QUANTIFYING NITROGEN LOADING AND CORRESPONDING EUTROPHICATION SYMPTOMS IN 7 BAYS IN EASTERN NEW BRUNSWICK, CANADA

by

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Submitted in partial fulfilment of the requirements
for the degree of Master of Science

at

Dalhousie University
Halifax, Nova Scotia
January 2015

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Abstract

Anthropogenic nitrogen loading has been identified as a significant cause of seagrass decline worldwide. In the Southern Gulf of St. Lawrence, eelgrass (*Zostera marina*) is the dominant macrophyte in shallow coastal bays and designated an ecologically significant species, yet is increasingly threatened by eutrophication. This thesis applied a Nitrogen Loading Model (NLM) to estimate the magnitude and sources of nitrogen loading in 7 bays in eastern New Brunswick and linked model outputs to eutrophication symptoms and eelgrass bed structure in each bay. Nitrogen loading rates and the proportion of wastewater loading were significantly correlated with eelgrass tissue nitrogen content and isotope signatures. Additionally, higher nitrogen loading rates and longer bay flushing time were linked to elevated eutrophic symptoms, including epiphyte cover and microphytobenthos concentrations, and differences in eelgrass bed structure. This research can inform effective and targeted nutrient management and land-use planning in the region.

List of Abbreviations and Symbols Used

Δ	Delta (Uppercase)- used for isotope notation
Δ	Delta (Lowercase)- used for Delta-N model notation
μ	micro (unit of measurement: $1x10^{-6}$)
BSS	Baie St. Simon Sud
BT	Bouctouche
C	Carbon
Chl*a*	Chlorophyll a
CN	Cocagne
C:N	Carbon: Nitrogen ratio
DAAF	Department of Agriculture Aquaculture and Fisheries (New Brunswick)
DEM	Department of Energy and Mines (New Brunswick)
DFO	Department of Fisheries and Oceans (Canada)
DNR	Department of Natural Resources (New Brunswick)
DOH	Department of Health (New Brunswick)
ESS	Ecologically Significant Species
HABs	Harmful Algal Blooms
HAC	Hierarchical Agglomerative Clustering
KB	Kouchibouguac
LM	Lamèque
N	Nitrogen
NB	New Brunswick
NEEA	National Estuarine and Eutrophication Assessment
NH_3	Ammonia
NH_4^+	Ammonium
NLM	Nitrogen Loading Model
nMDS	non-metric Multi Dimensional Scaling
NO_2^-	Nitrite
NO_3^-	Nitrate
NO_x	Nitrous oxides inclusive of Nitrate and Nitrite
P	Phosphorus
PEI	Prince Edward Island
PIM	Particulate Inorganic Matter
POM	Particulate Organic Matter
RB	Richibucto
SD	Standard Deviation
SE	Standard Error
SNB	Service New Brunswick
SST	Sea Surface Temperature
TB	Tabusintac
TPM	Total Particulate Matter
US	United States of America
USEPA	US Environmental Protection Agency

Acknowledgements

I would firstly like to thank my supervisor, Dr. Heike Lotze for her guidance, support, positivity, and drive which helped me complete this research. Additionally thank you to Inka Milewski of the Conservation Council of New Brunswick for her guidance, framework, previous research, and support with the nitrogen loading model. To the other members of the Lotze lab, Lauren Kay, Allison Schmidt, Nakia Cullain, Tyler Eddy, Scott McCain, Kristen Wilson, Mizuho Namba and others, you were essential components of the field survey, success, and enjoyment of this research and I extend my sincerest thanks. I would also like to recognize John Lindley for his help and support throughout the field surveys in 2013. Thank you to members of the New Brunswick Department of Environment and Local Government (Francis leBlanc) who provided me with wastewater effluent measurements that were integral to the model application. I thank the members of the New Brunswick Department of Natural Resources (Giselle Gaudet, Danny Crain) for supplying the digital data necessary for the completion of the model application. Thank you to Dr. M Niles, Dr. M Ouellette, Dr. S Doiron and Dr. M-J Mallait for supplying me with data on aquaculture lease area and density. A large thank you to the community watershed ground in the region, namely the Shediac Bay Watershed association, Broken River association, Friends of the Kouchibouguac, The Tabusintac Watershed Association, and Coalition pour la viabilité de l'environnement de Shippagan et les îles Lamèque et. Miscou. Lastly I would like acknowledge Dalhousie University and the National Science and Engineering Council of Canada for providing the funding that made this research possible.

Chapter 1: Introduction

1.1 Background

Seagrass ecosystems in coastal waters contribute important ecosystem services to both the marine environment and human populations, which derive a range of social, cultural, economic, and environmental benefits from them (Johnson et al. 2002, Hanson 2004). They sequester and store globally significant quantities of carbon, surpassing the capacity of tropical forests per square km (Fourqurean et al. 2012, Duarte et al. 2005). Other habitat services include nutrient cycling, provision of habitat for commercially important fish and invertebrate species, and sediment and coastline stabilization (Hemminga and Duarte 2000, Orth et al. 2006, Schmidt et al. 2011). Despite these critical benefits that seagrass ecosystems offer, they are threatened by primarily anthropogenic impacts including pollution, coastal development, invasive species, and changing ocean temperatures and pH levels (Lotze et al. 2006, Orth et al. 2006, Waycott et al. 2009, Short et al. 2011). Particularly nutrient loading has been identified as a major contributor to the reduction of seagrass health and cover worldwide, with losses of up to 90-100% recorded in some highly impacted areas (e.g. portions of Waquoit Bay, U.S., Hauxwell et al. 2003, Latimer and Rego 2010).

Although both phosphorus (P) and nitrogen (N) are essential and often limiting nutrients for seagrass growth, in marine and estuarine systems N is understood to be the limiting nutrient (e.g. Howarth and Marino 2006, Bricker et al. 2008). Excessive N loading resulting in eutrophication in eelgrass beds has been well researched and the following effects are generally proposed (see Nixon and Pilson 1983, Bowen and Valiela 2001a, Bricker et al. 2008): increased nutrient loading results in increased primary production in the water column and epiphytic algae, causing decreased light penetration within the water column, direct shading and smothering of eelgrass from algal overgrowth. In seagrass beds the responses can include reductions in shoot density, biomass, nutrient cycling and carbon storage capacity, and decreases in floral and faunal diversity of the seagrass habitat (e.g. Nixon and Pilson 1983, Bricker et al. 2008, Waycott et al. 2009, Schmidt et al. 2012).

In addition to the amount of nutrient enrichment, the residence time of water within a bay will also affect the impact of N loading on the ecosystem. Longer flushing times of coastal embayments are associated with higher Chl*a* concentrations in the water column and more frequent and severe algal blooms (Monsen et al. 2002, Valiela et al. 2004, Bricker et al. 2008). Therefore bays with longer flushing times are more susceptible to eutrophication as a result of N loading than systems that are more quickly cleared (Bricker et al. 2003, Latimer and Rego 2010). Bivalve aquaculture may also impact seagrass habitats, although the type of impacts differ depending on the distance of a seagrass bed from an active lease area and are not easily disentangled (McKindsey et al. 2006, Dumbauld et al. 2009, Vance 2013). For instance, near-field to a suspended aquaculture lease the watercolum may be depleted of phytoplankton, increasing light availability for submerged macrophytes like seagrass (Landry 2002). In some areas bivalve aquaculture has been suggested as a means of eutrophication mitigation for shallow systems because a higher biomass of bivalves would theoretically increase filtration of organic particulate matter. Furthermore nutrients would be removed from the system upon harvesting of the stock (e.g. Haamen 1996, Landry 2002). Yet the increased deposition of N rich detritus and waste from the cultured bivalves can increase the nutrient and organic content of the sediments directly below and beyond the lease (Grant 2005, McKindsey et al. 2006, Dumbauld et al. 2009). Shading from the cages within the leases can lead to the complete disappearance of seagrass from within the lease area (Skinner et al. 2014).

For the purpose of this research I define an increase in eutrophication symptoms and decrease in seagrass health as an increase in the prevalence in some or all of the quantifiable changes in annual algae and seagrass bed structure noted above. Large-scale monitoring programs such as SeagrassNet (www.seagrassnet.org, Short et al. 2006ab), the U.S. National Estuarine Eutrophication Assessment (NEEA, ian.umces.edu/neea, Bricker et al. 2008), and the Assessment of Estuarine Trophic Status (www.eutro.org) assess estuarine eutrophication and seagrass health in ecosystems throughout primarily the United States (U.S.) by measuring standard combinations of these and additional symptoms (e.g. eelgrass shoot width, seagrass growth rate, sediment redox potential) (Bricker et al. 2003, 2008, Short et al. 2006ab). These programs provide a framework with which to compare results of local or regional eelgrass habitat monitoring that employ the measurement of some or all of these quantifiable eutrophic symptoms and seagrass parameters (Bricker et al. 2003, Short et al. 2006a).

1.2 Context of Research

In the southern Gulf of St. Lawrence, shallow coastal bays have sandy or muddy substrate and are dominated by eelgrass (*Zostera marina*). Eelgrass is the only species of seagrass occurring here, and these habitats provide important three-dimensional habitat for commercially important vertebrate and invertebrate species in the region (Senpaq 1990, DFO 2009, Schmidt et al. 2011). In 2009, the Department of Fisheries and Oceans Canada (DFO) designated *Z. marina* an Ecologically Significant Species (ESS) due to the important positive influences it has in coastal ecosystems of Atlantic Canada, distinct from other macrophytes (DFO 2009, 2011). This helped encourage increased research efforts concerning this species, habitat areas, and management initiatives including nutrient loading (e.g. Budgen et al. 2014, Southern Gulf of St. Lawrence Coalition on Sustainability: http://www.coalition-sgsl.ca/). Previous research in the southern Gulf of St. Lawrence has identified nutrient loading as a principal cause of eutrophication with effects including documented declining eelgrass health (St Hilaire et al. 2001, Schmidt et al. 2012, Turcotte-Lanteigne and Ferguson 2013). Until now, however, there has been a lack of specific and quantifiable information regarding the sources and magnitude of nutrient loading in bays along the eastern coast of NB. Yet having a measure of the amount and sources of N entering from upland watersheds is essential to mitigate and reduce potentially negative impacts in coastal waters (e.g. Valiela et al. 1997ab, Beck et al. 2001, Johnes 2013).

This research is the first to quantify the magnitude and sources of N loading in eastern NB and investigate whether present levels of N loading are resulting in eutrophication and changes to eelgrass habitats in this region. I contextualize our results by comparing both our N loading estimates and the magnitude of eutrophic symptoms measured to other large-scale monitoring frameworks employing these and similar methods in the United States (e.g. Bowen and Valiela 2001a, Bricker et al. 2003, 2008, Latimer and Charpentier. 2010, Latimer and Rego 2010). The research presented in this thesis helps to identify which combination of factors increases the susceptibility of a bay to eutrophication, and therefore identifies which bays and eelgrass habitats are at a higher risk for negative impacts of nutrient loading. The results will allow managers to make targeted decisions about nutrient reduction (e.g. more stringent wastewater effluent treatment), with the goal of preventing deterioration of eelgrass habitats throughout this region.

1.3 Research Objectives and Thesis Structure

This thesis is structured around two research objectives that are linked:

a) Quantification of N Loading (Chapter 2): the first objective of this research project was to quantify the magnitude and sources of N loading to 7 bays in eastern NB which have previously been identified as being on a gradient of nutrient loading. I apply the Waquoit Bay Nitrogen Loading Model (NLM) to the seven watersheds in this region. Justification for this model selection is found in Chapter 2. I then verify the model results using eelgrass tissue characteristics including N isotope signatures and N tissue content in each bay. Additionally I estimate flushing time of each bay, and integrate it with measures of N loading to assess the combined effects of these factors on N content and isotope characteristics of eelgrass tissue. The results are discussed in the context of N loading and sources in Atlantic Canada and the United States.

b) Linking N loading to eutrophication symptoms and impacts to eelgrass habitats (Chapter 3): The second objective of this project was to assess whether the estimated annual contributions of N to each of these bays was impacting the health of eelgrass habitats. I conducted an extensive field survey in summer of 2013 to collect eelgrass samples and measure parameters indicative of eutrophication including primary productivity in sediments, water column, and annual algal cover. I used uni- and multivariate statistics to investigate and compare eutrophic symptoms and eelgrass bed structure at each site with respect to site characteristics, including estimates from the NLM, flushing time, sampling depth and bivalve aquaculture lease area and density.

In the general discussion (Chapter 4), I combine all results and discuss the overall eutrophic susceptibility of our eelgrass habitats and bays. I use the NEEA monitoring framework to rank our bays from low to high susceptibility in respect to each other, but also to those throughout the continental United States. I also review how the future impacts of climate change may interact cumulatively with eutrophication in this region. Finally I discuss management implications of our research.

Chapter 2: Land Use and Nitrogen Loading in Seven Bays Along the Southern Gulf of St. Lawrence, Canada

2.0 Abstract

Nitrogen loading from coastal watersheds is a principal factor associated with the decline in health and cover of eelgrass beds in receiving bays. Applying the Nitrogen Loading Model (NLM) framework developed in the Waquoit Bay region, we used site-specific information and mapping of land-use, human populations, wastewater production, and atmospheric deposition to estimate annual input of Total Dissolved Nitrogen (TDN) from different sources to 7 bays dominated by eelgrass habitats. Using physical characteristics of each bay and freshwater inflow we also estimated flushing time and the theoretical annual change in ambient nitrogen concentration Delta(Δ)-N in the bays. We aimed to validate our NLM by analyzing the link between estimated nitrogen loading, flushing time and nitrogen signals in estuarine primary producers using stable isotope and tissue content analysis of eelgrass tissues. Overall, total nitrogen load (kg TDN yr^{1}) was strongly dependent on watershed and bay size, while loading rate per unit watershed area was linked to human population density. Atmospheric deposition was the largest contributor of nitrogen to all bays except Lamèque, where seafood processing effluent was the greatest source. Stable isotope analysis of eelgrass tissue reflected this distinction, with high $\delta^{15}N$ values of 8-10 (indicating wastewater) in Lamèque compared to values of 2-6.5 in other bays. Tissue nitrogen content was positively related to nitrogen loading rate per ha or volume of the receiving bay, highlighting the influence of variable watershed:bay size ratio. Multiple regression identified a significant interaction between nitrogen loading rate per unit bay area and flushing time on eelgrass tissue nitrogen content and isotopes, pointing to mitigating effect quick flushing time of a bay can have on the expression of nitrogen enrichment in primary producers. Our results can inform watershed management and land-use planning, and easily be updated and applied to other bays. Due to the dominance of atmospheric nitrogen deposition in this region, further removal of forest cover and weltands, which have an increased capacity to assimilate and store nitrogen should be minimized, especially in riparian zones bordering watercourses and coastline.

2.1 Chapter 2 Introduction

2.1.1 General Introduction

Anthropogenic nitrogen (N) loading from coastal watersheds is one of the most influential degraders of macrophyte habitats in receiving bays and estuaries worldwide (Hauxwell et al. 2003, Orth et al. 2006, Waycott et al. 2009, Short et al. 2011). Specifically, the consequences of eutrophication on eelgrass (*Zostera marina*) beds are well documented (see Nixon and Pilson 1983, Bowen and Valiela 2001a, Bricker et al. 2008). Increased planktonic, epiphytic and benthic annual algae lead to decreased light penetration within the water column, direct shading and smothering from algal overgrowth, and increased consumption of oxygen at the sediment-water interface from microbial decay of the algal detritus. The resulting impacts on eelgrass beds can include reductions in shoot density and biomass, consequent reduction in nutrient cycling and carbon storage within the beds, and decreases in floral and faunal diversity of the eelgrass habitat. Yet the susceptibility to algal blooms and eutrophication can also be significantly influenced by the residence time of water within the bay or estuary (Ferreira et al. 2005, Valiela et al. 1997b). More extensive distributions, and more frequent episodes of eutrophic concentrations of chlorophyll a (Chl *a*) and harmful algal blooms (HABs) have been shown to occur in systems that have longer flushing and residence times (Monbet 1992, Ferreira et al. 2005, Bricker et al. 2008, Latimer and Charpentier 2010).

In eelgrass dominated bays in eastern NB both point sources (e.g. waste water treatment plants, seafood processing plants) and non-point sources (e.g. agricultural and turf fertilizers, septic systems, atmospheric deposition) of anthropogenic N have been identified as potential contributors to excessive N loading (e.g. Lotze et al. 2003, Therriault et al. 2008, Plante and Courtenay 2008). Yet there remains a lack of specific and quantifiable information regarding the sources and magnitude of nutrient loading in bays along the eastern coast of NB. A biologically verified quantitative estimate of the N loading from both point and non-point sources in individual watersheds is necessary to developing a management strategy for coastal habitat conservation and maintenance (Lajtha et al. 1995). Thus, the purpose of this chapter was to apply a N loading model (NLM) framework to a selection of watersheds and associated bays in eastern NB with various sizes, characteristics and human activities (Figure 1). We use this NLM to achieve the research objectives of: a) Estimating how much Total Nitrogen is entering each of these seven estuaries from both point and non-point sources over a year; b) Determine whether nutrient content and isotopic signatures of eelgrass tissue from each receiving bay can be used to reflect the estimates of the NLM.

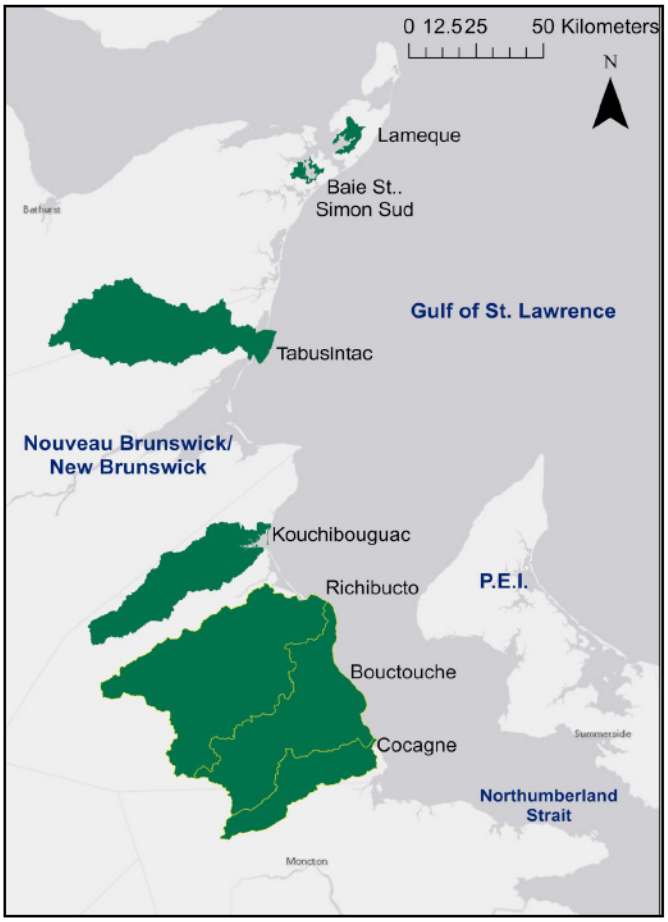

Figure 1. Map of seven watersheds in eastern New Brunswick for which a nitrogen loading model (NLM) was developed and groundtruthed in the associated bays and estuaries. Image created using ArcGIS (ESRI 2011).

2.1.2 Model Selection

Models to estimate N loading are varied and differ in their purpose, scope, precision, and applicability (Valiela et al. 1997a, Daniel et al. 2011). Spatially, N loading models can be classified as lumped, semi-distributed, and distributed. Lumped models (e.g. Valiela et al. 1997a, Fernandez et al. 2002) treat the entire watershed as a unit, over which watershed parameters and variables are averaged (Daniel et al. 2011). In semi-distributed and distributed models the basin is divided into a number of smaller units where hydrological and physical characteristics share similarity, and provide a higher resolution of localized loading (Daniel et al. 2011). For our purposes, we were interested in assessing N loading at an entire watershed scale and a model framework that would be accessible for use by community watershed groups. Furthermore, the limited data available for many of the watersheds we were interested in was more suitable for application of a lumped model.

In the eastern United States numerous models estimating N loading from point and non-point sources have been applied to catchments at both local and regional scales, including the Hydrologic Simulation Program Fortran (HSPF, http://www.epa.gov/ceampubl/swater/hspf), Net Total Nitrogen Inputs (NTNI, Howarth et al. 2012), Nutrient Export from Watersheds (NEWS, Mayorga 2010), SPAtially Referenced Regressions On Watershed attributes (SPARROW, McCrackin et al. 2013), Waiquot Bay Nitrogen Loading Model (NLM, Valiela et al. 1997a, 2000), and Estuarine Loading Model (ELM, Valiela et al. 2004, McCrackin et al. 2013). These models vary in their input requirements (e.g. in situ measurements vs. inferred parameters) and their outputs (e.g. inorganic vs. total N loading), but all have been shown to be effective at predicting N loads to coastal embayments from various land use patterns and point sources. In addition, some model outputs have shown good agreement (e.g. NLM and SPARROW, Valiela et al. 1997a, 2000).

