07	0.66	7.44	6.23	2.9	3.6
22	6.18	0.15	1.57	11.95	0.69	5.92	5.87	1.2	3
21	6.35	0.18	1.63	10.64	0.65	5.51	5.86	2.3	3.4
20	7.28	0.18	1.56	9.84	0.70	4.89	6.00	1.2	5.9
19	7.02	0.19	1.52	9.33	0.74	4.33	5.06	1.5	6.6
18	7.08	0.20	1.59	9.49	0.77	4.41	5.09	1.0	5.8
Anticosti	6.88	0.26	2.19	9.70	0.71	4.40	4.69	1.7	6.7
16	7.01	0.25	1.85	8.72	0.82	4.95	5.23	1.3	N.D.

*CI indicates OM reactivity (Schubert et al., 2005). The CI scale ranges from 0.2 for pure chlorophyll to approximately 1 for highly degraded OM.

[a] from Alkhatib et al., (in press.)

N.D.: not determined

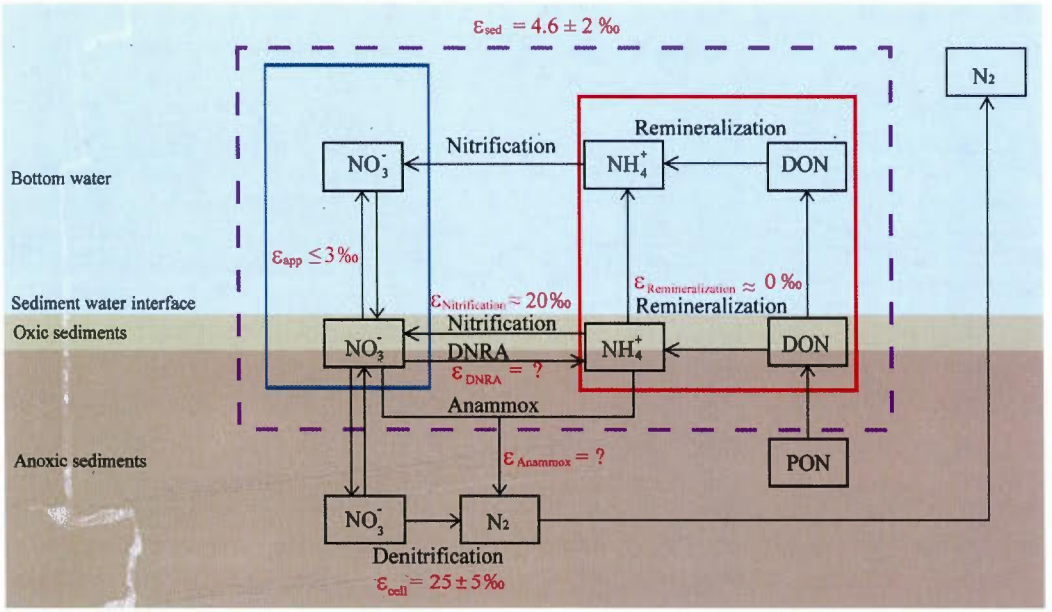

Fig. 3.1. Schematic overview depicting the most important processes that control benthic DIN/RDN exchange and associated N isotopic effects. ε_{cell} refers to the biological N isotope effects of denitrification, ε_{app} refers to the apparent nitrate N isotope effect of benthic nitrate reduction in the above-lying water column, and ε_{sed} refers to the N isotope effect of total dissolved nitrogen benthic exchange, considering both nitrate and RDN fluxes (see text for details).