There have also been some efforts to estimate nitrate and total N loading in the Southern Gulf of St. Lawrence, particularly in response to agricultural runoff and resulting severe eutrophication and anoxic events in estuaries on Prince Edward Island (PEI) (Nishimura and Jiang 2011, Jiang et al 2011, Grizard 2013). Most recently a nitrate loading model developed by Jiang et al. 2011 and refined and extended by Grizard (2013) utilizes land use patterns within a watershed coupled with weighted averages of nitrate concentrations and flow rate in river discharge. The model is expanded to watersheds without in situ measurements. The model links high intensity agriculture, characteristic of PEI, with high nitrate loads to receiving estuaries (Grizard 2013).

For the purposes of our research, we were interested in estimates of total N loading to a bay as well as the proportion of N loading from distinct point and diffuse sources within the associated watershed in eastern NB. Agriculture is not as dominant or intensive in this region, and instead point sources of nitrogen, such as seafood processing effluent, have been attributed to severe eutrophication events earlier this decade (Garron and Rutherford 2006, Plante and Courtenay 2008). Therefore, we applied the framework of the Waquoit Bay Land Margin Ecosystem Research (WBLMER) NLM originally constructed and field validated for Waquoit Bay, Massachusetts by Valiela et al. (1997a, 2000). This NLM is a lumped, steady-state model developed to estimate total dissolved nitrogen (TDN) loading, loss of TDN within the watershed, and remaining (excess) TDN entering a receiving coastal water body. We chose the NLM for the following reasons: 1) The NLM has been applied to numerous watersheds in the north eastern United States (Valiela et al. 1997a, 2000, Latimer and Charpentier 2010) and been compared with good agreement to results from other nutrient loading models (e.g. Latimer and Charpentier 2010); 2) The NLM framework is applicable to other coastal watersheds underlain by unconsolidated course-grained sediments, where the delivery of nutrients to receiving waters is primarily via groundwater flow, and land cover is primarily forested, residential, and agricultural (Valiela et al. 1997a); it is therefore applicable to our study watersheds in eastern NB (Rivard et al. 2008, Pronk and Allard 2013); 3) The predictive NLM consists of straightforward additive formulas to calculate the load (kg) of TDN that enters a bay each year from point sources and diffuse non-point sources in that watershed, making the model accessible for watershed groups and managers to both interpret and use; 4) The input data required are all either openly accessibly through municipal and provincial sources, or can be retrieved from governing bodies through direct communication; 5) In the absence of direct measurements of N contribution from individual sources, a model, such as the NLM, that allows the apportioning of N from different sources is vital for developing management strategies that will have the most direct impact.

Specific to this region we added the contribution of N from peat harvesting operations to the NLM framework, as this is an important industry in these watersheds. Peat harvesting can contribute significant nutrient and sediment quantities to receiving water bodies during the preparation of bogs into a harvestable resource (e.g. Klove 2001, Waddington et al. 2009). In NB peat bogs are drained of surface and interally contained water to allow the extraction of the peat by tractors. Therefore, water, nutrients (including N) and sediments, which have been progressively attenduated in the peat bogs since the end of the last glaciation event in the region (≈11000 before present), are released quickly, over the course of 3-10 harvesting seasons (Klove 2001). We consider peat harvesting a point source as the N rich drainage water is directed into holding ponds and then into a freshwater source leading to the bay from a single outfall or drainage ditch (St. Hilaire et al. 2003, Waddington et al. 2009).

2.1.3 Model Application

In our application of the NLM, the amount of TDN entering each bay is predicted by a) calculating the amount of N entering the bay directly from both point sources (municipal wastewater treatment facilities (MWWT), seafood processing plants, peat harvesting, atmospheric deposition on bay surface) and non-point sources (atmospheric deposition on watershed, turf and agriculture fertilizer application, septic systems); and b) using loss parameters defined in Valiela et al. (1997a) to estimate the amount of N from non-point sources lost in the terrestrial surface layer, vadose zone, and aquifer while moving through the watershed. The amount of N lost within the surface layer is dependent on the type and proportion of land-cover within each watershed (e.g. forest cover vs. housing and associated infrastructure). The full NLM model schematic is shown in Figure 2. Overall, the NLM framework provides estimates of total annual TDN loading to a bay, while also revealing which anthropogenic sources are the largest contributors. This is an important first step in understanding how to manage N sources within these coastal watersheds.

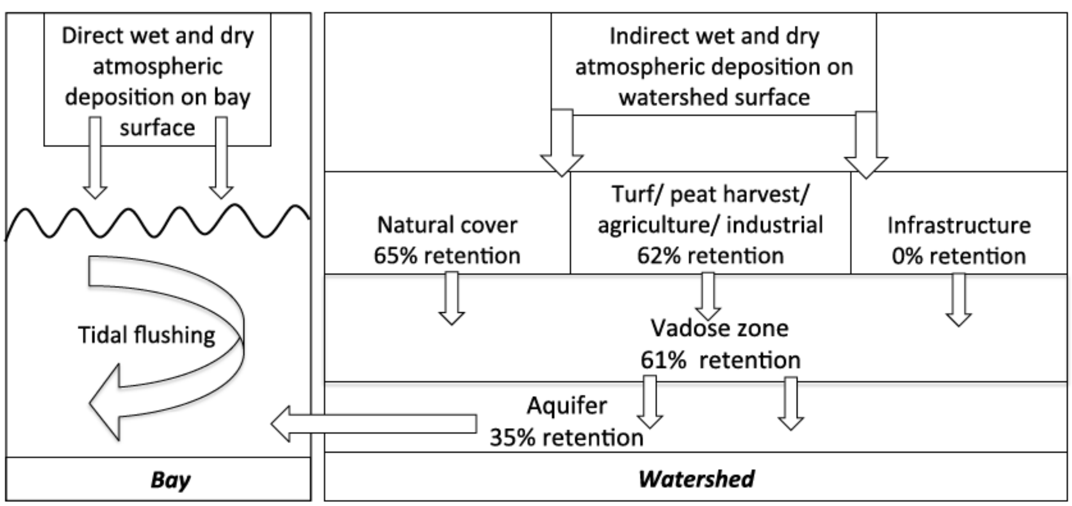

Figure 2. The fate of atmospherically derived nitrogen in watersheds in eastern NB. All direct wet (TDN) and dry (DIN) nitrogen deposited on the bay surface is assumed to be available for use/transformation/flushing within the bay. For indirect atmospheric deposition on the watershed, the percent of wet and dry (TDN) deposited nitrogen retained (i.e. not transported) in each layer

of the watershed is shown. Different losses occur in the surface layer depending on the type of land cover, while there is one loss parameter for the vadose zone and one for the aquifer zone. Image created in Microsoft Word.

Importantly, the final loading estimates from the NLM are in total dissolved N (TDN) and not dissolved inorganic nitrogen (DIN) species such as nitrate (NO_3^-) and ammonium (NH_4^+), which would be available to primary producers in coastal waters. Thus, Valiela et al. (2004) developed an Estuarine Loading Model (ELM), which uses the NLM framework to provide N loads but additionally applies further loss (burial and denitrification) and N-transformation parameters to represent DIN dynamics within different parts of a bay ecosystem (bare sediments, seagrass meadow, wetland cover, water column). The ELM also includes a measure of the flushing rate of the bay. Although estimates of DIN would be valuable for our study region, there was insufficient information regarding the bottom cover of bays in NB, which is an important component of the ELM (Valiela et al. 2004); therefore, we produce estimates of TDN loading but also calculated flushing time in each bay.

Residence time of the excess nutrients can have a significant role in determining how susceptible a coastal system may be to eutrophication (Bricker et al. 2003, 2008). We first calculated simple flushing time for each bay to provide an initial idea of how strong the tidal influence is at the bay-wide scale. We then integrated N loading and tidal flushing to estimate Delta (Δ)-N, the change in N concentration of the bay compared to theoretical oceanic ambient N concentration (Monsen et al. 2002, Bugden et al. 2014). The Δ-N method was created for the Southern Gulf region and links nitrate loading to anoxic events in 34 PEI estuaries (Bugden et al. 2014). The Δ-N model is based on the principle of a Continuously Stirred Chemical Reactor (CSCR, Monsen et al. 2002) and uses theoretical oceanic nitrate concentrations, watershed-specific nitrate loads and flushing characteristics of each bay. It estimates the difference (Δ) in bay vs. oceanic concentration of N that would exist after a certain number of tidal cycles in the absence of other biological, chemical or physical processes within the bay. In watersheds with low human nutrient impact, freshwater has a small/negligible effect on bay N concentrations compared to oceanic input. As N loads from watersheds increase, however, Δ-N becomes more positive. In PEI, a critical Δ-N range was identified between which anoxic events in estuaries are more likely to occur during the spring/summer/fall (Bugden et al. 2014). Nitrate loadings and eutrophic symptoms are in general higher and more severe in PEI than NB due to the more intensive agricultural land use (Schmidt et al. 2012, Grizard 2013). Anoxic events, although previously measured in several NB bays (Robichaud and Doiron 2011), are not as severe as those seen annually in PEI (Grizard 2013); however, other symptoms of eutrophication are present and persisting in NB (Lotze et al 2003, Schmid et al. 2012).

Lastly, to assess the efficacy of our NLM we used eelgrass tissue N content and isotopes to trace the magnitude of loading from different sources to eelgrass habitats within each bay. Previous studies have indicated a positive relationship between ambient available N and tissue N (e.g. Nixon and Pilson 1983, Hemminga and Duarte 2000), and that N from certain human activities or sources has specific stable isotope (SI) signatures (the ratio of $\delta^{15}N$ / $\delta^{14}N$) (McClelland and Valiela 1998, Valiela et al. 1997b, Kendall 1998, Middelburg and Nieuwenhuize 2001, Xue et al. 2009, Schubert et al. 2013). Groundwater influenced by nitrogenous waste from higher trophic levels, including human sewage or animal waste (e.g. MWWT, seafood processing) is normally enriched in $\delta^{15}N$ and has characteristic isotope values (NO_3^- $\delta^{15}N$) between +8 to +20 ‰ (McClelland and Valiela 1998, Cole et al. 2006, Schubert, et al. 2013). Wastewater has characteristically higher NO_3^- $\delta^{15}N$ signatures resulting from volatilization of ^{14}N rich ammonia during initial treatment (Macko and Ostrom, 1994). Anthropogenic nitrate deposited through atmospheric deposition, however, typically has NO_3^- $\delta^{15}N$ values between +2 to +6‰ (e.g. Lepoint et al. 2004, Cole et al. 2006). Groundwater runoff from synthetic fertilizer, which synthesizes natural N_2 from the atmosphere during manufacturing, generally has lower NO_3^- $\delta^{15}N$ values between -4 to +4‰ (Gormly and Spalding 1979, Kendall 1998, Cole et al. 2006). Eelgrass tissue can be used to track and relay the SI signatures present in their surroundings, and despite variable fractionation of nitrate that occurs during assimilation, it has been identified as an effective indicator of nitrate sources to coastal water bodies (McClelland and Valiela 1998). The positive relationship between NO_3^- $\delta^{15}N$ in groundwater and NO_3^- $\delta^{15}N$ in eelgrass tissue is particularly strong when wastewater inflow and septic systems are dominant loading sources (McClelland and Valiela 1998, 1998, Schubert et al. 2013). Thus, we use the tissue N SI signature as a qualitative means of assessing the relative contributions of N from the watershed and compare these data to the NLM results.

This research is timely and relevant because most NB bays still support extensive eelgrass habitat despite consistent eutrophication symptoms (Plante and Courtenay 2008, Schmidt et al. 2012, Turcotte-Lanteigne and Ferguson 2013). Increased eutrophication may result in a decline and ultimate loss of eelgrass habitat in affected bays, which has been documented in numerous New England bays where human impact is high (Bricker et al. 2003, 2008, Latimer and Charpentier 2010, Latimer and Rego 2010). Understanding the magnitude and sources of N loading is critical to develop best management practices, preventing further degradation or loss of eelgrass habitat. Estimates about the relative contribution of N from different human activities will allow local, regional and aboriginal governments, policy-makers, stakeholders, and community watershed groups to make effective decisions regarding how to best manage N loading to the specific bay under their jurisdiction (Johnes et al. 1996).

2.2 Methods

2.2.1 Study Area

This study focuses on seven watersheds and bays along the eastern coast of NB (Figure 1), which will be referenced by an abbreviation (Table 1) and in geographical order from south to north, beginning with Cocagne (CN). Each bay is shielded from the direct circulation of the Northumberland Strait and Gulf of St. Lawrence either by a sand dune spit (CN, BT, RB, KB, TB) or by barrier islands (BSS, LM) (Figure 1). Terrestrially, this area is underlain by unconsolidated sandy, muddy, or silty sediments deposited as glacial till during the late Wisconsonic glacial event (Rivard et al. 2008). Most of the freshwater recharge that reaches the bedrock aquifers travels through this surficial layer (Rivard et al. 2008). This region is characterized by semi-regular diurnal tides due to an amphidromic point near the western exit of the Northumberland Strait (Dutil et al. 2012), and has a modified continental climate (Koutitonsky et al. 2004, Turcotte-Lanteigne and Ferguson 2013). The Gulf itself has estuarine circulation forced by drainage from the Great Lakes and St. Lawrence River, and inflow of deep oceanic water from the North Atlantic through the Laurentian Channel. Along the NB coast surface water velocities are slow (0.06- <0.02 m s^{-1}), and the tidal current ranges from >0.12-0.04 m s^{-1} (Koutitonsky et al. 2004, Dutil et al. 2012).

In each watershed, one or two main rivers drain into the bay except in BSS, which is surrounded by wetlands but no single large freshwater inflow. Estuarine characteristics are present in the lower portions of these rivers and in the sheltered bays (Thibault et al. 2000, DELG 2002) . Eelgrass beds are historically and currently the dominant benthic macroflora throughout these shallow coastal bays (Patriquin and Butler 1976, Thibault et al. 2000). There are small urban centers (generally <5000 persons) throughout the region, but predominantly the population is rural and coastal (Table 1). In 2013, bivalve aquaculture (primarily the American oyster *Crassotrea virginica*) was active in all bays we assessed except for KB and LM.

Table 1. Overview of watershed characteristics including presence or absence of point sources of nutrient loading (Municipal waste water treatment [MWWT] and seafood processing plants). Bay volume is based on average depth from previous research in the region (Patriquin and Butler 1976, Gregory et al. 1993, Plante and Courtenay 2008, Robichaud and Doiron 2011). Watershed population estimates from NB DNR civic address data and Statistics Canada household data (GeoNB 2012, Statistics Canada 2014).

Site [abbreviation]	Watershed area (ha)	Bay area (ha)	Bay volume (m^3)	Population	Pop. density (pers/ ha)	MWWT plant	Seafood plant
Cocagne [CN]	33,246	2,438	2.8×10^7	12,041	0.36	No	Yes
Bouctouche [BT]	76,032	3,813	4.2×10^7	25,868	0.34	Yes	Yes
Richibucto [RB]	128,578	5,118	1.1×10^8	20,693	0.16	Yes	Yes
Kouchibouguac [KB]	53,042	1,458	2.2×10^7	3,368	0.06	No	No
Tabusintac [TB]	71,276	3,666	4.0x 107	5,015	0.07	No	No
Baie St. Simon Sud [BSS]	2,157	833	1.3×10^7	743	0.34	No	Yes
Lamèque [LM]	3,241	1,077	3.3×10^7	2,274	0.70	Yes	Yes

2.2.2 Nutrient Loading Model

2.2.2.1 Watershed and Bay Delineation and Land Use Cover

Service NB (SNB) provides a geographic database (GeoNB) for the entire province (GeoNB 2012), in partnership with numerous provincial government departments and groups. Included in the digital map products is the publically available hydrographic network for NB with information on watercourse and water body identification and parameters (e.g. length, surface area), coastal boundaries and watershed boundaries. We used these watershed boundaries to estimate watershed area (ha) for 4 of the study sites (CN, BT, RB, TB) as they sufficiently contained all freshwater inputs to each respective bay (Table 1). KB, BSS, and LM watersheds, however, all needed further subdivision. Using the ArcGIS 'ArcHydro' toolbox, the Digital Terrain Model (DTM) database from SNB, and watercourse data from GeoNB (ESRI 2011, ESRI 2013, GeoNB 2012) we created an elevation model to predict the flow of surface water in the drainage areas around these three bays. Although water table contours would have been more desirable for this purpose, they are not available for this region. Using surface contours we were able to delineate sub-watershed boundaries specific to each bay using the ArcHydro tools. Additionally, we used the GeoNB hydrographic data to get the surface area for the 7 bays of interest (Table 1) and included only portions of the bay with the designation of 'Tidal water body' (ESRI 2011, GeoNB 2012). The surface area was assumed to be the area of the water body at high-tide (G. Gaudet, NBDNR, pers. comm.).

The NB Department of Natural Resources (NB DNR) provided us with digital land cover and land use data for the region (G. Gaudet, NB DNR, pers. comm.). From these data we were able to extract information on the area of forest (harvested and non-harvested), wetlands, peat lands (harvested and non-harvested), settlement, recreation, industrial, infrastructure, and agricultural areas (Appendix 1: Table 1). Within each of these categories there was more specific land use information available, which we did utilize for determining N contribution from agriculture. The GeoNB and DRN digital data was sourced and verified between 2002-12, depending on the region (NB DNR 2012). As well, Irving Canada provided us with industrial forestry freehold land cover under their jurisdiction, which could be separated into non-forest (harvested) and forested (previous, and/or future harvest) land (G. Pattman, Irving Canada, pers. comm.). We clipped the layers of land use within the watershed boundaries previously defined, and retrieved the area of cover of each type of land use within each watershed (ESRI 2011).

2.2.2.2 Point Sources

Point sources of N are more straightforward to quantify than non-point sources because there is a direct entry point of sewage or discharge into the coastal water body and therefore no loss parameters included in the calculations.

Municipal Waste Water Treatment (MWWT)

TDN contained in wastewater effluent from a treatment facility was quantified using **Eq. 1** (Table 2) (Valiela et al. 1997a), with detailed information on individual treatment operations provided by the DELG in NB (Appendix 2: Table 1).

Seafood Processing Plants

To calculate TDN load from seafood processing plants, no loss parameters were applied to the effluent, as the concentration of TDN is measured post-treatment. We used **Eq. 2** (Table 2) (Valiela et al 1997a,) with site specific information from the DELG in NB (F. LeBlanc, pers. comm.) (Appendix 2: Table 2). In some instances the N concentration of effluent was not known/not measured during the processing of a specific species; in these cases we applied values from the literature based on the type of species processed (Appendix 2: Table 2) (AMEC 2004, Garron and Rutherford 2006)

Peat Moss Harvesting

To quantify the TDN runoff from peat harvesting operations we used the average surface water runoff coefficient (0.3642 ± 0.115 SD) from 11 different peat harvest operations in Canada (Manitoba, Quebec, Newfoundland) and the United States, where measures of surface run off from operations with similar regulations for sedimentation ponds and drainage are in place (Klove 2001, Joensuu et al. 2002, Waddington et al. 2009, Hynninen et al. 2011, Swystun et al. 2013) (Appendix 2: Table 3). We then averaged annual precipitation at each study site from Environment Canada monitoring stations over a10-year period consistent between all sites (1995-2005) (Appendix 2: Table 4). If a station was not available in a specific watershed, we took the average of adjacent watersheds. Finally, we used average concentration (0.372 mg L^{-1}) of total N recorded by St. Hilaire et al. (2004) in runoff from sedimentation ponds from three different sites within the St. Charles Peat Bog in the RB watershed between 1996-2001. We estimate TDN entering the bay after passing through sedimentation pond(s) as the product of runoff from total peat harvest area in a watershed and the concentration of TDN observed by St. Hilaire et al. 2004 (Table 2: **Eq. 3**, Appendix 2: Table 3).

Table 2. List of equations used in the NLM, flushing time, and ΔN calculations. Equations 1-10 are specific to the NLM (Valiela et al. 1997a), equation 11 was used for flushing time (Gregory et al. 1993) and equations 12-13 were used to calculate ΔN (Bugden et al. 2014).

Point Sources	
Eq. 1	*MWWT Nitrogen Loading (kg TDN /yr) = Daily effluent flow rate (L/day) x Concentration of TDN (mg/L) in effluent x Number of days per year in operation*
Eq. 2	*Seafood Processing Nitrogen Loading (kg TDN /yr) = Daily effluent flow rate (L/day) x Concentration of TDN (mg/L) in effluent x Number of days per year in operation*
Eq. 3	*Peat Harvest Nitrogen Loading (kg TDN/yr) = 0.3642 (runoff coefficient) x Total annual precipitation (mm) x 0.372 (Concentration of Nitrogen in water draining from sedimentation ponds and buffer zone (mg/L))*
Non-point sources	
Eq. 4	*Septic System Nitrogen Loading (kg TDN/yr) = 4.19 kg TDN/person/year* x Average persons/house x Number of houses >200 m x 0.6 0 (proportion of N reaching vadose zone) x 0.65 (proportion of N reaching the aquifer) x 0.65 (proportion of N reaching bay seepage face)* *For houses <200 m from shoreline, don't apply last loss parameter (assume no retention of N in aquifer- see methods).*
Eq. 5	*Agricultural Fertilizer Application (kg/yr) = (Manure N 0.75 accounting for volatilization in storage (kg TDN /yr) + (Synthetic Fertilizer N 0.61 accounting for volatilization after application) (kg TDN /yr)) x (Area of synthetic and manure fertilizer use (ha))*
Eq. 6	*Turf and Lawn Fertilizer application (kg/yr) = (150 kg TDN/ha/yr) x (Settlement area (ha) x 0.3) x 0.98 (proportion of property owners reporting a lawn or garden) x 0.3725 (proportion of these properties that report application of fertilizer containing nitrogen) x 0.61 (proportion of fertilizer remaining for uptake and watershed transport after volatilization)*
Eq. 7	*Sum of Fertilizer Nitrogen Loading (kg TDN/yr) = Applied fertilizer Nitrogen from agriculture and turf (kg TDN/yr) x 0.61 (proportion of N reaching vadose zone/ not lost in surface vegetation) x 0.39 (proportion of N reaching the aquifer/ not lost in vadose zone) x 0.65 (proportion of N reaching bay seepage face/ not lost in aquifer)*
Eq. 8	*Indirect Atmospheric Nitrogen Loading to bay (kg TDN/yr) = Dry or wet deposition (kg TDN/yr) x proportion of N reaching vadose zone (depends on surface type) x 0.39 (proportion of N reaching the aquifer/ not lost in vadose zone) x 0.65 (proportion of N reaching bay seepage face/ not lost in aquifer)*
Eq. 9	*Direct Atmospheric Nitrogen Loading to bay (kg TDN/yr) = Dry or wet deposition (kg TDN/yr)*
NLM- Total loading	

Eq. 10	*Total Nitrogen Loading (kg TDN /yr) = Sum of point and non-point contributions of TDN*
Flushing time	
Eq. 11	$$\frac{-12.42}{\ln\left(\frac{\text{Bay Volume}^{**}}{\text{Bay Volume}^{*} + \text{Tidal Volume}}\right)}$$
Delta(Δ)- N	
Eq. 12	$\Delta N = Ne - No = (Qf \times (Ngw - No)) / (Qo + Q_f)$ ***
Eq. 13	$\Delta N \sim L / (Qo + Qf)$ ****

* From USEPA estimate (USEPA 2002)

** Calculated using average depth from previous research in the region (Patriquin and Butler 1976, Gregory et al. 1993, Plante and Courtenay 2008, Robichaud and Doiron 2011).

*** Where:

> *Qo* is the rate of inflow of oceanic water at nitrogen concentration *No*
> *Qf* is the rate of inflow of freshwater (ground water) at nitrogen concentration *Ngw*
> *Qe* is the rate of outflow of estuarine water at nitrogen concentration *Ne*
> Or, if *Ngw* > *No*, or fresh water nitrogen concentration is much greater than the oceanic concentration due to terrestrial and atmospheric anthropogenic nutrient addition.

**** Where L is our predicted loading to the bay (from NLM), *Qo* and *Qf* are as above.

2.2.2.3 Non-point/ Diffuse Sources

Loss Parameters

The NLM considers N from non-point sources (septic systems, agriculture and turf fertilizer, atmospheric deposition) to enter the coastal water body through primarily groundwater transport, and not overland transport/surface runoff. Exceptions to this would occur throughout the year, for instance during heavy rainfall events or spring melting. N from non-point sources that is transported by groundwater is subject to retention, dilution and transformation as it traverses the vegetative layer, vadose zone (saturated soil layer), and the aquifer. Therefore, we apply loss parameters supplied by the WBLEMR NLM to represent this loss of N between initial entrance into/onto the watershed and entrance into the receiving bay (Valiela et al. 1997a, 2000). These loss parameters are explained for each source below, and shown in Table 2.

Septic systems

Septic system loading calculations were done using the per-capita method (see below), and were split into two components based on the proximity of a civic address to the water's edge (Koppelman 1982, Valiela et al. 1997a). Losses of N within septic systems can be variable due to the age of the system, integrity of the system, water flow into the system, and the soil/ground type the system is installed in (Valiela et al. 1997a, Bowen and Valiela 2001a, NB DOH 2013). The WBLEMR model uses both available literature values and in-situ results and estimates the average loss of TDN within the leaching field as a result of denitrification, volatilization, and adsorption of ammonium to sediment particles (40% loss, Table 2: **Eq. 4**) (Valiela, et al. 1997a). Of the wastewater N that does extend in plumes beyond the leaching field into the unsaturated zones, 35% may be diluted, transformed or lost while the plume maintains integrity (≈200 m), while the remainder can be transported into groundwater (Table 2: **Eq. 4**). With additional distance from the septic leaching field, wastewater N may enter the aquifer, or disperse within the vadose zone towards the seepage face. Within the aquifer, additional uptake and retention of wastewater N may occur from denitrification and general dilution, and this additional loss term is estimated to be 35% (Table 2: **Eq. 4**) (Valiela et al. 1997a). Therefore, houses <200 m to the bay edge are likely a greater source of wastewater N than houses further removed.

Using ArcGIS we first identified the number of civic addresses (registered private properties in a municipality or rural service district) outside of a municipal area serviced by MWWT. We then identified the civic addresses that were <200 m from the water's edge, and those >200 m (ESRI 2011, NB DNR 2012). As per the per-capita method we multiplied the number of civic addresses by the average number of people per household in each watershed (Statistics Canada 2014), and then multiplied this figure by the USEPA average N loading to a septic system per person, per year (4.19 kg TDN $person^{-1}$ yr^{-1} , (USEPA 2002)) (Table 2: **Eq. 4**). For houses >200 m, the TDN load was multiplied by the loss factors 0.60 **x** 0.65 **x** 0.65, which represents the proportion of TDN not retained/consumed in the septic tank, vadose zone, and aquifer, respectively, while it is transported to the bay seepage face. For houses <200 m the last loss factor (0.65) was omitted as the wastewater is not expected to disperse into the aquifer in the 200 m between the septic system and seepage face (Valiela et al. 1997a) (Appendix 2: Table 5).

We took into account the increased use of seasonal dwellings in the summer due to tourist influx. Based on numbers of tourists and non-owner occupied dwellings in each region we estimate that 10% of all civic addresses in each watershed were only used for half the year, effectively reducing the annual N contribution of these residences in half (Statistics Canada 2014, NB Tourism 2013, 2014). Using civic address data may overestimate households on septic systems within a watershed, as some civic addresses may not have a building on them. Others that are non-households may be commercial, industrial or recreational sites that have a septic system present and in use by persons residing both within and outside of the watershed.

Agriculture and turf fertilizer addition

To determine N loading from fertilizer use within the watershed, we estimated 1. fertilizer addition to agriculture (Table 2: **Eq. 5**), 2. fertilizer applied to turf/lawn (Table 2: **Eq. 6**), and 3. the sum of all fertilizer use (Table 2: **Eq. 7**). Existing regulations in NB prevent farming and land alteration activities such as manure storage and fertilizer application from taking place within a 30 meters landward of coastal features/waterways (DELG 2002). We therefore calculated fertilizer N loading as a non-point source, assuming that all agricultural and turf/lawn fertilizers are subject to volatilization once applied, uptake by the vegetative layer, loss within the unsaturated zone and loss within the aquifer (Valiela et al. 1997a, Latimer and Charpentier 2010) and applied these respective loss rates to the sum of all fertilizer input (see below).

1. Agricultural fertilizer inputs:

To quantify TDN from agriculture we first had to distinguish between two principal sources: synthetic fertilizers and manure. Statistics Canada reports the agricultural area within each local district which have either synthetic or manure fertilizer applied (Statistics Canada 2011).

To estimate TDN loading from manure fertilizers, we used values from Yang et al. (2011) who estimated the amount of TDN in manure produced in the province by area each year (kg TDN ha^{-1} yr^{-1}). We account for manure N as much of the feed surpluss to grass for grazing (grain etc) for cattle and other livestock in these watersheds is brought in from outside the region (Yang et al. 2011). Because manure is often stored before it is applied as a fertilizer there is some amount of N lost due to volatilization. We took the average proportion of N lost from different storage facilities (e.g. open, unlined pit vs. covered and lined shed, average = 25% loss due to volatilization) (Appendix 2: Table 6), and multiplied remaining proportion by TDN in original manure produced to get an estimate of TDN applied to surface pasture or crops as fertilizer from manure (Table 2: **Eq. 5**) (Yang 2006, Huffman et al. 2008, Yang et al. 2011).

To quantify TDN loading from synthetic fertilizer we first used the NB fertilizer application guidelines to estimate the quantity of fertilizer applied (kg TDN ha^{-1} yr^{-1}) to each type of crop within the province (NB DA 2001, Appendix 2: Table 7). Due to the variety of crops present in each watershed we took the average recommended fertilizer N application amount for all crop types (Statistics Canada 2011) and multiplied this value by the area in each watershed that has reported synthetic fertilizer application (Canada 2012) (Table 2: **Eq. 5**) (Valiela et al. 1997a). Whereas volatilization of nitrates and ammonia occurs principally in storage for manure fertilizer, volatilization of up to 39% of synthetic fertilizer N occurs after application and before uptake by vegetation or transport beneath the soil surface. Therefore, we multiplied the total volume of synthetic fertilizer applied in each watershed by 0.61 to account for this loss (Valiela et al. 1997a, Table 2: **Eq. 5,** Appendix 2: Table 6).

2. Turf and lawn fertilizer inputs

To calculate the fertilizer contribution from lawn and garden practices we first had to estimate the surface area of the province that was in use as a lawn or garden. We used ArcGIS and settlement areas delineated by Service NB to estimate total settlement area within each watershed (ESRI 2011, GeoNB 2012). Using Google maps satellite imagery we then estimated the average proportion of property that was lawn and or garden throughout the region (30%), and multiplied total settlement area by this proportion (Google maps, 2014). Next, Statistics Canada provided information on the proportion of households that have a garden or lawn and the number of these households that apply synthetic or organic fertilizer N. Between 2005-2011, 98% of households reported having a lawn or garden, and the proportion of these households that applied organic or synthetic fertilizers containing N was 37.25% (Statistics Canada 2012). Finally, we used the recommended fertilizer application guidelines for NB of 150 kg TDN ha^{-1} yr^{-1}(NB DA 1989) and multiplied this by the proportion of settlement area with a lawn or garden where fertilizer application is reported (Table 2: **Eq. 6**, Appendix 2: Table 8).

3. Sum of all fertilizers

Loss parameters were applied to the sum of all applied fertilizer (manure, synthetic, turf/lawn) within a watershed to account for losses/uptake/transformation of N in the vegetative layer (39% retained, 61% transported to vadose zone), vadose layer (61%

retained, 39% transported to aquifer), and aquifer (35% retained, 65% transported to bay) (Table 2: **Eq. 7**).

Atmospheric deposition

N from the atmosphere can be deposited through wet deposition (precipitation) and dry deposition (aerosolized particulate matter) on both the terrestrial and water surface of a watershed (McClelland and Valiela 1998, Valigura et al. 2001, Castro and Driscoll 2002). N loading from terrestrial deposition is referred to as indirect loading, as this N must traverse the watershed vegetative or surface layer, unsaturated zone, and aquifer before reaching the bay seepage face (Valiela et al. 1997a) (Figure 2). Direct atmospheric deposition refers to N deposited on the surface of the receiving body of water, and no loss terms are applied to this N source as it is considered to be immediately available for biological use, transformation or removal through tidal flushing within the bay (Bowen and Valiela 2001b, Valiela et al. 2004, Castro et al. 2013)

1. Indirect atmospheric deposition

We used the Canadian National Atmospheric Chemistry Database (NatChem 2012) to get average wet deposition rates of nitrate (NO_{3-}) and ammonium (NH_4) from weekly monitoring sites along the east coast of NB. Two stations were located within our study region: Harcourt provided deposition estimates for the southern watersheds (CN, BT, RB, KB) and Petit-Paquetville for the northern watersheds (TB, BSS, LM). We used the average weekly nitrate and ammonium deposition volumes between 1992-2008 (Appendix 2: Table 9). Nitrate deposition has been fluctuating but generally decreasing since 2002 in both locations, while the rates of ammonium deposition are less than nitrate, and show a more stable trend (NatChem 2012).We used the average rate between 1992-2008 instead of taking only recent deposition values as we wanted to account for currently undefined residence times of N within the watersheds of this region. We adjusted precipitation and N deposition estimates according to regional rates of evapotranspiration, which will reduce the volume available for both overland and groundwater transport (Shiau 1968, Lajtha et al. 1995).

Though nitrate and ammonium is measured in wet deposition along the eastern coast of NB, no sites measured particulate dry deposition or DON. Because atmospheric deposition is site-sensitive and can be affected by salt content of coastal air, local climate, wind and both local and far-field contributing activities, we did not use measured dry deposition available from the west coast of NB. Instead, we used a 1:1 ratio for wet to dry deposition on terrestrial surfaces based on research throughout New England and Eastern Canada (Valiela et al. 1997a, 2000, Valigura et al. 2001). This could slightly overestimate dry deposition in some areas because of the increased surface area of natural land cover versus unremarkable surfaces like pavement and rooftops (Valiela et al. 2004). To estimate the proportion of DON deposited through wet deposition we used a relationship between DIN, TDN, and DON averaged from literature data, which assumes DON is 30% of TDN (the other 70% is assumed to be DIN) (Valigura et al. 2001, Valiela et al. 2004, Latimer and Charpentier 2010). We did not include DON in any dry deposition estimates due to a deficit of knowledge and available research at this time. Therefore, dry deposition estimates only reflect the 1:1 wet:dry ratio of NO_{3-} and NH_4 measured at the NatChem stations.

To apply appropriate loss parameters to atmospheric N within the watershed we compiled information on land use within each watershed (see 2.2.1 above, Figure 3, 4, Appendix 1: Table 1). For instance, naturally vegetated land will consume and retain a proportion of deposited N, while runoff from impervious surfaces generally directs deposited N to freshwater and the vadose zone without losses at the soil surface (Figure 2) (Valiela et al. 1997a). Thus, we applied loss parameters at the vegetative layer, vadose zone, and aquifer to N deposited on the watershed surface from wet and dry deposition. At the surface layer, the NLM assumes 65% retention (35% transported to vadose zone) on surfaces with natural cover, 62% retention (38% transported to vadose zone) on agricultural, peat harvest, cleared forestry lands, and industrial lands, and 0% retention on infrastructure lands (Figure 2, Table 2: **Eq. 8**). N that has leached through the surface/vegetative layer is then subject to loss/transformation in the unsaturated zone (61% lost, 39% transported to aquifer), and aquifer (35% lost, 65% transported to bay) (Table 2: **Eq. 8**, Appendix 2: Table 9).

2. Direct atmospheric deposition

Direct deposition required no loss parameters in the model, and therefore is more straightforward (Figure 2). There are no regional velocity deposition estimates for Atlantic Canada, however, meaning the airborne concentration of particulate and aerosol

N cannot be computed to a deposition rate in this region. Therefore, we based rates of direct wet deposition on indirect wet deposition and literature data (Valigura et al. 2001, Castro and Driscoll 2002, Valiela et al. 2004, Castro et al. 2013). We assumed that rates of wet deposition of NO_3, NH_4, and inferred DON (see above) on the bay surface were equal to rates on land (Valiela et al. 2004). Similar to indirect dry deposition, we did not include DON in the quantification of direct dry deposition. We reduced the rate of DIN direct dry deposition to 70% of indirect dry deposition based on literature data to account for the decreased surface area available of the water relative to land cover (Appendix 2: Table 9) (Valigura et al. 2001, Castro and Driscoll 2002).

2.2.2.4 NLM output calculations

Once applicable loss parameters were applied to all non-point sources we added the volume of TDN from all point and non-point sources within a watershed to produce a cumulative estimate for the amount of N entering each bay (Table 2: **Eq. 10**). To calculate N loading rates we used the total annual N load (kg TDN yr^{-1}) and divided by the respective area (ha) of the watershed or bay, or the estimated volume (m^{-3}) of each bay.

We obtained estimates of error for some sources of N loading based on data provided for multiple years and from multiple sources. For instance, we took the average nitrate and ammonium deposition from precipitation between the years of 1992- 2008, and therefore have the measured variation in N deposition over this time period. Similarly, most MWWT and seafood processing facilities were able to provide effluent nutrient characteristics for multiple years for most facilities, allowing us to use an average value for N concentration in effluent in our calculations, and to calculate the variance in loading over the time period (Appendix 2: Table 1,2). Because we did not have a runoff coefficient for peat harvest operations in NB, we took the average coefficients from other North American operations on the Canadian Shield. The minimum and maximum N loading from peat harvesting represent the standard deviation in both this runoff coefficient and precipitation amounts between 1995-2005 (Appendix 2: Table 3,4). Because other estimates of N concentrations and contributions from different sources (agriculture, turf and agricultural fertilizers, septic systems) were taken from the literature or Statistics Canada, with no range or error rate provided, we were not able to calculate a range in potential loading for these sources.

2.2.3 Bay Susceptibility

2.2.3.1 Tidal flushing time

To estimate the flushing time for each bay we used methods specified by Gregory et al. (1993), which calculate the time required to reduce the concentration of a tracer throughout a bay to a third of its initial concentration (Table 2: **Eq. 11**). This required information of high tide bay volume, tidal volume, and tidal period (Appendix 2: Table 10). We calculated the bay volume using surface area from GeoNB hydrogeographic data, and available average depth measurements from previous research and reports of the area (Patriquin and Butler 1976, Gregory et al. 1993, Plante and Courtenay 2008, Robichaud and Doiron 2011, GeoNB 2012). The tidal volume was calculated using a simple tidal prism, incorporating the mean tidal range and surface area of each bay, and the tidal period was assumed to be semi-diurnal (12.42 hrs) based on the amphidromatic point in the north west area of the Northumberland Strait (Appendix 2: Table 10) (Gregory et al. 1993, Dutil et al. 2012, NB DNR 2012). We do note that tides in this region can deviate from a semi-diurnal pattern. For the scope of this research, however, we were interested in a bulk flushing time estimate and use the semi-diurnal tidal cycle as the average cycling time, similar to previous and related research in this region (e.g. Gregory et al. 1993, Robichaud and Doiron 2011, Dutil et al. 2012).

2.2.3.2 Terrestrial freshwater recharge

To quantify the annual input of freshwater into each bay we used the annual recharge volume of groundwater from each watershed. Recharge volume was calculated by multiplying annual average rainfall minus evapotranspiration estimated for this region by Shiau (1968) by the area of each watershed (Lajtha et al. 1995, Valiela et al. 1997a). This produces a prediction of annual freshwater discharge into the receiving water body. We used precipitation data from Environment Canada monitoring stations to represent precipitation in that watershed and closest surrounding watersheds (Appendix 2: Table 11). The freshwater recharge was then used in the estimate of Delta-N (see 2.3.3).

2.2.3.3 Delta (Δ)-N

To integrate N loading and flushing time we applied the Δ-N framework (Monsen et al. 2002, Bugden et al. 2014). We estimated Δ-N using our NLM results (TDN loading yr-1), annual average freshwater recharge, and tidal prism estimates (Table 2: **Eq. 12,13**). The amount of N entering the bay from the watershed is the NLM estimate for kg TDN $watershed^{-1}$ yr^{-1}. The volume of the bay replaced by the tide each cycle is calculated by multiplying the bay surface area (high tide) and the tidal range (Patriquin and Butler 1976, Gregory et al. 1993, Plante and Courtenay 2008, Robichaud and Doiron 2011, NB DNR 2012). The tidal flushing component of the model treats the bay as a Continuously Stirred Chemical Reactor (CSCR, Monsen et al. 2002), making the assumption that all water entering the bay from freshwater and tidal sources is completely and evenly mixed. The change in ambient N concentration in a bay is estimated by dividing the N load (kg TDN $watershed^{-1}$ yr^{-1}) by the sum of the rates of oceanic and freshwater inflow, producing the theoretical difference in N concentration in the bay from oceanic N concentration (Appendix 2: Table 12). If N loading is negligible, Δ-N will be close to zero; the larger the N inputs from a watershed (reflecting larger inputs from anthropogenic sources), the more positive Δ-N will be (Bugden et al. 2014).