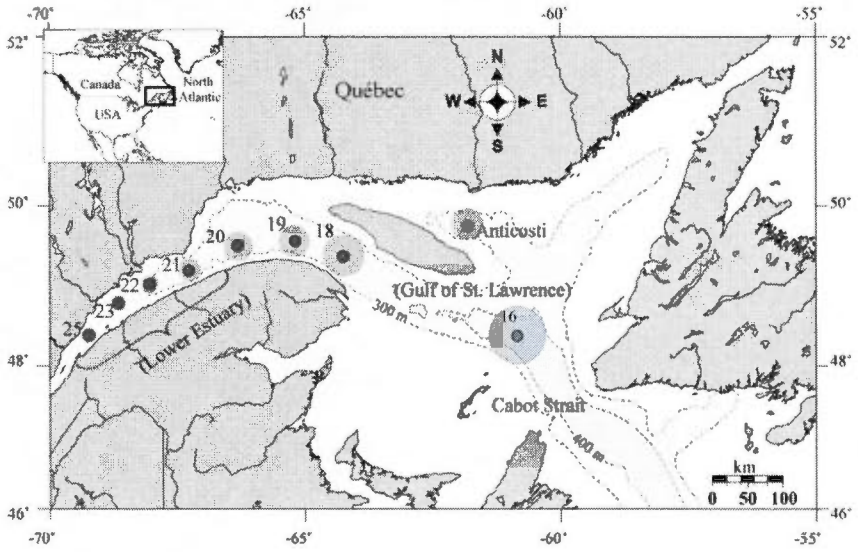

Fig. 3.2. Map showing the sampling locations in the St. Lawrence Estuary and the Gulf of St. Lawrence. Bathymetric contours outline the Laurentian Channel along the 300 and 400 m isobaths. The size of shadowed circles around study sites denotes the relative DO concentrations. For absolute values of bottom water DO see Table 3.1.

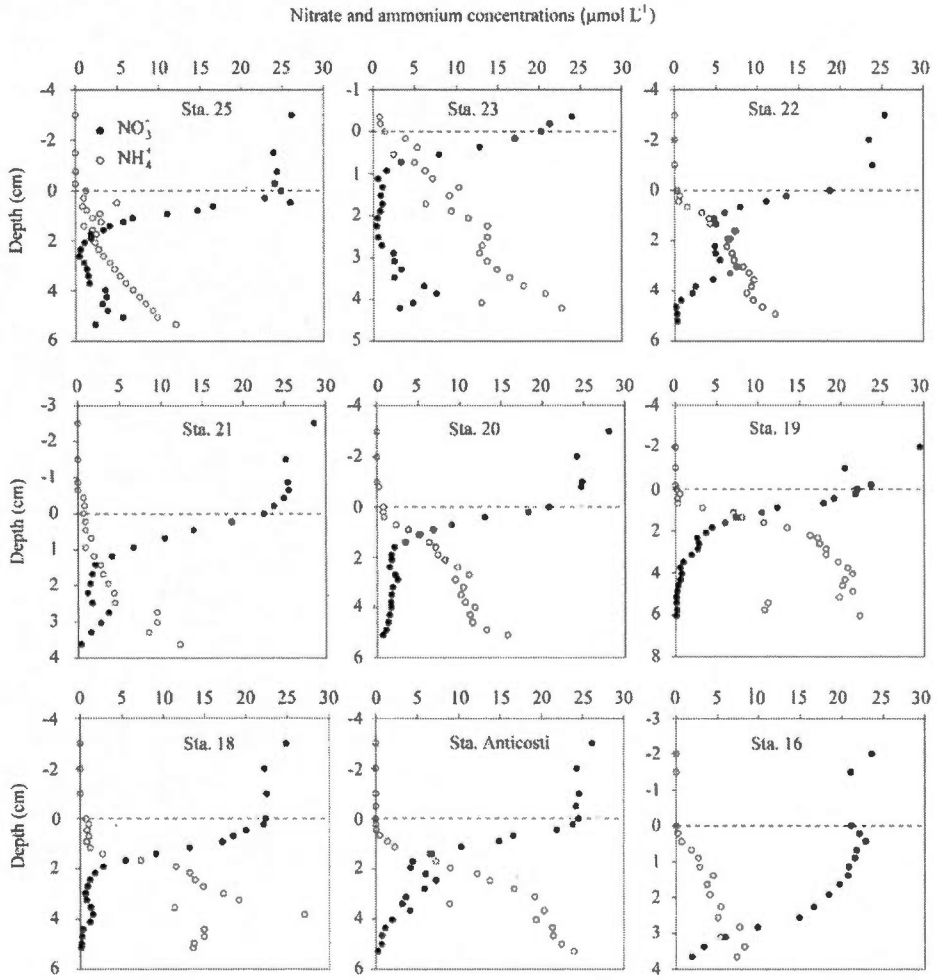

Fig. 3.3. Pore water nitrate and ammonium concentration profiles from locations in the St. Lawrence Estuary and the Gulf of St. Lawrence.