2.2.4 Field Data

2.2.4.1 Sampling and processing

Eelgrass tissue samples were collected in all seven bays in spring, summer and autumn 2013 at long-term sampling locations (Lotze et al. 2003, Schmidt et al. 2012). For spring (June 4-5th) and autumn (October 16-17th) sampling, snorkelers collected 3 root and shoot tissue samples haphazardly within a 400 m^2 (50 m x 4 m) area parallel to shore. Each sample contained between 10-15 shoots and connected roots and rhizomes. A hand-held GPS allowed us to sample within the same 400 m^2 throughout the year. For the summer sampling (July 29–August 12), we collected eelgrass tissue at all sites while conducting fieldwork for a related research project using slightly different sampling methodology. We laid two 50 m long transects, 4 m apart, parallel to shore (maintaining the 400 m^2 sampling area using coordinates stored in the GPS). Sediment cores (0.0365 m^2) were taken at 0, 30 and 50 m on transect 1, and at 5, 25, and 45 m on transect 2. The root, rhizome and shoot eelgrass tissue removed in the cores was separated from the sediment and used for tissue analysis). All tissue samples were rinsed, placed in labeled bags, and stored on ice until it was returned to the lab, where it was stored in a fridge (4°C) for a maximum of 7 days until it could be processed.

In the lab, we first separated the roots/rhizomes from the shoots, keeping these portions separate for the rest of the processing and analysis. We carefully removed all epiphytic algae and invertebrates with freshwater and a razor blade. Cleaned tissue was dried in the oven at 80°C for 48 hours. Following desiccation it was ground up using a mortar and pestle, and stored in airtight glass vials in a cool, dark drawer. Approximately 5.0 mg of dried and ground sample was encapsulated in tin (Sn) foil and send to the University of California Davis Stable Isotope facility for analysis of % tissue N and N isotopes ($\delta^{15}N$).

2.2.4.2 Linking NLM to field data

NLM predictions, including total N loads, N loading rates, flushing time and Δ-N were compared to tissue characteristics (tissue N and N isotopes) using simple linear regressions. Tissue values were first analyzed by season to assess seasonal differences in relationships between model results and tissue characteristics, and then grouped to assess the relationships between model results and average tissue characteristics throughout the entire growing season. Additionally, we used simple regression to compare wastewater N loading with N isotope values from eelgrass tissue. Lastly, using multiple linear regression we tested whether multiple factors explained the variance in (seasonally averaged) tissue N content and isotope characteristics, namely the interactions between N loading rates per unit bay area from the NLM and estimated flushing time (hrs). The assumptions of linear regression were tested by examination of residual plots testing normality, linearity and homogeneity of variance. Since assumptions were met data were not transformed.

2.3 Results

2.3.1 Land Use Patterns

The land use patterns for each watershed (Figure 3, 4, Appendix 1: Table 1 and Figure 1) illustrate some regional patterns, as well as differences between watersheds. The five larger watersheds (CN, BT, RB, KB, TB), are mainly covered by forests, wetlands, and natural scrublands, with the greatest proportion in TB (96%), KB (93%) and RB (92%). In these five larger watersheds human activity, including farming, peat harvesting and settlement areas are concentrated near to the main river and the coastal areas and comprise ≤16% of the overall watershed size (Figure 3,4). Across all 7 bays, BT has the largest proportion of watershed area dedicated to agriculture (10.4%), followed by LM (9.3) and CN (6.5%). In contrast to the larger watersheds, BSS and LM have higher population densities, less natural land cover, and more land dedicated to peat harvesting, agriculture and settlement (Figure 3,4, Appendix 1: Table 1). Land cover within the 30m riparian zone of each watershed is similar to land cover within the rest of that watershed (Appendix 1: Figure 1). Forest and scrubland dominates the riparian zone in the large watersheds, while peat harvest land constitute a large proportion of the riparian zone in BSS (56%) and LM (39%); these peat extraction fields are required to have drainage systems and settling ponds to prevent the direct flow of runoff into waterways (Ouellete et al. 2006, Waddington et al. 2009, NB DEM 2013).

The watersheds with the larger civic centers (BT, RB, LM) are host to fish processing plants and MWWT facilities (Table 1), with the MWWT being almost exclusively different forms of secondary treatment (Francis Leblanc, NB DNR, pers. comm.). Fertilizer (synthetic and manure) is applied to 10-100% of agricultural lands depending on the watershed, and pastureland accounts for 0-60% of agricultural land (Statistics Canada 2011). Cattle is the most dominant livestock type, and produce the majority of the organic fertilizer in the form of manure (Huffman et al. 2008, Statistics Canada 2011). We estimate that lawn and turf fertilizer is applied to less than 2% of watershed area, except in LM where ≈4% of the watershed is estimated to be fertilized for non-agricultural purposes (Appendix 2: Table 6).

The size of bays are not directly associated with the upland watershed size (Table 1), and the two smallest watersheds (BSS and LM) have a notably smaller watershed:bay ratio (2.6 and 3.0 respectively), compared to the larger watersheds CN (13.6), BT (19.9), RB (25.1), KB (36.4) and TB (19.4).

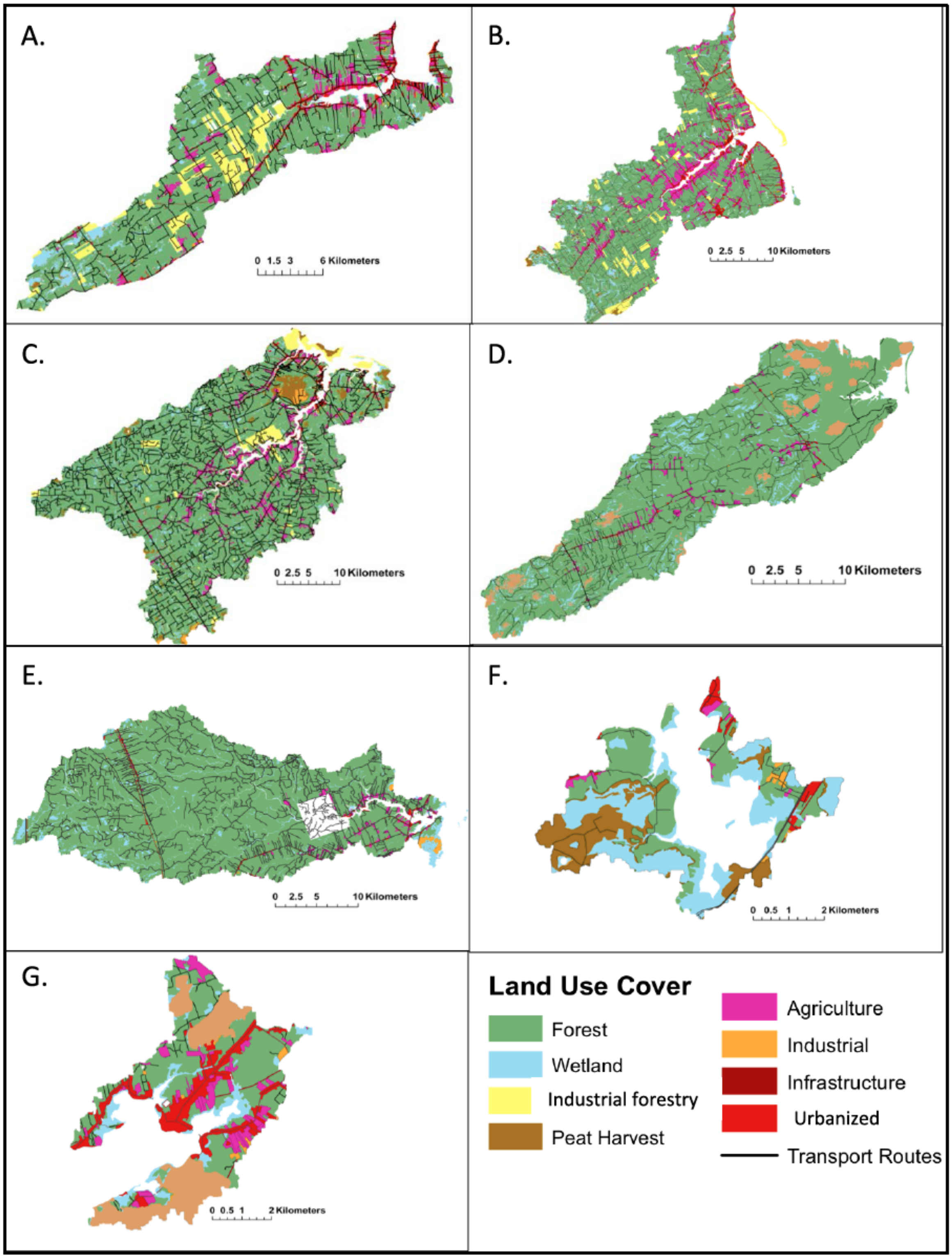

Figure 3 Land use patterns in the 7 watersheds assessed in eastern New Brunswick. A) Cocagne (CN); B) Bouctouche (BT); C) Kouchibouguac (KB); D) Tabusintac (TB); E) Baie St. Simon Sud (BSS); F) Lamèque (LM) (ESRI 2011, NB DNR 2012).

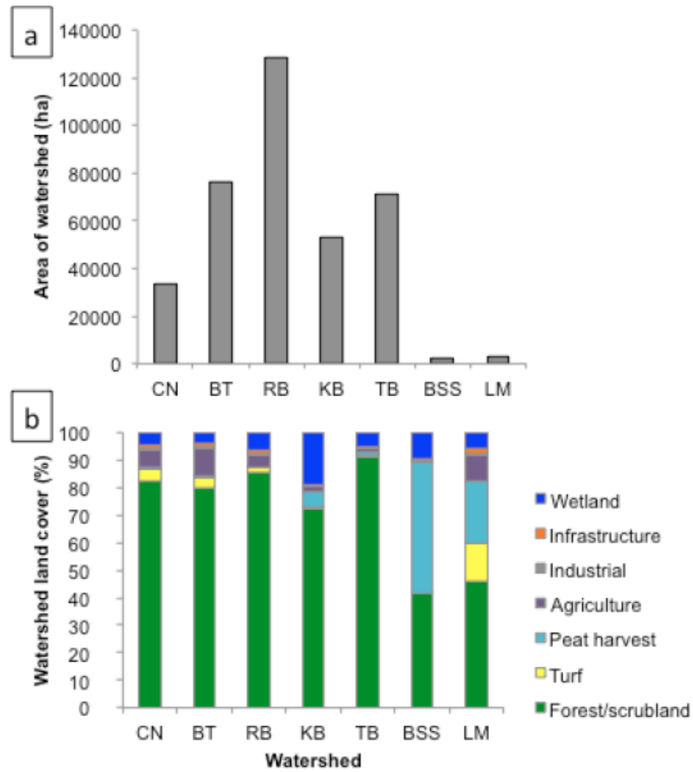

Figure 4 a) Terrestrial area in 7 watersheds in Eastern New Brunswick. b) Watershed land cover (%) for each land use considered in the NLM. Agricultural land includes both animal and non-tree plant husbandry.

2.3.2 NLM Output- Predicted Nitrogen Loading

Estimated TDN loads (kg y^{-1}) is highest in RB, followed by BT, and lowest in BSS (Figure 5a, Table 3). In contrast, the estimated N loading rates per unit watershed area (yields) are highest in LM, followed by BSS, CN, BT, and RB (Table 3). The yield in LM (20.9 kg TDN ha watershed^{-1} yr^{-1}) is more than double that of the other watersheds, and is >10 times higher than in TB and KB (Table 3). KB has the lowest predicted yeild (1.84 kg TDN ha watershed^{-1} yr^{-1}). Despite the relatively yeilds in BSS, the small watershed:bay area ratio here results in the lowest estimated N loading rates per unit bay area (19 kg TDN ha bay^{-1} yr^{-1}) compared to the other study sites (Table 3). Although watershed:bay area ratio is also small in LM, the estimated loading rate per unit bay area is the highest of all 7 bays (62 kg TDN ha bay^{-1} yr^{-1}). The larger watersheds (CN, BT, RB, KB, TB, Figure 1) have intermediate loading rates per unit bay area, between 35-55 kg TDN ha bay^{-1} yr^{-1} (Table 3). Total N load (kgTDN yr^{-1}, including direct deposition to bay surface) is closely related to watershed size (Figure 6a), while N yields are strongly positively correlated with population density (Figure 6b).

Table 3. Estimated total nitrogen load per year and nitrogen loading rates per area of watershed, area of bay surface and volume of bay (± range of loading given range in atmospheric deposition, freshwater recharge, MWWT and seafood processing). Flushing time and nitrogen load are components of Δ-N, the net change in nitrogen concentration in the bay over the course of one year. Positive Δ-N indicates a net increase in average ambient nitrogen concentration in the bay. See input data in Appendix 2.

	CN	BT	RB	KB	TB	BSS	LM
N load (kgTDN yr^{-1})	96,330 *±29,028*	198,753 *±58,724*	266,108 *±96,749*	79,958 *±30,841*	130,802 *±45,466*	15,773 *±6,803*	67,223 *±7,632*
N loading rate (kgTDN ha $watershed^{-1}$ yr^{-1})	2.90 *±0.87*	2.50 *±0.77*	2.07 *±0.75*	1.51 *±0.58*	1.84 *±0.64*	7.31 *±3.15*	20.74 *±2.36*
N loading rate (kgTDN ha bay^{-1} yr^{-1})	39.51 *±11.91*	49.76 *±15.40*	51.99 *±51.99*	54.84 *±21.15*	35.68 *±12.40*	18.94 *±8.17*	62.42 *±7.09*
N loading rate (kgTDN m^3 bay^{-1} yr^{-1})	0.0034 *±0.001*	0.0045 *±0.001*	0.0024 *±0.001*	0.0037 *±0.001*	0.0033 *±0.001*	0.0012 *±0.001*	0.002 *±0.002*
Flushing time (hrs)	31.58	33.15	66.74	52.54	30.22	30.32	53.79
Recharge volume (m^3 yr^{-1})	1.7 x10^8	3.9 x10^8	6.7 x10^8	2.7 x10^8	3.5 x10^8	1.0 x10^7	1.5 x10^7
Δ-N (mgL^{-1})	0.005	0.007	0.008	0.0094	0.0045	0.0017	0.0055

The land use patterns in each watershed are reflected in the predicted proportions of TDN entering each bay from different sources (Figure 4, 5b). Indirect atmospheric deposition, that is wet and dry N deposition on the watershed surface, is the largest source of N in the large watersheds. Direct atmospheric deposition (wet and dry deposition on the surface of the bay) is the largest contributor in BSS (Figure 5b). LM is the only watershed where a point source constitutes the majority of the N loading: the shellfish and fishmeal processing plant produces an estimated 39 104 kg TDN yr^{-1} in its effluent, which is discharged into Lamèque Bay (Appendix 2: Table 8). This is more than 7 times the amount of N released from fish processing facilities in the other watersheds (Figure 5b). Septic systems contribute less than 15% to N loading in any watershed; the highest contributions from septic systems are in the watersheds with higher populations, particularly CN, BT, and RB where many civic addresses are within 200 m of the shoreline (Figure 5b, 6b, Appendix 2: Table 6). MWWT contributes <5% of total N loading in CN, BT, and RB, however in LM more of the population in the watershed is serviced by MWWT than septic systems resulting in a higher proportion of loading from MWWT and a lower proportion from septic systems (Figure 5b). The small populations and limited infrastructure in KB and TB are reflected in N sources there (Table 1, Figure 5b). In these watersheds atmospheric deposition is estimated to contribute 94% of loading in KB, and 90% in TB, with the remainder of the loading coming from septic systems, peat harvest, and fertilizer additions to turf and agriculture (Figure 5b).

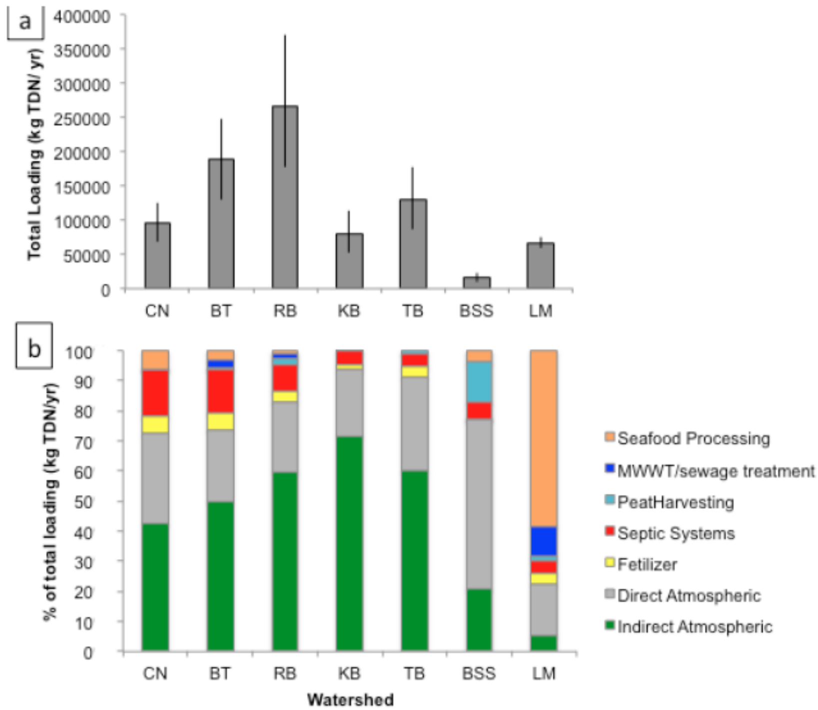

Figure 5. a) Mean total dissolved nitrogen (TDN) load to each bay over one year. Error bars represent the minimum and maximum loading given the variability in precipitation, evapotranspiration, freshwater recharge, and concentration of nitrogen in MWWT and seafood effluent; b) Percent of total load from each source considered in the NLM.

2.3.3 Bay Susceptibility

Flushing time estimates reflect the volume of each bay, as average tidal amplitude (a component of the tidal prism calculation) is similar throughout the entire region (±0.35m) (Dutil et al. 2012). RB has the longest estimated flushing time of 67 hrs, followed by LM (54 hrs) and KB (53 hrs, Table 3). The other four bays have shorter and similar flushing times of 30-33 hrs (Table 3).

Freshwater recharge was calculated as a component of the Δ-N model (Appendix 1: Table 10). The watershed with the highest predicted recharge volume was RB (2.8 x 10^8 m^3 yr^{-1}), while the smallest estimate was for BSS (1.1x10^7 m^3 yr^{-1}) (Table 3).

The Δ-N model yielded positive values for all bays, meaning there is a theoretical increase in ambient N concentration in each bay as a result of terrestrial and atmospheric N loading within that watershed and bay throughout the year (Table 3). The highest estimated Δ-N was in KB (0.0094), while the smallest was in BSS (0.0017). For reference, the Δ-N range associated with estuaries in PEI experiencing anoxia during the summer was an order of magnitude higher at 0.06-0.10 (Bugden et al. 2014).

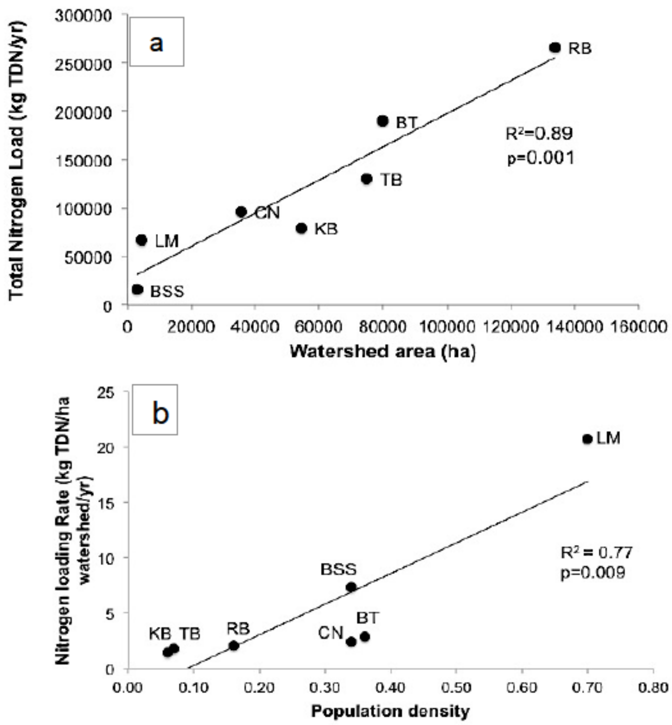

Figure 6. Relationship between a) watershed size and total N load (y = 1.7662x + 28104) and b) population density and N loading rate per ha of watershed (y= 27.609x - 2.4553) in 7 watersheds in eastern New Brunswick.

2.3.4 Field Verification

N isotope ratios differed significantly between above ground (AG) and below ground (BG) tissue (Studentized 2-tailed t-test of equal variance, $p<0.05$) and AG and BG components were therefore assessed separately. Still, overall isotopic patterns between sites were similar for both components with much higher values in LM than at other sites. Lowest δ^{15}N values were recorded in CN and BT (Figure 7a).

Tissue N content was significantly different for AG and BG eelgrass components (Studentized 2-tailed t-test of equal variance, $p<0.001$). Seasonally averaged AG tissue from CN and LM displayed the highest N content, and BSS the lowest. Tissue N from

BT, RB, KB and TB was similar (Figure 7). The highest average BG N content was from CN, while RB, KB and TB had the lowest BG tissue N content, however overall average BG tissue N was lower and less variable between sites than AG tissue (Figure 7b).

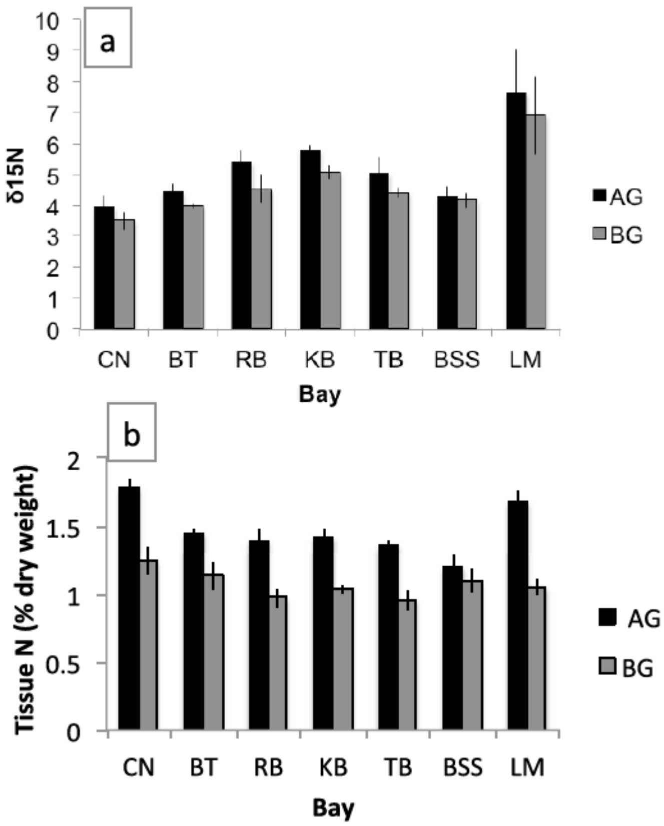

Figure 7. a) Average nitrogen isotope ($\delta^{15}N$) values for AG and BG eelgrass tissue sampled in June, August, and October 2013. Error bars show standard deviation (n=12/site); b) Same as a) but showing AG and BG tissue N content (% dry weight).

We found significant positive relationships between predicted N loading rates per ha of bay or watershed and NO_3^- $\delta^{15}N$ in AG and BG tissue averaged across all seasons (Figure 8, Table 4), and in summer and fall measurements individually (Appendix 3: Table 1). There was also a significant positive correlation between estimated flushing time of each bay and seasonally averaged, summer and fall NO_3^- $\delta^{15}N$ in AG and BG tissue. A significant linear relationship between Δ-N and average AG $\delta^{15}N$ measurements was also identified.

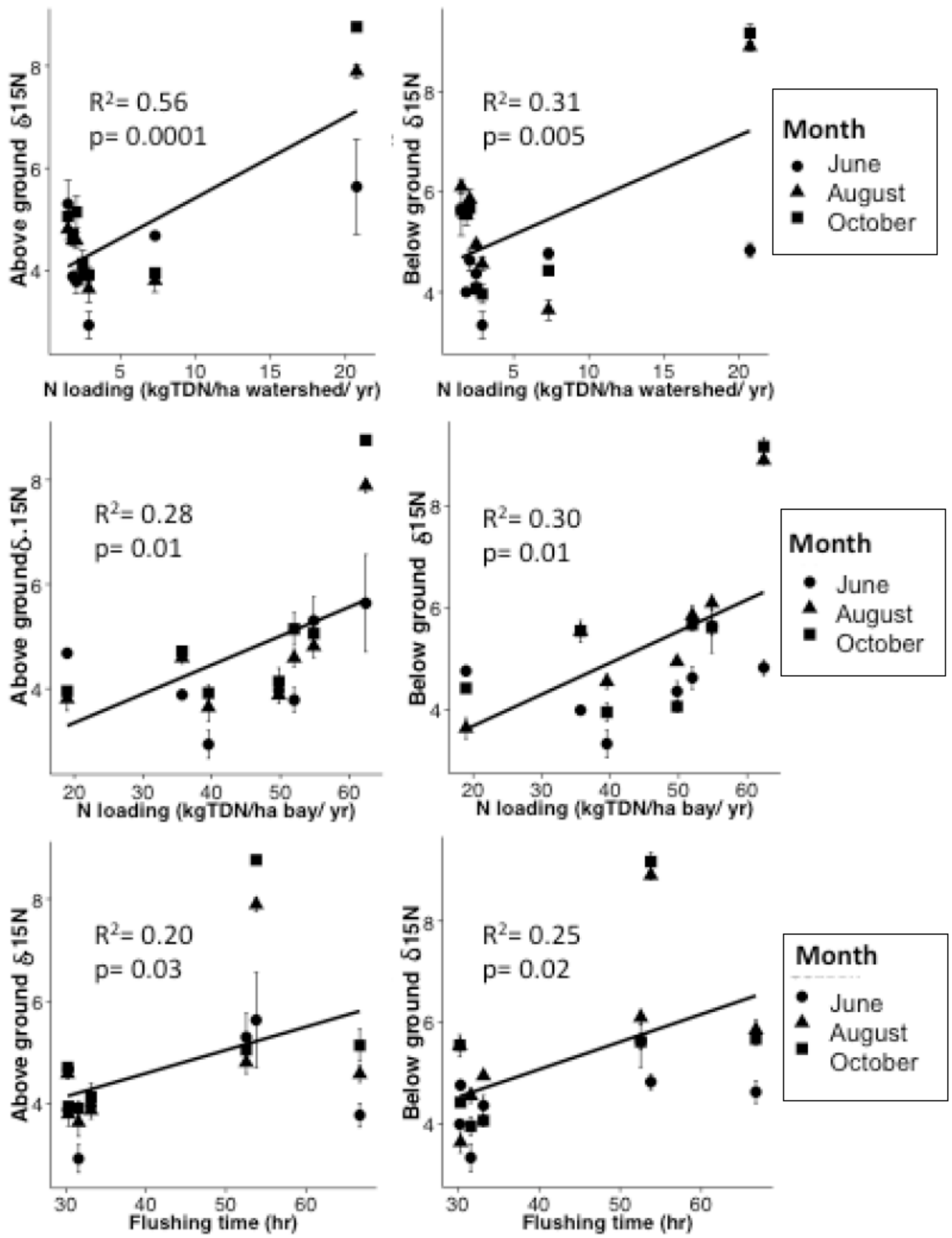

Figure 8. Relationships between AG (left column) and BG (right column) $\delta^{15}N$ and nitrogen loading rates per ha of watershed (top) per ha of bay surface (middle), and flushing time (bottom). The trendlines show the relationship between average tissue isotope values from all seasons and the model result, and samples from different seasons (mean ± SE) are denoted by symbol shape.

Additionally, there was a significant relationship between wastewater (MWWT, septic systems, seafood processing plants) loading rates per ha bay and ha watershed and average AG and BG NO_3^- $\delta^{15}N$ values (Figure 9, Table 4) as well as summer and fall independently (Appendix 3: Table 1). There were no identifiable relationships between N isotope values from spring tissue and estimates from the NLM, but there were significant relationships between spring AG and BG tissue and flushing time and Δ-N (Appendix 3: Table 1).

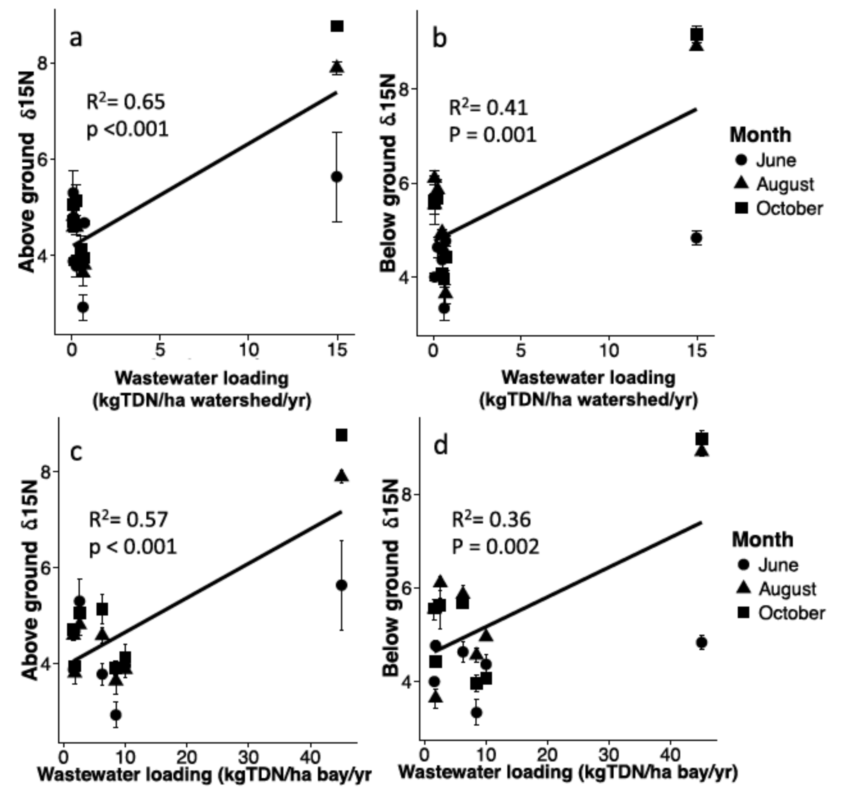

Figure 9. Relationships between AG and BG $\delta^{15}N$ and wastewater nitrogen loading rates (per ha of watershed, per ha of bay surface). The trendlines shows the relationship between average tissue values from all seasons and the model result, and samples from different seasons (mean ± SE) are denoted by different symbols.

Regarding % tissue N content, we found a significant positive relationship between N loading rates per bay area and volume and AG tissue % N sampled in summer, as well as a slightly non-significant relationship with flushing time (Figure 10, Appendix 3: Table 2). There was a significant relationship between N loading per unit bay area and seasonally averaged AG tissue N (Table 4), but in contrast, AG tissue from spring and fall did not show strong relationships. The same was true for BG tissue N content, which showed no strong relationships with any results of the NLM, flushing time, or Δ-N estimates (Table 4, Appendix 3: Table 2).

Multiple regression analysis illustrated a significant relationship between NO_3^- $\delta^{15}N$ values in both AG and BG eelgrass tissue from all seasons, as well as summer and fall individually, and the interaction between N loading rate (kg TDN ha bay^{-1} yr^{-1}) and flushing time (Table 5, Appendix 3: Table 3). There were also significant relationships between seasonal averages, as well as summer AG tissue N and the interaction between N loading rate (kg TDN ha bay yr^{-1}) x flushing time (Table 5: Appendix 3: Table 4). No relationship between the interaction of N loading rate and flushing time was found for N content or isotope values in spring tissues, nor for BG tissue content. We tested interactions between other outputs of the NLM and flushing time in multiple regressions using Tissue N and Tissue N isotopes as dependent variables. There were no significant interactions between Total N load yr^{-1}, N loading rate per unit watershed area, or N loading rate per m^3estuary with flushing time, and we do not show these non-significant results.

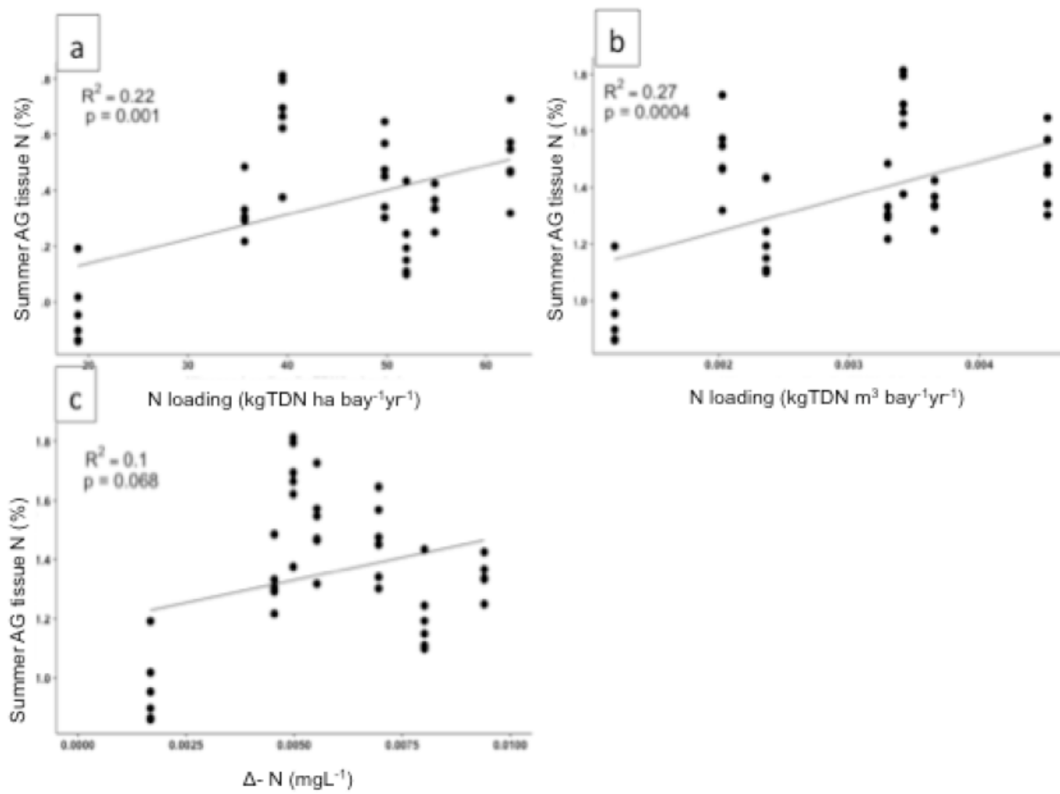

Figure 10. Relationships between nitrogen content (%) of all AG eelgrass tissue sampled in summer 2013 and a) Nitrogen loading rate per unit bay area, b) Nitrogen loading rate per m3 bay, and c) Δ-N.

2.4 Discussion

The goal of this research was to quantify the magnitude and identify the main sources of N loading from seven watersheds to their respective bays in eastern NB using the NLM framework adapted from the Waquoit Bay region (Valiela et al. 1997a, 2000). Additionally, we evaluated whether the estimates produced from the NLM were indicative of N tissue content and isotope signatures in eelgrass beds that dominate these shallow bays. Our NLM results indicate that atmospheric deposition is the dominant contributor of N in this region, most notably in the larger watersheds. Yet there is a clear footprint of human settlement and land use in CN, BT, BSS and LM, with >20% of N loads from human activities. This is shown by higher estimated N loading rates per ha of watershed and reflected in higher tissue N content in eelgrass sampled in the associated bays. N isotope characteristics are correlated with N loading rates, particularly wastewater loading into each bay. This indicates these macrophytes can reflect the proportion of N loading coming from human and animal wastewater.

Table 4. Results of simple linear regression using estimates from the NLM, Δ-N and flushing time as independent variables and seasonally averaged AG and BG N stable isotopes and AG and BG tissue nitrogen (% dry weight) from eelgrass samples collected in June, August, October, 2013. Relationships with a significant p-value (<0.05) are shown in bold. For results for individual seasons see Appendix 3: Tables 1-2.

Model Prediction	AG δ^{15} N (all seasons)					BG δ^{15} N (all seasons)			
	df	F-stat	Residual st. error	Adj. R^2	p-value	F-stat	Residual st. error	Adj. R^2	p-value
Load (kg TDN yr^{-1})	1,79	0.11	1.55	-0.01	0.74	2.68	1.36	0.02	0.11
Loading rate (kg TDN ha $watershed^{-1}$yr-1)	**1,79**	**46.72**	**0.37**	**0.36**	**1.47×10^{-9}**	**73.81**	**0.99**	**0.48**	**6.12×10^{-13}**
Loading rate (kg TDN ha $bay^{-1}yr^{-1}$)	**1,79**	**50.06**	**1.22**	**0.4**	**5.08×10^{-10}**	**28.86**	**1.18**	**0.26**	**7.63×10^{-7}**
Loading rate (kg TDN m^3 $bay^{-1}yr^{-1}$)	1,79	2.435	1.56	0.02	0.12	**6.78**	**1.32**	**0.068**	**0.01**
Δ-N (mg/L)	1,79	**6.54**	**1.49**	**0.06**	**0.01**	1.37	1.37	0.01	0.25
Flushing time (hrs)	1,79	**30.62**	**1.32**	**0.27**	**3.85×10^{-7}**	**19.95**	**1.23**	**0.19**	**2.61×10^{-5}**
Wastewater load (kg TDN yr^{-1})	1,79	**17.03**	**1.38**	**0.17**	**9.0×10^{-5}**	**19.6**	**1.27**	**0.19**	**3.0×10^{-5}**
Wastewater loading rate (kg TDN ha $watershed^{-1}$yr-1)	1,79	**130.9**	**1.11**	**0.62**	**$<2.2 \times 10^{-16}$**	**74.76**	**0.92**	**0.45**	**4.3×10^{-13}**
Wastewater loading rate (kg TDN ha bay^{-1}yr-1)	1,79	**97.13**	**1.15**	**0.55**	**2.1×10^{-15}**	**65.28**	**0.99**	**0.44**	**5.6×10^{-12}**
Wastewater loading rate (kg TDN m^3 $bay^{-1}yr^{-1}$)	1,79	**25.78**	**1.34**	**0.24**	**2.5×10^{-6}**	**21.37**	**1.22**	**0.2**	**1.4×10^{-5}**
	AG % Tissue Nitrogen (All Seasons)					BG % Tissue Nitrogen (All Seasons)			
Load (kg TDN yr^{-1})	1,79	0.02	0.29	-0.01	0.90	0.93	0.27	-0.01	0.34
Loading rate (kg TDN ha $watershed^{-1}$yr-1)	1,79	3.85	0.29	0.03	0.05	0.02	0.27	-0.01	0.89
Loading rate (kg TDN ha $bay^{-1}yr^{-1}$)	1,79	**9.89**	**0.28**	**0.10**	**3.0×10^{-3}**	0.11	0.27	-0.01	0.74
Loading rate (kg TDN m^3 $bay^{-1}yr^{-1}$)	1,79	2.65	0.29	0.02	0.11	0.24	0.27	-0.01	0.56
Δ-N (mg/L)	1,79	1.19	0.29	0.01	0.28	0.25	0.27	-0.01	0.62
Flushing time (hrs)	1,79	0.19	0.29	-0.01	0.67	1.42	0.27	0.01	0.24

Table 5. Multiple regression results of nitrogen loading rate per unit bay area, flushing time, and the interaction of these factors related to average AG and BG isotope content, and AG and BG N content of eelgrass tissue sampled across seasons in June, August and October. For results for individual seasons see Appendix 3: Tables 3-4. Relationships with a significant p-value (<0.05) are shown in bold.

	AG $\delta^{15}N$ (‰) All seasons							BG $\delta^{15}N$ (‰) All Seasons						
	df	Coeff	SE	p-value	F-stat	Overall Adj. R2	Overall p-value	df	Coeff	SE	p-value	F-stat	Overall Adj. R2	Overall p-value
Flushing Time	3, 78	**0.16**	**0.02**	**1.57×10^{-12}**	**29.02**	**0.51**	**1.05×10^{-12}**	**3, 78**	**0.13**	**1.72×10^{-2}**	**1.87×10^{-10}**	**24.65**	**0.47**	**2.78×10^{-11}**
Loading rate (kg TDN ha bay^{-1} yr^{-1})		**2.0×10^{-5}**	**5.33×10^{-6}**	**6.64×10^{-4}**					**1.17×10^{-5}**	**4.94×10^{-6}**	**0.021**			
Flushing time x Loading rate		**-5.25×10^{-7}**	**1.04×10^{-7}**	**2.56×10^{-6}**					**-4.08×10^{-7}**	**9.66×10^{-8}**	**6.64×10^{-5}**			
	AG Tissue N (%) All seasons							BG Tissue N (%) All Seasons						
Flushing Time	3, 78	**0.01**	**4.88×10^{-3}**	**0.01**	2.42	0.05	0.07	3, 78	-1.86×10^{-3}	4.63×10^{-3}	0.69	0.55	-0.02	0.65
Loading rate (kg TDN ha bay^{-1} yr^{-1})		**3.32×10^{-6}**	**1.40×10^{-3}**	**0.02**					-1.50×10^{-7}	1.33×10^{-6}	0.91			
Flushing time x Loading rate		**-7.149×10^{-8}**	**2.72×10^{-8}**	**0.01**					-1.32×10^{-9}	2.6×10^{-8}	0.96			

Our results further indicate a significant interaction between N loading and flushing time on eelgrass tissue N content and N isotope values, suggesting that both the amount of N loading and tidal flushing impact the availability of N to primary producers. Thus, bays with high N loading rate but short flushing time may be as susceptible to eutrophication as bays with low N loading rate but long flushing time, whereas bays with high N loading rate and long flushing time may be the most vulnerable.