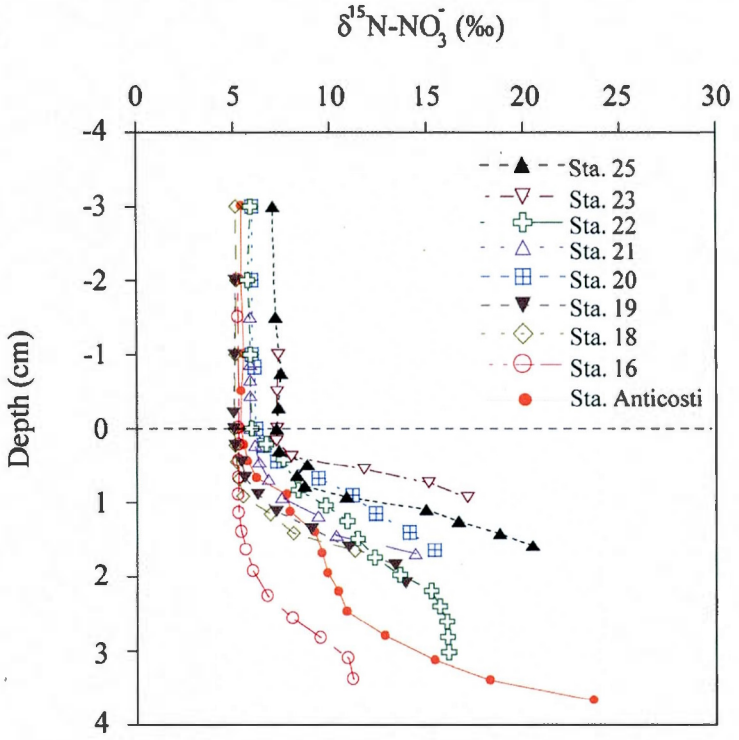

Fig. 3.4. Pore water nitrate $\delta^{15}N$ profiles from locations in the St. Lawrence Estuary and the Gulf of St. Lawrence.

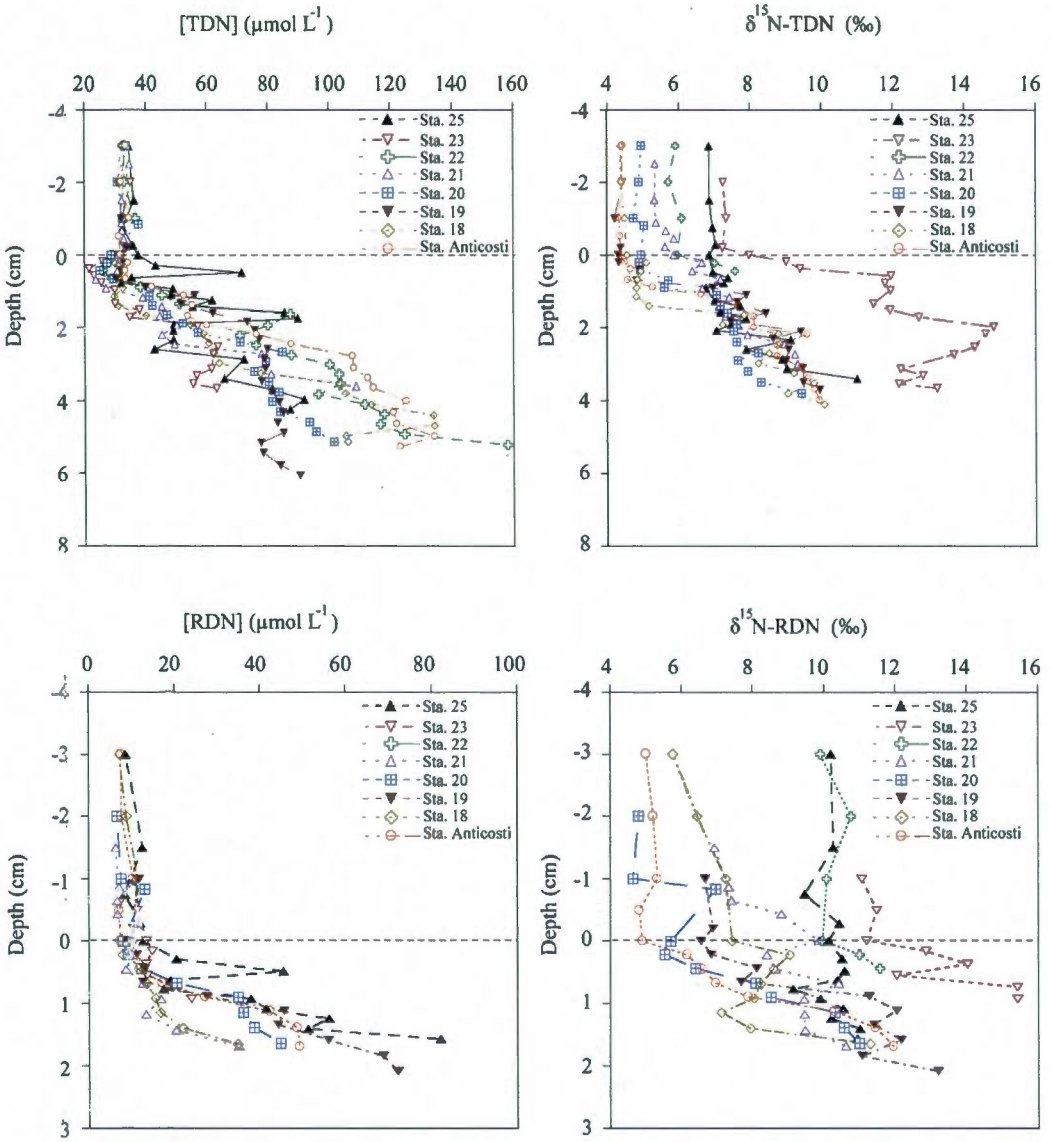

Fig. 3.5. Depth distribution of total dissolved nitrogen (TDN) (upper panels) and reduced dissolved nitrogen (RDN) (lower panels), and $\delta^{15}N$ of TDN and RDN in pore water from sediments in the St. Lawrence Estuary and the Gulf of St. Lawrence.

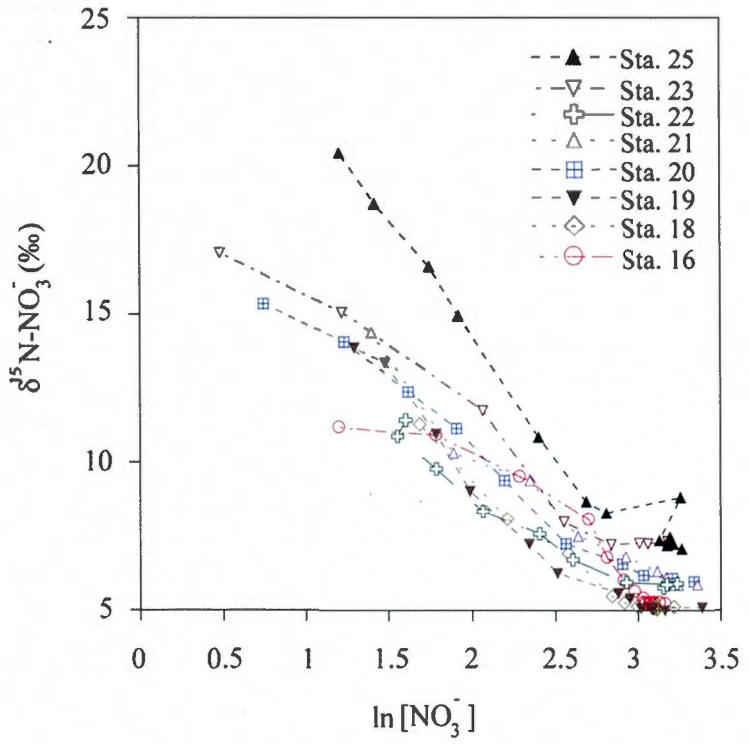

Fig. 3.6. Change of the nitrate $\delta^{15}N$ as function of the natural logarithm of the pore water nitrate concentration. The slopes of the linear regression lines correspond to the nitrate N isotope effects of net nitrate consumption within the sediments ($\varepsilon_{Rayleigh}$). For the St. Lawrence sediments $\varepsilon_{Rayleigh}$ ranged between +3‰ and +7‰.