2.4.1 Nitrogen loading and sources

Our estimated N loads reveal some interesting trends for the overall region as well as between the 7 bays, dependent on the size of the watershed and bay, land use and population density. Overall, atmospheric deposition, both indirect and direct, is the largest source of N in bigger watersheds (CN, BT, RB, KB, TB, Figure 5) resulting in a strong relationship between watershed size and total N loads (Figure 6a). This is not an unexpected result given that land use area is used to determine atmospheric deposition; however, the strength of the relationship illustrates how dominant atmospheric deposition is compared to other sources.

In eastern NB, nitrate is the primary species of inorganic N in atmospheric deposition with approximately 6 times more nitrate than ammonium in precipitation consistently throughout the last 2 decades (Appendix 2: Table 2) (NatChem 2012). It is likely that reactive N deposited through wet and dry deposition is largely transported from outside the region, from areas with higher population densities and industrial practices (Valigura et al. 2001, Bowen and Valiela 2001b). Conversely, volatilization of ammonium from local agricultural practices contributes relatively little to overall N loading here, which reflects the small concentration of livestock in this region relative to dense agricultural regions in south western Ontario and Alberta (Yang 2006, Huffman et al. 2008, Yang et al. 2011). Overall, our estimates of atmospherically deposited N are smaller than those predicted by the NLM in Waquoit Bay and other regions in New England (e.g. Bowen and Valiela 2001b). Whereas estimates for the northeast United States are on average 12 kg TDN ha watershed^{-1} yr^{-1}, ours do not exceed 11 kg and are generally between 8-10 kg TDN ha watershed^{-1} yr^{-1}. We propose our estimates are lower due to lower ammonium deposition in this region, but not minimal because of the transport of airborne nitrate from Central Canada and the United States (Bowen and Valiela 2001b, Castro and Driscoll 2002).

Despite the predominance of atmospheric deposition, our estimated N loading rates per unit watershed area show a clear footprint of human settlement and activities within watersheds, namely in BSS and LM (Figure 5b, 6a, Table 3). These are the two smallest of our seven watersheds and have a larger proportion of land cover designated for settlement and infrastructure, peat harvesting, and industrial activities (Figure 3,4, Appendix 1: Table 1). These land use patterns are reflected in the higher proportion of N from point sources, including peat harvesting (BSS) and wastewater from MWWT and seafood processing (LM). Conversely, the five larger watersheds are predominantly forested and naturally vegetated (scrubland, wetland, unexploited peatland). Although overall watershed populations in these bigger watersheds exceed those in BSS and LM, population densities are lower (RB, KB, TB) or similar (BT, CN) to those in BSS and LM (Table 1). The higher N loading rates per unit watershed area in watersheds with higher population densities (BT, CN, BSS, LM) indicate that N loading in excess of atmospheric contribution is a product of human activities (Table 1, 3, Figure 6b). The relationship we found between population density and N loading per unit watershed area is similar to the relationship between increased housing development and N loading rates in Waquoit Bay (Short and Burdick 2007).

Eelgrass tissue NO_3^- isotopes from LM reflect the higher N loading rates per area of watershed and per area of bay, particularly the higher proportion of loading from wastewater (MWWT, septic, seafood processing) (Figure 7a, 9, Appendix 1:Figure 1). Summer and fall tissue isotope characteristics from LM were much higher than values from other bays and reached 8-10‰, indicating tissues are incorporating the nitrogen signal from wastewater sourced N (McClelland and Valiela 1998, Cole et al. 2006). This highlights the potential of these primary producers to integrate N signals from terrestrial sources in NB, as has been shown in coastal habitats of New England, the Baltic Sea, and elsewhere (Voss and Struck 1997, McClelland and Valiela 1998, Voss et al. 2000, Cole et al. 2006). Results of tissue NO_3^- $\delta^{15}N$ analysis are less distinct in the other six bays, and the majority of values fall within a range indicative of atmospheric deposition and synthetic or organic fertilizers (2-6‰) (e.g. Lepoint et al. 2004, Cole et al. 2006). This reflects the NLM estimates for these watersheds, where atmospheric deposition is the primary source of N loading (Figure 5b). We note that while the results of NO_3^- isotope analysis of eelgrass tissue agree with our results from the NLM, isotope values representative of atmospheric deposition and fertilizer N are not clearly distinct from values typical of background levels of NO_3^- $\delta^{15}N$ in bedrock and pristine groundwater (McClelland and Valiela 1998, Lepoint et al. 2004, Cole et al. 2006, Xue et al. 2009). We also acknowledge that isotopes are principally related to nitrate availability in a bay and not other species of N (e.g. NH_4^+, DON), and that there are physical, chemical and biological processes that could contribute to variable fractionation of NO_3^- in groundwater and marine producers (Middelburg and Nieuwenhuize 2001). Still, we highlight the distinctions in tissue N isotope ratios and sources of N in each bay and that both high proportions of anthropogenic N in wastewater and atmospheric deposition may dominantly influence the nitrogen isotope signal of freshwater entering these bays.

2.4.2 Tidal Influence and Bay Susceptibility

Estimates from the NLM (kg TDN ha watershed^{-1}yr^{-1}, kg TDN ha bay^{-1}yr^{-1}, kg TDN m^{3}bay^{-1}yr^{-1}), flushing time, Δ-N, and their relationships with tissue characteristics of eelgrass in each of the bays indicate that bay size and tidal flushing are important factors influencing the terrestrial or atmospherically derived N available to primary producers. We compared our simple flushing time estimates to more spatially explicit models of flushing for the whole RB estuary (Guyondet 2013). Of the water renewal estimates proposed for the three distinct areas of the RB bay and estuary (Main Harbor, North Arm, and Baie du Village, Guyondet 2013), our estimate of flushing time for RB is within the range for the Main Harbor (5-20 days), but underestimates flushing time proposed for the other two arms of the bay where renewal estimates are longer by 10-20 days. Therefore, we note that our estimates could generally represent flushing time in the main bay of each study site we assessed (where eelgrass samples were collected), but not necessarily the estuarine portions or portions very removed from the channel of tidal inflow to each bay.

The significant positive relationship between % tissue N of seasonally averaged and summer eelgrass shoots/blades with N loading per bay area or bay volume indicates that bay size may affect the legacy of N loading and thereby the concentration of N available to eelgrass (Figure 10). Moreover, the significant interaction between N loading and flushing time on tissue N content is a further indication that bay size in combination with hydrodynamics influences the amount of N available to eelgrass and other primary producers. The lack of a significant relationship between N loading and spring and fall tissue N content (Appendix 3: Table 2) may be explained by seasonal nutrient dynamics: spring runoff of snowmelt and ground thaw, and fall decomposition of organic matter within eelgrass beds, can create a surplus of watercolumn N in these seasons, possibly diluting the signal from land based N loading. In comparison, tissues sampled in the summer may give a better idea of the ambient N available to eelgrass following spring blooms when nitrogen usually becomes limiting (in the absence of excessive anthropogenic N loading) (Hemminga and Duarte, 2000). Unfortunately, because the NLM produces an estimate of total annual loading we are not able to comment directly on the loading rate in different seasons. In contrast to AG tissue N content, we found no strong relationships between N loading rates and BG N content. Eelgrass roots and rhizomes primarily take up NH_4^+ from sediment pore water as NO_3^- concentrations are low there, limited by the lack of oxidation of ammonium in sediment with anoxic characteristics (Hemminga and Duarte 2000). Therefore, BG tissue may not directly reflect the ambient N content of the water column, which in addition to ammonium may have higher nitrate concentrations from atmospheric deposition and land runoff. Additionally, although roots and rhizomes are important for acquiring N from sediments, shoots can take up more than 50% of the N used by the plants directly from the water column. Therefore AG tissue may better reflect the ambient DIN available in the water column, while BG tissue may neither reflect the total N uptake nor the proportion of nitrogen species attenuated by the plant (Hemminga and Duarte 2000).

When integrating N loading and flushing time into estimates of Δ-N, our model produced positive Δ-N values for each bay, indicating a theoretical increase in ambient N concentration over the course of one year. We cannot comment directly on their magnitude (large or small) as we use TDN loading estimates and not nitrate loading, as was done for the Δ-N application in PEI (Bugden et al. 2014). Still, our values for Δ-N are a level of magnitude lower than those in PEI that are associated with anoxic endpoints (Δ-N = 0.06-0.10 mg L^{-1}). The majority of N loading in PEI is from intensive fertilizer application, and resulting blooms of annual algae and wide-scale anoxia can be sustained and severe (Bugden et al. 2014). Our Δ-N results do not show strong relationships with tissue N or isotopes from our bays. Based on our results it may not be a sensitive enough indicator for this region where nitrogen loading and Δ-N values are much lower than in PEI. At this time we propose that our use of individual factors for nitrogen loading and flushing time in multiple regression is a more effective indicator of nitrogen enrichment in our bays than combining these factors into one metric (Δ-N). Alternatively, different endpoints than tissue characteristics may prove more useful for interpreting the results of Δ-N for NB bays in future research.

2.4.3. Comparison of Bays in Eastern NB

Based on land use patterns, predicted N loading and estimated potential for tidal flushing we can draw some overall conclusions for each of the seven bays assessed. KB and TB are watersheds with a high proportion of natural vegetation: KB contains Kouchibouguac National Park and TB a RAMSAR wetland protection area. Both these bays have exhibited low eutrophic symptoms over the past decade (Lotze et al. 2003, Schmidt et al. 2012). According to our NLM, N yeild is the lowest in KB followed by TB, reflecting the small human footprint in both these watersheds. Because of its small bay size and high watershed:bay size ratio, however, KB has a relatively high N load per unit bay area. In contrast, despite a high loading per ha of watershed in BSS, the loading per unit bay area is low, due to a small watershed:bay size ratio. This may be a reason why BSS showed low eutrophic signs according to Schmidt et al. (2012) and highlights the dilution potential of terrestrial N loads in a larger receiving water body. Compared to the other six bays, LM has both high N loading per unit watershed area and per unit bay area. Since tighter regulations on effluent treatment and discharge from seafood processing facilities in LM have come into place (2003-2007), the severe occurrences of *Ulva* blooms in the summer have been nearly eliminated (Plante and Courteney 2008). Still, this site continues to exhibit more symptoms characteristic of eutrophication relative to KB TB and BSS (Schmidt et al. 2012, Chapter 2), illustrating the continued impact of N loading from wastewater in this bay, and perhaps residual N stored in sediments (Figure 5b). The remaining three bays (CN, BT, RB) have somewhat intermediate N loading per ha watershed and per ha bay. Despite larger bay surface areas, these bays have higher loading per unit bay area as well as longer flushing times than TB and BSS, which may indicate their higher risk for eutrophication. Indeed, eutrophic symptoms have been documented in eelgrass habitats in the BT and CN throughout the last decade (Lotze et al. 2003, Schmidt et al. 2012) and within estuarine portions of RB (Turcotte-Lanteigne and Ferguson 2013). Previous spatially explicit modeling of hydrodynamic patterns in RB has identified that the longer flushing time is a hazard for eutrophication, especially in portions of the bay near to freshwater inflows with high nutrient and sediment loads (St Hilaire et al. 2004, Turcotte-Lanteigne and Ferguson 2013).

2.4.4 Broader Context

One of the benefits of using the NLM framework is that it has had wide-scale application to numerous estuaries in the continental U.S. (e.g. Bowen and Valiela 2001a, Latimer and Charpentier 2010, Giordano et al. 2011). The results from our application of the NLM fit within the gradient of N loading rates predicted by Latimer and Charpentier (2010) for 74 watersheds of various size and N loading rates. Predicted loading rates per ha bay and per ha watershed for TB and BSS correspond to the minimum or 25th percentile (below average) of loading rates predicted in New England and other US estuaries. LM on the other hand has a loading rate per ha of watershed that is in the 75th percentile (above average), and loading rates per unit bay area in the 50th percentile. Loading rates per unit watershed area here are similar to those predicted for Delaware Bay (20.7 kg TN ha watershed^{-1} yr^{-1}), but due to the small watershed:bay ratio in LM, loading rates per unit bay area in LM are 100 x lower than those to Delaware Bay. We see the opposite case in BT and KB, where N loading rates per unit watershed area are similar (BT) or below (KB) the minimum observed loading rates in the U.S., but loading rates per unit bay area are in the top 50th percentile. Indeed loading rates in BT and KB are almost 2x higher than those in Buzzards Bay (17kg TN ha bay^{-1} yr^{-1}), which at this loading rate exhibits annual eutrophication events and shown declines in eelgrass bed area in the inner estuarine portions (Latimer and Charpentier 2010, Latimer and Rego 2010).

Previous applications of the NLM in New England have also linked eutrophication and loss of eelgrass habitat to a threshold value of N loading rates per ha of watershed. For instance, Bowen and Valiela (2001a) proposed a loading of 20 kg TDN ha watershed^{-1} yr^{-1} as a threshold for the survival of eelgrass meadows in the Waquoit Bay region. Latimer and Rego (2010) observed high variability among estuaries of different size and different flushing capacities, but at loading rates of 50 kg TN ha watershed^{-1} yr^{-1} they observed significant declines in eelgrass cover in most estuaries, and at a threshold value of 100 kg TN ha watershed^{-1} yr^{-1} they found ubiquitous loss of eelgrass bed cover in respective estuaries. In general, the watersheds and receiving bays we assessed fall within the mid to low range of the nitrogen loading gradient seen in the 74 estuaries assessed by Latimer and Charpentier (2010). Notably however, our highest predicted loading rates were in LM and exceeded the 20 kg TDN ha watershed^{-1}yr^{-1} threshold value noted above, yet were well below the higher threshold values of 50 and 100 kg TDN ha watershed^{-1}yr^{-1}. Our estimates of N loading are consistant with estimates from other applications of the NLM yet as none of these bays in NB are experiencing N loading rates as high as 50 or 100 kg TDN ha watershed^{-1}yr^{-1} we cannot directly comment on the applicability of previously proposed thresholds of N loading to this region. We are additionally limited in proposing a threshold loading value that is linked to loss of eelgrass cover within our bays as bay-scale eelgrass cover has not yet been quantified for all these bays, although there are currently research efforts addressing this (Northumberland Strait Environmental Monitoring Partnership, Southern Gulf of St. Lawrence Coalition on Sustainability (http://www.coalition-sgsl.ca/), pers. comm.). We can note that although the N loading experienced by these watersheds in NB may not have yet elicited the significant loss of canopy cover across a bay (pers. comm. regarding ongoing research, Dr. Mark Skinner) they are not benign. Indeed, we link higher nitrogen loading rates per unit bay area to increases in tissue nitrogen and to bays (CN, BT, RB, LM) that have previously exhibited heightened symptoms of eutrophication including increased annual algae, higher water column primary production and reductions in eelgrass biomass (Lotze et al. 2003, Schmidt et al. 2012, Turcotte-Lanteigne and Ferguson 2013). These symptoms of eutrophication may be a precursor to canopy loss if loading levels are increased or not mitigated.

2.5 Conclusions and Management Implications

Using the NLM framework adapted from the Waquoit Bay region (Valiela et al. 1997a, McClelland and Valiela 1998), we were able to estimate N loading to seven bays in eastern NB and quantify the different point and non-point sources. Moreover, our analysis of eelgrass tissue nutrient content and isotopes was useful for validating and interpreting our estimates from the NLM as well as flushing time calculations for each bay (Valiela et al. 1997b). We found that N isotopes in both AG and BG eelgrass tissue were effective in reflecting increased proportion of N loading from human and animal wastewater (as estimated in the NLM). Previous research in this and other regions has shown that higher tissue N content of coastal producers is associated with an increase in ambient N concentration and other symptoms of eutrophication (Valiela et al. 1997b, Hemminga and Duarte 2000, Lee et al. 2004, Kennish and Fertig 2012, Schmidt et al. 2012). Therefore, while N stable isotopes in eelgrass tissue are valuable for distinguishing whether wastewater is a dominant N source, N tissue content is useful as an indicator of nutrient enrichment and eutrophication status in the associated bays.

We suggest that bays with significant sources of N additional to atmospheric deposition (e.g. seafood processing plants, MWWT, septic systems), and bays with a reduced capacity to remove excess N through tidal circulation and flushing are at a higher risk of exacerbating eutrophic condition in eelgrass habitats. Based on our estimates from the NLM, integrated with flushing time, CN, BT, RB, and LM are bays of concern, and we suggest that management directives should focus on preventing increases in N loading from existing sources. At this time, TB and BSS are at a reduced risk of eutrophication, a reflection of low human impact within the watershed in TB and a small watershed:bay area ratio in BSS. Although KB is one of the least impacted watersheds in terms of the human footprint, the large watershed:bay ratio and slow flushing time of the bay imply that it may be more susceptible to N loading than TB and BSS.

Management of N sources that originate from within the watershed are perhaps easier to tackle than those from outside. For instance, increasing the wastewater treatment capacities of a seafood processing plant or municipal sewage can reduce N concentrations in effluent, thereby reducing N loading into the receiving bay. On the other hand, airborne N species, particularly nitrous oxides (NO_x) may originate from outside a watershed, region or even country. Yet, given the large amount of N loads from atmospheric deposition, management action is still necessary. The 1991 Canada-United States Air Quality Agreement is an example of cross-boundary management, and in both countries nitrous oxide emissions have been reduced by over 40% from 1992 levels (USEPA 2012, Environment Canada 2013). This cross-boundary management strategy has successfully resulted in a decrease in nitrate deposition in precipitation in eastern NB (Appendix 2: Figure 1), although decreases in nitrate deposition have not been recorded in all intended areas under the agreement (Rammoth 2001). Both Canada and the United States have met the targets set in the original agreement, therefore further decreases in NO_x are not certain (NATchem 2012, USEPA 2012, Environment Canada 2013). Ammonium emissions are more likely to travel shorter distances before deposition. The major sources of volatized ammonium include fertilizers, household and livestock animals, and wastewater effluent (e.g. Castro et al. 2003). Volatilization could be reduced through ensuring manure and wastewater treatment holding areas are covered; however, the N incorporated in solid wastes is still likely to enter the watershed through other means, such as by use as a fertilizer, or in effluent discharge following primary or secondary treatment. Therefore, focusing on landscape characteristics and N attenuation capacity of the terrestrial watershed may be one of the most effective methods of mitigating both atmospherically derived N and surface runoff of N from agriculture, commercial peat operations, and recreational areas that use fertilizers (Hill 1996, Galloway et al. 2003, Waddington et al. 2009, Hynninen et al. 2011). At the landscape scale ecosystems such as wetlands and riparian buffer zones have an inherently elevated capacity to conserve N as a result of their position near the terrestrial and watercourse interface, and also because they are characterized by wet and anaerobic soils that make denitrification favorable (Hill 1996, Driscoll et al. 2003). Therefore, protecting remaining wetland areas as well as re-establishing and maintaining riparian buffer zones in accord with the existing NB Clean Water Act would be a beneficial initial action to prevent further increases in N loading (GNB 1989). This would be especially relevant for BSS and LM given that the buffer zones in these watersheds are characterized by less forest or wetland (24.4% in LM, 48% in BSS) than in the larger watersheds (Appendix 1: Figure 1). Future research could address seasonal precipitation rates and concentrations of N in point source effluents, thereby assessing when the majority of loading is happening. This would help disentangle the seasonal responses to N loading in these eelgrass habitats

This study provides a quantification of the magnitude and different sources of annual N loading representative of the time period 2002-2012 to seven bays in eastern NB. Additionally we provide simple and user-friendly estimates of hydrodynamics and influence of tidal flushing on nutrient loading in these bays through flushing time and Δ-N calculations (Gregory et al. 1993, Bugden et al. 2014). The NLM, flushing time, and Δ-N utilize information and digital data that is either publically available or accessible through the provincial Department of Natural Resources, and thus can easily be updated in future years or extended to other watersheds and bays in NB and beyond. Our results provide a baseline assessment of nutrient sources, loading and eutrophication risk, which can aid in community watershed management and land use planning at the watershed and regional scale, with the aim to balance human impacts and conservation of important coastal habitat.