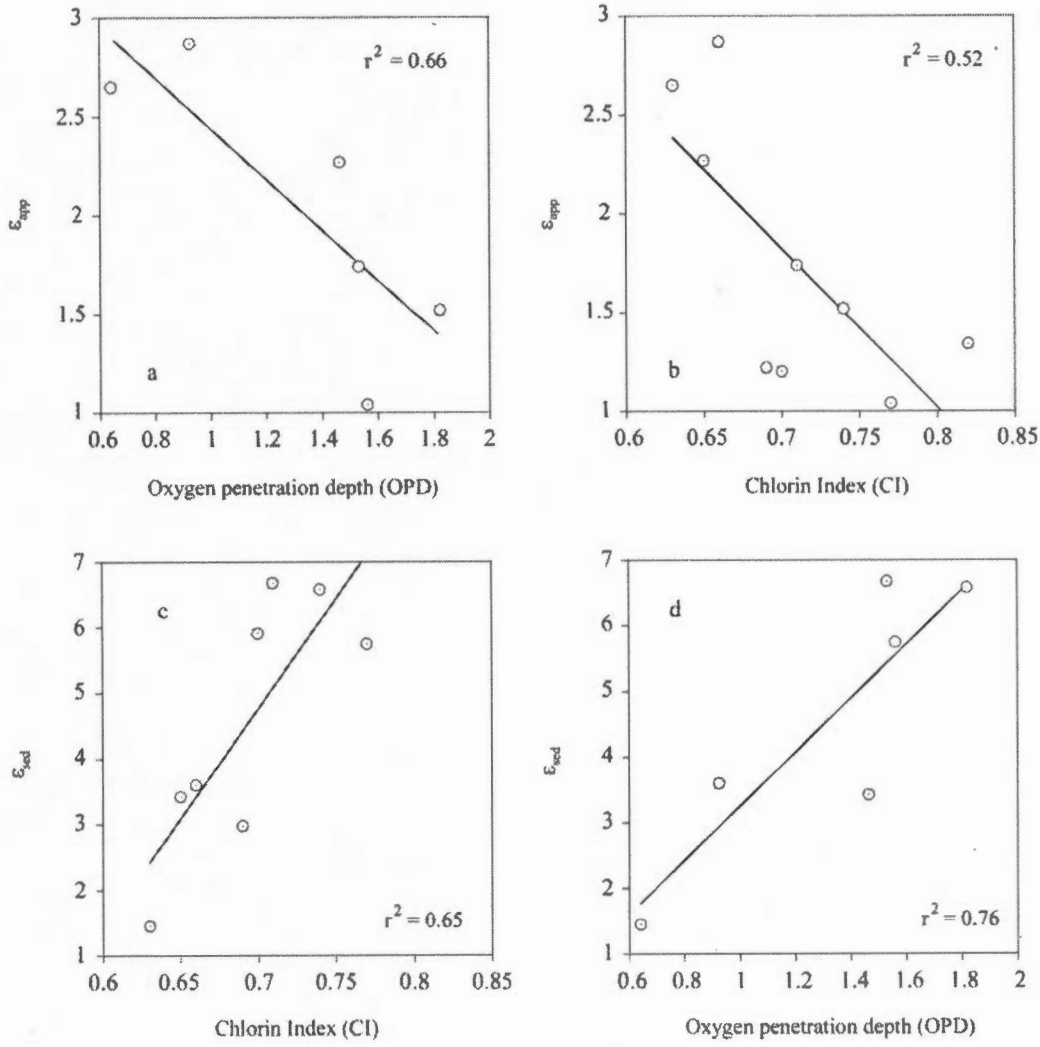

Fig. 3.7. Correlation between ε_{app} and a) the Chlorin Index (CI) and b) the oxygen penetration depth (OPD), as well as between ε_{sed} and c) CI and d) oxygen penetration depth (OPD). CI indicates OM reactivity (Schubert et al., 2005). The CI scale ranges from 0.2 for pure chlorophyll to approximately 1 for highly degraded OM.

GENERAL CONCLUSION

A major goal of marine biogeochemistry in the coming decade will be to accurately predict the response of the marine sediment processes to the high inputs of land derived nutrients and both particulate and dissolved organic matter (POM and DOM) resulting from climate-driven shifts in precipitation and runoff. These inputs will most likely induce higher pelagic algal primary productivity and as a result higher inputs of POM to estuarine and coastal sediments, with consequences on the development of hypoxia, potentially exacerbating coastal eutrophication by returning significant amounts of nutrients and DOM to the water column as sediment flux. The effectiveness of such predictions will depend greatly on our quantitative understanding of the fate of this POM, and of the mechanisms that control its diagenesis in the sediments.