Chapter 3: Linking Estimates of Nitrogen Loading to Eelgrass Structure and Eutrophication Symptoms Across 7 Bays in Eastern New Brunswick, Canada

3.0 Abstract

Nitrogen loading is a principal cause of coastal eutrophication and declines in seagrass health and cover globally. Using results from a Nitrogen Loading Model (NLM) and large-scale field surveys, we aimed to link quantitative estimates of anthropogenic nitrogen loading to eutrophic symptoms and eelgrass bed structure across 7 bays in eastern New Brunswick, Atlantic Canada. Results indicate that primary symptoms of eutrophication, including increases in epiphytic macroalgae and microphytobenthos exist in a gradient between bays. Carbon isotope ratios in eelgrass tissue were strongly positively related to eutrophic primary production symptoms, consistent with selective uptake capacities for ^{12}C over ^{13}C in phytoplankton and eelgrass. Changes in eelgrass bed structure and biomass were less pronounced, suggesting that secondary symptoms of eutrophication, such as wide-scale loss of eelgrass habitats are not yet occuring at present loadings. Multivariate ordination analysis revealed correlations between nitrogen loading rate per bay area and tidal flushing time with eelgrass structure and eutrophic symptoms at each site, with nitrogen loading rate showing the strongest relationship. Our study is the first to link quantitative estimates of nitrogen loading to eutrophication symptoms in bays in eastern NB.

3.1 Introduction

Coastal ecosystems that contain seagrasses are some of the most biologically productive areas on the planet, support diverse floral and faunal communities, and provide significant ecosystem services for the marine environment and human populations (Hemminga and Duarte 2000, Hanson 2004, Larkum et al. 2006, Lotze et al. 2006). Despite the recognized importance of these habitats, anthropogenic activities like nutrient loading and habitat destruction can negatively impact them, causing a reduction in their functioning capacity and declines in cover and abundance (e.g. Orth et al. 2006, Waycott et al. 2009, Short et al. 2011). Globally, it is estimated that between 29-65% of historical seagrass habitats are no longer functioning or present as such (Lotze et al. 2006, Orth et al. 2006), with some regions having >90% losses (e.g. Waquoit Bay, Buzzard Bay, Northern Adriatic; Hauxwell et al. 2003, Lotze 2010, Lotze et al. 2011).

Nitrogen (N) loading from anthropogenic sources in coastal watersheds and atmospheric deposition is recognized as a principal threat to eelgrass beds (e.g. (Hauxwell et al. 2003, Latimer and Rego 2010, Short et al. 2011). Quantifiable symptoms indicative of eutrophication in eelgrass habitats include increased epiphytic and benthic annual algae cover in/on eelgrass beds, anoxic sediment, higher chlorophyll a (Chl *a*) and particulate matter (TPM) concentrations in the water column and sediments, and the production of hydrogen sulfide (H_2S) at the sediment water interface (Bricker et al. 2003). The measurable effects of eutrophication on eelgrass beds include reduced shoot density, increased canopy height (to try to compensate for reductions in light penetration), reduced % cover of beds, overall reductions in biomass, and increased tissue N content, which may reflect the higher quantity of available ambient N (Bricker et al. 2003, Short et al. 2006a, Short and Burdick 2007). For the purpose of this research we define an increase in eutrophication and decrease in eelgrass health as an increase in the prevalence in some or all of these quantifiable metrics (Bricker et al. 2003, 2008, Short et al. 2006ab).

Eelgrass (*Zostera marina*) is the dominant macrophyte in shallow soft-sediment estuaries and bays of the Southern Gulf of St. Lawrence and the Atlantic coast of Canada (Senpaq 1990, DFO 2009, 2011). Previous research in the Southern Gulf of St. Lawrence has shown that bays along the coasts of New Brunswick (NB), Nova Scotia (NS), and Prince Edward Island (PEI) vary in the level of eutrophication and health of eelgrass habitats (e.g. Lotze et al. 2003, Plante and Courtenay 2008, Schmidt et al. 2012, Vandermuelen et al. 2014). A survey by Lotze et al. (2003) linked qualitative impacts of increasing nutrient loading with declines in eelgrass bed cover, increases in epiphytic and benthic algal cover, and increases in Chl *a* in eelgrass habitats of seven bays in NB. Schmidt et al. (2012) re-surveyed six of the same bays five years later and found similar trends between bays. The authors also identified that tissue N content of roots and shoots was a good predictor of other symptoms of eutrophication for the region, and allowed for a binary distinction between the sites with 'high' and 'low' eutrophication (Schmidt et al. 2012).

Many methods to measure and model N loading to estuaries exist, and vary in the type and quantity of input data, in situ measurement, and modeling complexity required (see Chapter 2). The Nitrogen Loading Model (NLM) framework developed for Waquoit Bay (Valiela et al. 1997a, 2000) was applied to 7 watersheds and bays in eastern NB to quantify N loading to the receiving bays with continuous eelgrass beds. The NLM results provide estimates of total dissolved N (TDN) loading from point and non-point sources from the respective watersheds. Point sources include municipal wastewater treatment effluent (MWWT), seafood processing plants, and peat harvest drainage, while the non-point sources are atmospheric deposition, agricultural and turf fertilizer runoff, and septic systems. The framework utilizes land-use and precipitation data, as well as information on concentration, flow volume and operation time of point sources of N (see Chapter 2).

The susceptibility of a bay to algal blooms and eutrophication can also be significantly influenced by the bathymetry, tidal amplitude and period, and freshwater inflow. More extensive distributions, more frequent occurrences and higher concentrations of Chl *a* and harmful algal blooms (HABs) have been shown to occur in systems that have longer flushing and residence times (Monbet 1992, Ferreira et al. 2005, Bricker et al. 2008). Concurrent with the development of the NLM for this region, a tidal prism model was applied to estimate flushing time of each of the bays providing a relative measure of bay susceptibility to N loading among them (see Chapter 2).

Bivalve aquaculture, primarily for the American oyster (*Crassostrea virginica*) is a prominent industry in many coastal bays in eastern NB. *C. virginica* has high clearance rates, and can be size selective in the particles they uptake, thereby influencing the pelagic (through filtration) and benthic (through biodeposition) production and nutrient characteristics close to aquaculture leases (Newell 2004, McKindsey et al. 2006). Important factors of aquaculture that can impact eelgrass habitats include stocking density, hydrodynamic patters and flushing time, and perhaps most importantly distance from an active lease (near-field to estuary scale, e.g. Dumbold et al. 2009, Skinner et al. 2013, Vance 2013). Underneath and near-field to aquaculture operations biodeposition of N rich organic waste from bivalves (benthic organic and nutrient loading, Hatcher et al. 1994, Grant 2005), direct shading, or the overwintering of cages on the bay floor (Skinner et al. 2013, 2014) can result in significant losses of seagrass habitat (McKindsey et al. 2006, Dumbold et al. 2009, Skinner et al. 2013, 2014). On the other hand, at the bay-wide scale bivalve aquaculture has been suggested as a mitigation tool for shallow eutrophic systems to increase filtration of organic particulate matter and removal of nutrients from the system upon harvesting (e.g. Haamen 1996, Landry 2002 Dame 1996, Vance 2013).

Other variables may further complicate the interaction between aquaculture and eelgrass habitats. The impacts of cultured bivalves may depend on the type of gear and associated density (e.g. suspended cages or lines which have lower stocking density than bottom cages), the extent of the farms, and the species of phytoplankton available for filter feeders (as size preference can alter the proportion of inorganic particles in the water column) (McKindsey et al. 2006, Comeau 2013). Hydrodynamic and oceanographic patterns which are variable between systems may also exert a significant effect on the local impacts of aquaculture leases on eelgrass habitats, in some instances preventing the negative effects of biodeposition and benthic nutrient loading (Mallet et al. 2006, Bastien-Daigle et al. 2007). In eastern NB, a recent study concluded that the shift from bottom to suspended aquaculture results in a decreased stocking density and a decrease in the grazing potential of individual oysters, effectively reducing the overall filter feeding capacity of the operations and negating the top-down control of phytoplankton at the bay scale (Comeau 2013). To account for the presence of bivalve aquaculture in our study bays, and potentially observable far-field effects on the eelgrass habitats, we included variables of cultured bivalve lease area and density in conjunction with N loading and flushing time in our assessment of eelgrass health and eutrophication.

The goal of this research was to test linkages between eutrophication symptoms in eelgrass habitats with estimates of N loading and the potentially mitigating factors of bay flushing time and bivalve aquaculture in 7 bays along the eastern coast of NB (Figure 1). We utilized recently calculated (see Chapter 2) estimates of (a) N loading (based on the Waquoit Bay NLM framework, Valiela et al. 1997a, 2000), (b) flushing time (based on a tidal prism model, Gregory et al. 1993) and (c) information on lease area and bag counts for oyster aquaculture (NBDAAF 2014) for all 7 bays. We performed a large-scale field survey in summer 2013 to assess eelgrass habitat characteristics and symptoms of eutrophication in each bay building on methods used by Lotze et al. (2003) and Schmidt et al. (2012). Because our field surveys incorporate measurements used in other large-scale monitoring efforts we can compare the eelgrass bed structural health (SeagrassNet, Short et al. 2006ab) and severity of eutrophication in our bays (NEEA, Bricker et al. 2003, 2008) to other regions in a global context. Finally, we analyzed whether spatial trends in eelgrass characteristics and eutrophic symptoms can be explained by site characteristics including estimates of nutrient loading, flushing time, and bivalve aquaculture. This work provides quantitative information on eelgrass habitat characteristics and eutrophication symptoms at a regionl scale and the multiple factors influencing them. Such information is essential to inform coastal planning and ecosystem-based management, and will allow decision makers to develop a site- and regional-scale approach to the management of human activities and conservation of seagrass habitats (Bricker et al. 2003, Short and Burdick 2007).

3.2 Methods

3.2.1 Site Selection and Description

Six of the bays assessed in this study (Cocagne (CN), Bouctouhe (BT), Kouchibouguac (KB), Tabusintac (TB), Baie St. Simon Sud (BSS), Lamèque (LM), Figure 1) are part of a long-term research effort assessing the health of eelgrass beds throughout eastern NB (Lotze et al. 2003, Schmidt et al. 2012). For our study we added Richibucto (RB) because there has been a large amount of previous research conducted on watershed characteristics, bay hydrodynamics, and historical and cultured bivalve populations (St Hilaire et al. 2001, Guyondet et al. 2005, Turcotte-Lanteigne and Ferguson 2013). All seven bays are shallow, soft-sediment embayments that contain eelgrass as the dominant macrophyte. All sampling sites (Figure 1, Table 1) are easily accessible from a roadway and representative of the average depth of the bay. In all bays there is a channel where the incoming and outgoing tidal water moves at a faster current, and these channels are meters deeper than the rest of the bays (Patriquin 1976, Gregory et al. 1993, Turcotte-Lanteigne and Ferguson 2013). Sampling depth did not exceed 3m at high tide at any of the sampling locations.

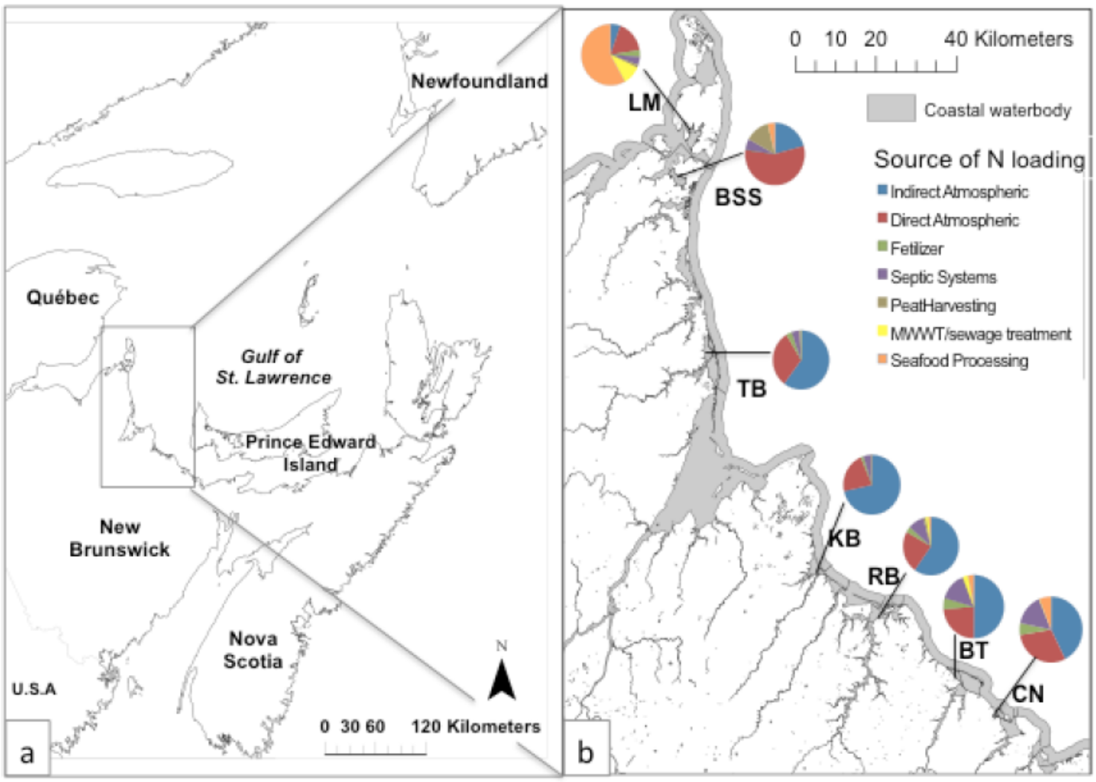

Figure 1. a) Study area in the Southern Gulf of St. Lawrence. b) Sample sites were in the bay portion of the estuaries, accessible from shore, and had continuous eelgrass beds. Maps made in ArcMap with digital data provided by the NB Department of Natural Resources and GeoGratis Canada (ESRI 2011, NB DRN 2012, GeoGratis 2013). The right panel also shows the proportion of sources of total N load to each bay in which we sampled in 2013 as estimated by the NLM. Map created in ArcMap (ESRI 2011) and Microsoft Excel 2010.

3.2.2 Data Collection

Site characteristics for each bay including total N load, N loading rates, flushing time, and Δ-N (Table 1) were taken from the previous application of the NLM model detailed in Chapter 2. Statistics on oyster aquaculture were accessed through the NB Department of Agriculture, Aquaculture and Fisheries. Except where noted, the data we present is representative of active lease area and bag counts in years 2012-14 as no major changes in stocking were anticipated (NBDAAF 2014). Stocking density (bags/ha) per lease and per bay was calculated by dividing the total bag count by the active lease area and the bay area. Sampling depth within the eelgrass beds we assessed were recorded on SCUBA dive computers during the field survey (see 2.3).

3.2.3 Field Survey

3.2.3.1 Spring and Fall Survey

Spring sampling took place between June 4-5th and fall sampling between October 16-17th 2013 at all 7 sites (CN, BT, RB, KB, TB, BSS, LM). Sampling took place within the same 400m^2 area as previously sampled and GPS recorded in 2007 by Schmidt et al. (2012). Because Richibucto was not sampled using this design in 2007 we selected a site of similar depth and distance from shore that had a continuous eelgrass bed of at least 50m width. During both spring and fall sampling we took water and eelgrass tissue samples within an hour on each side of high tide at all sites. Eelgrass samples were collected to assess elemental Carbon (C) and nitrogen (N) content and $\delta^{13}C$ and $\delta^{15}N$ of eelgrass shoots (above ground (AG)) and roots/rhizomes (below ground (BG)). Snorkelers haphazardly collected three large handfuls of eelgrass shoots and connected roots and rhizomes using their hands. All tissues were placed in labeled zip lock bags, stored on ice, and transported back to the laboratory for processing following fieldwork.

To assess water column integrated phytoplankton and TPM concentrations, the same snorkelers collected three, 1L water samples using a pipe sampler (weighted tubing 2m long, 3.25cm diameter) at high tide. Samples were immediately transferred to opaque thermos bottles and taken to shore. Within an hour of collection water samples were filtered for Chl *a* (μgL^{-1}) using a hand-held pump in a dark vehicle. For Chl *a*, a 70mL subsample of each water sample was pushed through the pump and a 0.7-mm Whatman GF/F filter (2.5cm diameter). For TPM (mgL^{-1}) enough water was filtered through the hand pump to create a noticeable change in colour on pre-ashed (6h at 400°C) and weighed 0.7-mm Whatman GF/F filters (2.5cm diameter). Filters were then rinsed twice with 10mL of ammonium formate to dissolve any salt accumulation. All filters for Chl *a* and TPM were immediately placed in pre-labeled cryovials and stored in a liquid nitrogen dewar at -80°C for transport back to the lab.

3.2.3.2 Summer Survey

From August 5-12th of 2013 we conducted a more extensive field survey that followed and expanded upon the sampling design introduced in 2003 (Lotze et al. 2003) and 2007 (Schmidt et al. 2012). At each site, we assessed eelgrass canopy structure (shoot density, canopy height, percent cover, above and below ground biomass), tissue CN content, epiphytic and benthic annual algal cover, and water column chlorophyll a (Chl *a*) and total particulate matter (TPM- both particulate organic (POM) and inorganic (PIM) matter). We added the analysis of above (AG) and below ground (BG) $\delta^{15}N$ and $\delta^{13}C$ stable isotopes as well as microphytobenthos Chl*a* concentration, and sediment organic content. All sampling occurred within 2 hours of high tide when DFO tidal stations in each or adjacent bays predicted tidal high as ≥ 0.9m.

We laid two 50m transects, parallel and 4m apart, within the same 400m^2 area as in 2007 and during spring and fall sampling (see above). Using SCUBA, eleven 0.5 x 0.5m^2 quadrats were placed in 5m intervals along the two transects, with quadrates being placed at 0, 10, 20, 30, 40, 50m on transect 1, and at 5, 15, 25, 35, 45m on transect 2. Within each quadrate a diver counted shoot density, measured average canopy height, and estimated the percent cover of eelgrass and bare sediment, as well as percent cover of benthic and epiphytic (on eelgrass shoots) algae. Additionally, in 6 quadrats (0, 30, 50m on transect 1; 5, 25, 45m on transect 2) the same diver used a small syringe core (1.5cm in diameter) to extract the top 2cm of surface sediment and microphytobenthos (volume of sample $\approx$3.53mL). Three syringe samples were taken from each quadrat and then on shore combined into a single sample for later fluorescent analysis to provide a more integrated measure of microphytobenthos (Seuront and Spilmont, 2002). The combined samples from each site were placed in individual labeled, plastic cryovials and stored first in liquid nitrogen and then a freezer (-20°C) until analysis in the laboratory (Riaux-Gobin et al. 1987). In the same 6 sampling quadrats a larger sediment core (0.0314m^2) was pushed $\approx$15-20cm into the sediment, closed off and pulled out, capturing the eelgrass shoots, roots and rhizomes contained in the core volume. On shore the eelgrass shoots were counted and the roots, rhizomes and shoots removed from the cores, placed in labeled bags and laid on ice for transport back to the laboratory. Once in the lab the eelgrass was stored in a fridge (4°C) for a maximum of 7 days until it could be further processed.

Water column primary production and particulate matter samples were collected using the same methods as the spring and fall sampling. The same individual performed the filtration for all sites in each season.

3.2.4 Laboratory Analysis

Back in the lab, eelgrass shoots were separated from the roots/rhizomes, keeping these portions separate for the rest of analysis. We carefully removed all epiphytic algae and invertebrates from the shoots with freshwater and a razor blade. Cleaned above (AG) and below (BG) ground tissue was weighed for biomass (wet weight, g m^{-2}) and then dried in the oven at 80°C for 48 hours and weighed again (dry weight, g m^{-2}). Following desiccation, dried tissue was ground up using a mortar and pestle, and stored in airtight glass vials in a cool, dark drawer. Tissue samples from spring, summer and fall were sent to the University of California Davis Stable Isotope facility for analysis of % tissue nitrogen (N) and carbon (C), and nitrogen ($\delta^{15}N$, ^{15}N:^{14}N) and carbon ($\delta^{13}C$, ^{13}C:^{12}C) stable isotopes. Tissue C and N were presented in mg and % of dry weight (% DW).

TPM filters were dried (24h at 60°C), weighted then combusted (6h at 400°C) and weighed again to estimate POM and PIM. POM content (g) is calculated by subtracting the ash weight from the dry weight, and PIM is the remainder. Chl*a* was measured using the Welschmeyer technique (Welschmeyer 1994). Chl*a* on filters was digested in 10mL of 90% acetone at -20°C for 24 hours and the extract was measured in a Turner Designs 10-005R fluorometer.

Microphytobenthos Chl*a* was measured similar to the water column Chl*a*. Prior to fluorometry frozen sediment samples were placed in labeled glass scintillation vials with 10mL of 90% acetone, vortexed for 1 minute and digested at -20°C for 24 hours. The following day samples were vortexed for one minute, placed in falcon tubes and centrifuged for 30 minutes at 3250rpm (T. Whitsit, Dalhousie, pers. comm.). The supernatant was subsequently pipetted into clean scintillation vials and measured in a Turner Designs 10-005R fluorometer.