In this dissertation I presented a comprehensive geochemical dataset on sedimentary particulate organic matter (POM) composition and quality, concentrations and fluxes of dissolved organic nitrogen (DON) and carbon (DOC) and the inorganic nitrogen species (NH_4^+ and NO_3^-), and the N isotopic composition of nitrate and the total dissolved nitrogen in the sediment porewater profiles. The purpose of this study was to investigate the sedimentary POM reactivity and factors that determine the distribution of reactive POM in the sediments along the St. Lawrence estuarine-marine gradient. In addition, this study aimed to assess the interactions and links between POM reactivity and nutrient and DOM diffusive fluxes, their $^{15}N/^{14}N$ distribution, and the total sediment N isotope effect. Although this study was originally motivated by the need to better understand hypoxia in the bottom waters of the St. Lawrence Lower Estuary, the implications of our results are not limited to the St. Lawrence, but rather address fundamental questions on POM diagenesis, DOM preferential degradation and significance, and the N budget and balance both at regional and global oceanic N cycle scales.

This study has been the first to assess and quantify sedimentary POM reactivity along the St. Lawrence terrestrial marine gradients. The sedimentary POM reactivity and the importance of POM sources versus processing in determining the sediment POM characteristics and reactivity along the St. Lawrence estuarine-marine gradients are presented in the first chapter of this thesis. The various compositional characteristics of the studied sediments suggest a trend towards less reactive material from the Upper Estuary towards the Gulf, while geochemical evidence highlights the transition of a more terrestrial to a more marine-dominated sedimentation along this transect. The terrestrial OM has traditionally been considered to be less reactive than the aquatic autochthonous marine OM, but in this study we provide an example of how the processing of OM in the water column and within the sediments was much more important than the source of OM in determining the reactivity of sediment OM. This in turn provides further insight into the role that terrestrial OM plays in the productivity of coastal marine systems. In addition, several lines of research from the Gulf of Mexico point out the potential importance of relatively reactive terrestrial OM in driving hypoxic conditions in stratified coastal regions, where continental runoff can carry significant amounts of terrestrial OM (Green et al., 2006; Sarzenski et al., 2008). The results of my thesis research suggest that relatively unaltered land-plant material from the St. Lawrence River and other tributaries discharge into the Lower St. Lawrence Estuary, where it contributes to the relatively high sediment oxygen consumption and thus to the maintenance of hypoxic DO levels in the water column. Our results also suggest that dissolved oxygen and OM vertical fluxes play a role in determining the concentration of specific components in the sediments (chlorins and amino acids), but do not necessarily influence the total $\%C_{org}$ in surface sediments. However, the role of DO on sediment OM diagenesis seems to be modulated by the reactivity of OM supplied to the sediments. In this regard, the exposure time of organic particles to oxic conditions within the sediments (OET; which is a function of both bottom water $[O_2]$ and OM vertical flux), correlated significantly with OM reactivity in the sediments. This suggests

that bottom water [O_2] *per se* is not necessarily the correct metric with which to examine these effects, and that OM degradation rate and extent were largely controlled by the "quality" of available organic substrate, as opposed to the relative supply of different electron acceptors.

In the second chapter of this thesis I explore the DOM in porewaters along the LC using samples collected by two methods, the whole core squeezing (WCS) and the Rhizon membrane methods. While diffusive fluxes of DON increased as a function of POM reactivity and vertical flux along the LC, DOC diffusive fluxes did not vary significantly along the LC, under different conditions of sediment POM reactivity, vertical OM flux, and bottom waters [DO]. This suggests that production and consumption of labile DOC components proceed at similar rates irrespective of what the overall benthic activity is. The observation that [DOC] decreases as the bottom water mass moves westwards along the LC further confirms the close coupling between DOC production and consumption inside the sediments, and implies that the sediments along the LC function as a sink for bottom water OM, including DOC. This trend may reflect C-limitation of benthic bacteria at the hypoxic/low [DO] locations (Sta. 25, 23, 22, and 21), as the DOM molar carbon to nitrogen ratio (C/N_{DOM}) was substantially lower than the Redfield ratio (~6.6; Redfield 1958) in those sites. It has been previously suggested that initial hydrolysis of freshly deposited POM in surface sediments produces DOM with C/N ratios lower than that of the deposited POM itself (Blackburn et al., 1996; Burdige and Zheng 1998; Weston et al., 2006). The discrepancies between the sedimentary C/N of DOM and C/N of POM (C/N_{DOM}-C/N_{POM}) along the LC transect suggest that C- vs. N fractionation is correlated with POM reactivity and its oxygen exposure time (OET). Reactive POM that is apparently of low C/N is available for hydrolysis independently of [DO], while effective hydrolysis of refractory or less reactive POM with higher C/N may require higher exposure to DO.

DON has been seldom considered in studies of estuarine and coastal nitrogenous fluxes. Previous benthic studies have focused on inorganic N fluxes and have not included DON fluxes as a source of available N to pelagic productivity. In this study we show evidence that DON fluxes out of the sediments along the LC transect are the dominant source of N from the sediments and are thus a significant component in the sedimentary N budget, likely playing a major role in shaping pelagic productivity. DON fluxes accounted for 30-64% of the total sediment denitrification rates, and for 60 to 150% of the total nitrate fluxes within the sediments along the LC. The DON fluxes along the LC are within the range reported by other studies from different continental shelves and coastal marine environments, and extrapolation of these DON flux rates suggests that the total benthic DON flux accounts to 48 Tg N yr^{-1}. This estimation is ~1.5 times higher than the estimated riverine DON input to the ocean, and half to one third of the total oceanic biological N_2 fixation. Therefore we argue that benthic DON fluxes represent an important source of reduced N to estuarine systems and to the oceanic N budget as a whole.

The third chapter investigated the N-isotope fractionation effect associated with denitrification and organic matter remineralisation in the sediments of the St. Lawrence Estuary. The apparent nitrate N isotope effect of benthic denitrification at the scale of sediment-water exchange (ε_{app}) has been suggested to be lower than the organism-level N isotope effect for denitrifiers in cultures (ε_{cell}; 20‰ and 30‰; Granger et al., 2008), if denitrification is limited by the rate of diffusive nitrate supply to the active denitrification sites within the sediments (Brandes and Devol, 1997; Lehmann et al., 2004, 2007). While the ε_{app} values that we report in Chapter 3 confirm the systematic under-expression of ε_{cell} that has been shown in other environments (ε_{app} <3‰), our study further shows that that ε_{app} values vary as a function of sediment POM reactivity and oxygen penetration depth (OPD).

More generally, these results clearly demonstrate that it is ultimately the combined net flux of N, including the sediment water exchange of reduced dissolved N species (i.e., RDN = NH_4^+ + DON, and TDN = NO_3^- + RDN) what shapes the isotopic impact of benthic N elimination on the water column of a marine environment. The isotopic effect of RDN fluxes has not been data previously addressed using empirical observations, and to date, ammonium and DON efluxes from sediments have not been investigated in terms of their N-isotopic composition. Partial nitrification may, while generating ^{15}N-depleted nitrate within the porewater DIN pool, produce porewater ammonium that is strongly enriched in ^{15}N. If this ammonium escapes to the water column, it can shunt significant amounts of "heavy" fixed N to the water column, an aspect that has until today been neglected in global or regional N isotope balances.

This thesis presents the first estimates of the combined sedimentary N isotopic effect (ε_{sed}) that incorporate actual measurements of $\delta^{15}N$ of porewater RDN. We show that in the St. Lawrence sediments, the combined benthic N transformations yield a flux of ^{15}N-enriched RDN that can significantly enhance ε_{sed}. Calculated ε_{sed} values were within the range of 4.6 ± 2 ‰, and were related to organic matter reactivity and oxygen penetration depth in the sediments. These parameters all modulate the amount and the $\delta^{15}N$ of the regenerated N that is ultimately denitrified. The mean value for ε_{sed} in the St. Lawrence sediment is larger than assumed by earlier work, questioning current ideas with regards to the balance of the modern N budget in this system. More generally, these values of ε_{sed} result in inconsistencies with current N isotope budgets, because they imply a greater sediment-to-water column denitrification ratio and, thus a greater overall imbalance in the global N budget than currently considered.

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