Sediment samples were analyzed for percent organic content at the Bedford Institute for Oceanography (M. Wong, pers. comm.). Samples were weighted (wet weight), dried (24h at 60°C), weighed (dry weight), and combusted (6h at 400°C) before the ash weight was taken. Organic content (g) was calculated by subtracting the ash weight from the dry weight, and percent organic content was calculated by dividing the organic weight by dry weight and multiplying by 100.

3.2.5 Statistical Analysis

The two questions we wanted to answer about regional patterns of eutrophication within eelgrass habitats with statistical means were: a) Which sites differ with respect to eelgrass bed structure and eutrophic symptoms and b) What site characteristics (NLM model estimates, flushing time, Δ-N, bivalve aquaculture lease area, bag count, and stocking density) are most related to combinations (multivariate distance matrix) of eelgrass parameters and eutrophic indicators from field surveys.

a) Eelgrass Bed Structure, Water Column and Sediment Characteristics

We tested whether eelgrass bed structure, tissue, sediment and water column variables differed between sites using permutational univariate analysis of variance (PERMANOVA) with site (bay) as the independent factor. We used seasonal averages (June, August and October samples) for tissue elemental content (C,N), tissue isotopes (C,N), and water column parameters (Chl*a*, TPM, PIM, POM). All other variables were only assessed in August. The analysis was conducted with the ADONIS function in statistical package *"vegan"* for 'R' (Anderson 2001, Oksanan et al. 2013). PERMANOVA tests the null hypothesis (with *a-priori* chosen significance level of $\alpha = 0.05$) that the centroids of the groups (sites in this case) in the pre-defined distance space (Euclidean) are equivalent for all groups. If H_o were true the observed differences among group centroids would be similar in magnitude to differences obtained by random allocation of observations at each site (through permutation) to groups (Anderson and Walsh 2013). Like ANOVA, PERMANOVA estimates the variation for independent factors as sums of squared fixed effects (divided by respective degrees of freedom) and actual variance components for factors (Anderson 2001). These parameters are presented in squared units of Euclidean distance, but by square root transforming them ($\sqrt{V}$) back to their original units we can interpret the proportion of variance explained by each factor, and therefore their relative importance in explaining overall variance in the model (Anderson 2001, Schmidt et al. 2012).

When parametric assumptions of variance homogeneity and normality were met we used both parametric (ANOVA) and non-parametric (PERMANOVA) statistics and compared results. Protected post hoc t-tests (Tukey's Honestly Significant Differences test) were used to determine which sites were different from each other in terms of eelgrass bed structure, tissue, sediment and water column variables.

PERMANOVA makes the assumption that there is homogeneity of multivariate dispersion among groups (or homogeneity of variance within groups for univariate analysis) (Anderson 2001, Anderson and Walsh 2013). We use the beta-disperse (analogous to PERMDISP for PERMANOVA) function in the "*vegan*" package (Oksanan et al. 2013) to test this assumption using the H_o that the average within-group dispersion, as measured by the average (Euclidean) distance of objects to the group centroid is equivalent among groups (sites). To assess whether assumptions of ANOVA were met (homogeneity of variance, normality, linearity) we looked at residual plots in R. If assumptions were violated we performed transformations on the dependent variable ($\log_{10}$, square and quarter root) and re-ran tests to see if assumptions were met.

We assessed whether different measures of primary production that can be indicative of eutrophication (microphytobenthos, epiphyte cover) are correlated with carbon isotope ratios of eelgrass tissue to evaluate whether carbon isotope ratios could be used as an integrated predictor of primary productivity. We also looked at the relationship between tissue C:N ratio and Tissue N content (%) in AG and BG tissue across sites to see whether eelgrass tissues were N limited.

b) Linking Differences in Eelgrass Beds and Eutrophic Symptoms to Site Characteristics (Nutrient Loading, Flushing Time and Bivalve Aquaculture)

The BIOENV procedure (Clarke and Ainsworth 1993) allows for the comparison of distance/dissimilarity matrices between two sets of data. These matrices are structured so that columns are variables, and rows are objects (e.g. in our case n=6 multivariate objects per site corresponding to the six quadrats at each site in which all variables except water parameters were sampled; see below). The matrices can both contain biological/community variables (BIO-BIO analysis) or environmental/site characteristic variables (ENV-ENV), or one matrix of each type of variables (BIO-ENV). We used the function *bioenv()* in the *R* package *"vegan"* (Oksanan et al. 2013) to perform an ENV-ENV procedure to select the best subset of site variables (all NLM outputs, flushing time, Δ-N, aquaculture bottom lease area, aquaculture bags ha^{-1}, and sampling depth) that maximize the rank correlation coefficient (Spearmans ϱ/ *Rho* statistic) with objects containing variables of eutrophication symptoms and eelgrass bed structure at each sample location (also ENV characteristics). We used different combinations of these eutrophic/eelgrass variables (eelgrass shoot density, canopy height, %cover, AG and BG biomass, tissue N, δC^{13}, δN^{15}, microphytobenthos, epiphytic and benthic algae %cover, sediment organic content) in multiple applications of the ENV-ENV to illustrate which site characteristics maximize the rank correlation of (a) all eelgrass/eutrophic symptoms assessed together, and (b) subsets of symptoms such as eelgrass bed structure, eelgrass tissue, sediment characteristics, and annual algae. The "best" subsets of site characteristics are selected by computing the correlation between objects in the normalized Euclidean distance matrix of eutrophic/eelgrass variables and all possible Euclidean distance site characteristic matrices (Clarke and Ainsworth, 1993, Oksanan et al. 2013). Eutrophic percent-cover variables were arc-sin transformed prior to normalization, and site characteristics were $\log_{10}$ transformed. We assessed seasonal water column variables (Chl*a*, PIM, POM) separate from other variables measured in the quadrats, therefore they are not included in these multivariate analyses. In all the multivariate analyses we use only summer AG and BG (not averaged) tissue characteristics (C, N content, δC^{13}, δN^{15}) as they correspond to all other measurements taken from the same six quadrats at each site.

The rank correlation coefficient (Spearmans ϱ/ *Rho* statistic) has a value between -1 to +1, with -1 indicating complete opposition of variables, 0 inferring the absence of any match between the objects in the two matrices, and +1 meaning complete agreement between the objects in the two matrices. This is an exploratory analysis and not a test of significance (Clarke and Ainsworth, 1993, Oksanan et al. 2013). Therefore, we then used the *adonis()* function in the *"vegan"* package for *R* (Oksanan et al. 2013) to identify whether the "best" site characteristics identified through the ENV-ENV procedure explain the variance in eelgrass characteristics and eutrophication symptoms observed in the sampling locations in each bay (Anderson 2001). We used a two way PERMANOVA to examine the effects of the "best" site characteristics on the normalized Euclidean distance matrices combining eutrophic symptoms/eelgrass characteristics. We performed multivariate analysis on different combinations of variables (see results), and then explored the effect and interaction of the "best" site characteristics for each eelgrass/eutrophication variable individually. As described above, we tested the null hypothesis (with *a-priori* chosen significance level of $\alpha = 0.05$) that the centroids of the groups in the pre-defined distance space (Euclidean) are equivalent for all groups, and tested for the homogeneity of multivariate dispersion/homogeneity of variance using the *beta-disper()* function in the *R* package *"vegan"* (Oksanan et al. 2013, Anderson and Walsh 2013).

To assess which sites had more similarity in eelgrass habitat structure and expression of eutrophic symptoms (e.g. to see whether eelgrass habitats at sites with high nutrient loading were more similar to each other than those with low loading) we used hierarchical cluster agglomeration (HCA) analysis on the objects of the normalized Euclidean distance matrix of eutrophic/eelgrass variables (the combination of variables that produced the best fit in the ENV-ENV analysis: Eelgrass shoot density and canopy height, epiphytic and benthic algae % cover, summer AG and BG Tissue N, $\delta^{15}N$, $\delta^{13}C$, microphytobenthos). Percent cover variables were arc-sin transformed prior to normalizing, and water column variables were not included. We overlaid the distances from hierarchical cluster agglomeration on the non-metric Multi-Dimensional Scaling (nMDS) 3D ordination of the same objects to illustrate similarity/dissimilarity between multivariate objects from the 7 sample sites using *ordispider()* in *"vegan"* for *R*. NMDS finds a non-parametric ordered (monotonic) relationship between the dissimilarities of objects in a matrix, and the Euclidean distance between objects and positions of these objects in low dimensional space (number of axes< number of variables). The function aims to maintain the distance between objects in the matrix in the ordination, and low stress values indicate the success (Oksanan et al. 2013). Analysis and visualization was done using the *hclust()* and *metaMDS()* functions in *R* package *"vegan"*, and "*ggplot2"* package for *R* (Wickham 2009, Oksanan et al. 2013). We overlaid vectors of those site characteristics that maximized the rank correlation of eutrophic/eelgrass symptoms, as identified by ENV-ENV on the nMDS ordination: the vectors point in the direction where the linear change in the site characteristic is fastest, and the length of the vector indicates the strength of the correlation between the site characteristic and the ordination axis score. Note, vector fitting is effectively a regression; however, we assumed that the assumptions of linear regression are not met so we applied a permutation test to create a random response variable and compared this to fitted values from the model to get an R^2 value and an indication of fit. The significance of the relationship is tested by applying the H_0 that there is no relationship between the site characteristic and the ordination axis scores (coordinates along ordination axes where an object is positioned) and we *a-priori* assigned a significance level of p=0.05 (*bioenv(), envfit()* in *"vegan"* for *R*, *"ggplot2"* for *R,* Wickham 2009, Oksanan et al. 2013).

3.3 Results

3.3.1 Site Characteristics and NLM Estimates

Below is a brief overview of the model estimates from the NLM, flushing time and Δ-N (based on Chapter 2) that we used as site characteristics in our analysis (Table 1). Total N loading was correlated with watershed size, and reflected the large proportion of atmospheric N deposition in the watershed (Figure 1, and see Chapter 2). N loading rates per unit watershed area were highest in LM (20.7 kgTDN ha watershed^{-1}yr^{-1}) and BSS (7.3 kgTDN ha watershed^{-1}yr^{-1}), and were around 2.0 kgTDN ha watershed^{-1}yr^{-1} in the other 5 watersheds. N loading rates per unit bay area differed from loading rates per unit watershed area due to the variable watershed:bay size ratios: highest loading rates were again in LM (62.47 kgTDN ha bay^{-1}yr^{-1}), while the lowest were in BSS (18.97 kgTDN ha bay^{-1}yr^{-1}). The other watersheds had intermediate loading rates between 35-54 kgTDN ha bay^{-1}yr^{-1}.

RB had the longest estimated flushing time from the tidal prism model (67h, Table 1). LM and KB had intermediate flushing time estimates over 50h, while CN, BT, TB and BSS had quicker estimated flushing near 30h. The Δ-N estimates were not distinct between sites, and were all within the same order of magnitude. KB (0.009) had the highest estimated Δ-N, BSS (0.002) the lowest, and other watersheds intermediate values (Table 1).

Of the 5 bays only LM and KB do not currently have any active aquaculture leases (Table 1). For the other 5 bays we present data that was representative of active lease area, bag counts, and stocking density in the years 2012-14 (except where noted), as no major stocking or lease changes were anticipated for this time. Largest active lease (260ha) and highest bag counts were found in RB (Table 1). CN and BSS had approximately 150ha of active lease, while TB and BT had less allocated area (~100ha). Stocking density per bay area (bags ha bay^{-1}) was similarly low in BT and TB, and intermediate in CN. The highest stocking densities were in RB and BSS, however, within lease stocking density (bags ha lease^{-1}) was much higher in RB, accounting for the higher proportion of surface area consumed by active lease in BSS relative to RB (Table 1).

Table 1. Location and site characteristics of sampling areas in 7 bays in eastern NB where summer surveys were conducted. Nitrogen load, loading rates, flushing time and ΔN for each bay were calculated in Chapter 2. Latitude and Longitude are shown in decimal degrees. Data on oyster aquaculture was sourced from the NB Department of Agriculture, Aquaculture and Fisheries and represents lease area and bag counts between 2012-14 as no major changes were anticipated between these years. Errors show difference between max and min loading based on variation in atmospheric deposition between 1992-2008, and wastewater effluent in provided data (See methods, 2.2.2).

Site	CN	BT	RB	KB	TB	BSS	LM
Latitude North	46.369	46.699	46.693	46.842	47.382	47.796	47.732
Longitude West	-61.603	-67.677	-64.815	-64.938	-64.940	-64.675	-64.776
Sampling depth (m)	1.00	0.75	1.20	0.75	0.80	1.50	1.00
N load (kg TDN/ yr)	96,330 ±29,028	189,753 ±58,724	266,108 ±96,749	79,958 ±30,841	130,802 ±45,466	15,773 ±6,804	67,223 ±7,632
N loading rate (kg TDN/ ha watershed/yr)	2.90 ±0.87	2.50 ± 0.77	2.07 ± 0.75	1.51 ± 0.58	1.84 ± 0.64	7.31 ± 3.15	20.74 ± 2.36
N loading rate (kg TDN/ ha bay/yr)	39.51 ± 11.91	49.76 ± 0.77	51.99 ± 18.90	54.84 ± 21.15	35.68 ± 12.40	18.94 ± 8.17	62.42 ± 7.09
Flushing time (h)	31.58	33.15	66.74	52.54	30.22	30.32	53.79
ΔN (mg/L)	0.005	0.007	0.008	0.009	0.005	0.002	0.006
Aquaculture active lease area (ha) [% bay area] (2012-14)	132.10 [5.4]	103.50 [2.7]	260.40 [5.1]	0 [0]	97.04* [2.6]	156.19* [18.7]	0 [0]
Aquaculture bag count (# bags/bay) (for 2012-14)	42,250	41,190	132,148	0	32,533*	25,865*	0

Aquaculture bag density (# bags ha bay^{-1}) (for 2012-14)	17.33	10.80	25.82	0	12.15*	23.54*	0
Aquaculture bag density (# bags ha active lease^{-1})	319.83	399.72	507.48	0	459.07*	125.46*	0

* Data is average of 2012, 13, 14 as there was some change in stocking density for these years.

3.3.2 Field Survey Results

During summer sampling water temperature ranged from 19.6-22.5°C at all sites. Salinity was also similar between sites, ranging from 23.9-27.2. Because there was no statistically identifiable difference of temperature or salinity between sites we did not include these variables in our multivariate analyses.

Results of the summer field survey are shown in Figures 2-9. Eelgrass canopy height was similar in CN, RB, TB, BSS and LM, while shoots were significantly taller in BT than BSS and LM, and significantly taller in KB compared to all other sites except BT (Figure 2a, Appendix 4: Table 1, 2, 3). Shoot density of eelgrass beds was significantly higher in BSS and higher (but not significant) in LM compared to BT, RB, KB and TB. Shoot density in CN was intermediate and neither significantly different from the beds in BSS and LM, nor the sample beds in the other 4 bays (Figure 2b, Appendix 4: Table 2,4). Percent cover of eelgrass was similar between all sites with near 100% cover in all quadrats in KB (98±1.2%) and BSS (100±0.5%). Some periodic patchiness was evident in TB (72±9.7%) and LM (85±9.5%), but overall there was no significant difference in cover between sites (not shown- see Appendix 4: Table 1). BG eelgrass biomass was higher than AG biomass at all sites, and no significant difference between sites was found. The lowest AG biomass per m^2 was sampled in CN, and KB had the highest sampled AG biomass (significantly higher than CN, but no other sites) (Figure 2c-d, Appendix 4: Table 2,5).

Epiphytic algae% cover was significantly lower in KB and TB and higher in RB compared to several other sites (Figure 3a, Appendix 4: Table 2,6). Benthic algal cover was lower throughout, with highest values in TB and LM, but no significant differences between sites (Figure 3b, Appendix 4: Table 2). Microphytobenthos Chl*a* concentration was lowest in KB and TB and highest in RB (Figure 3c, Appendix 4: Table 2,7), a similar pattern to epiphytic algae cover (Figure 3a). Sediment organic content was highest in TB and BSS, while CN, BT, RB and KB had similar proportions of organic matter in sediment (Figure 3d, Appendix 4: Table 2,8).

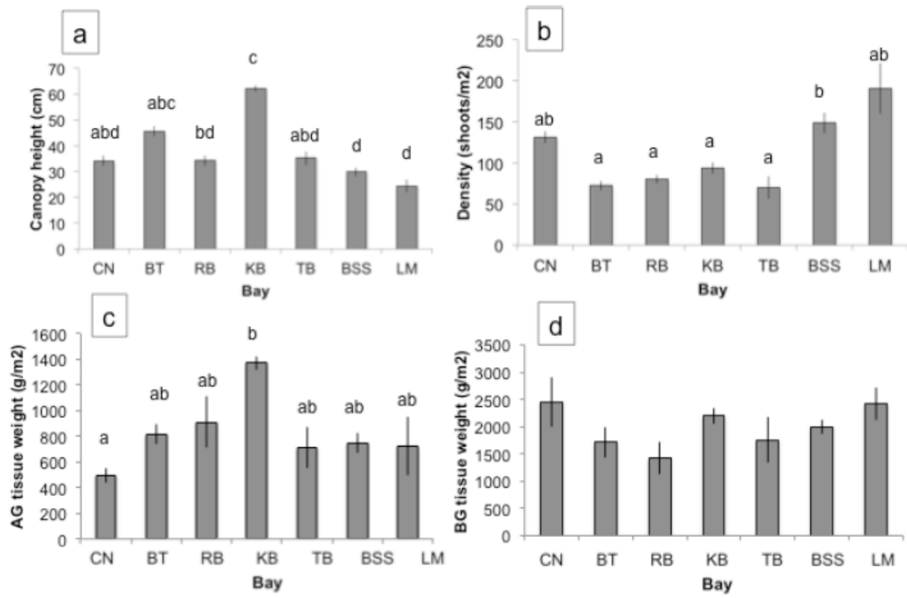

Figure 2. Average measurements (± SE) of eelgrass a) canopy height (n=11), b) shoot density (n=11), c) above ground (AG) tissue biomass (wet weight) (n=6) and d) below ground (BG) tissue biomass (n=6) taken in summer 2013 from sampling sites in 7 bays in eastern NB. Significant differences ($p<0.05$) between sites are indicated by letters. No significant difference in BG tissue biomass was detected among sites.

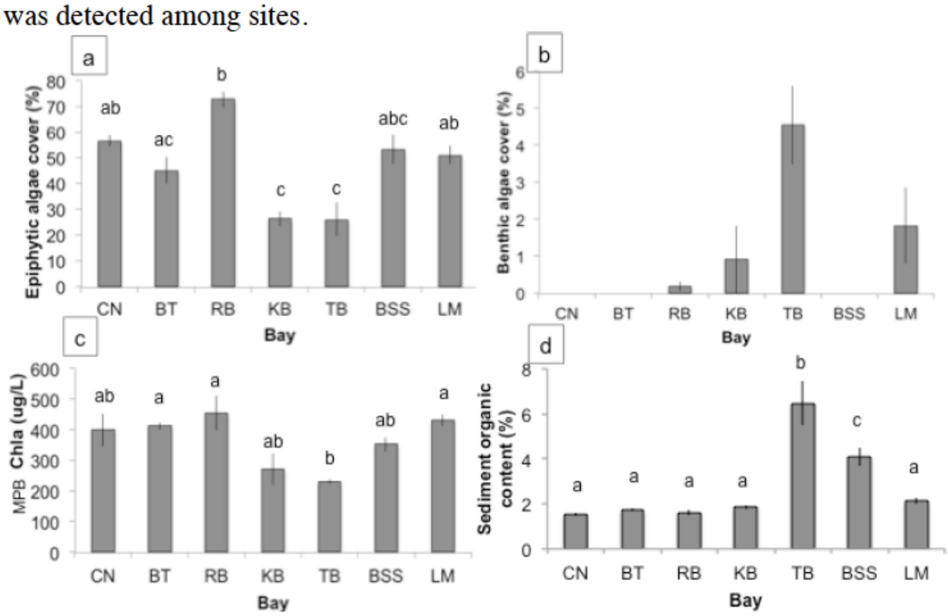

Figure 3 Average (±SE) measurements of a) epiphytic aglae % cover and b) benthic algae % cover (n=11), c) microphytobenthos (sediment Chl*a*, n=6), and d) sediment organic content (n=6) taken in summer 2013 from sampling sites in 7 bays in eastern NB. Significant differences ($p<0.05$) between sites are indicated by letters. No significant differences in benthic algae % cover among sites was detected.

In general, the spring, summer, and fall water samples collected in KB and RB had higher Chl*a*, and BSS consistently had very low concentrations (Figure 4a). Generally PIM constituted a larger proportion of TPM than organic matter, especially in KB and BSS where POM concentrations were the lowest among sites (Figure 4b-d). The high within site variation, and likely too few replicates caused the assumption of homogeneity of variances to be violated even when data was transformed. Therefore we cannot comment with confidence whether there was a significant difference in water column productivity parameters between sites).

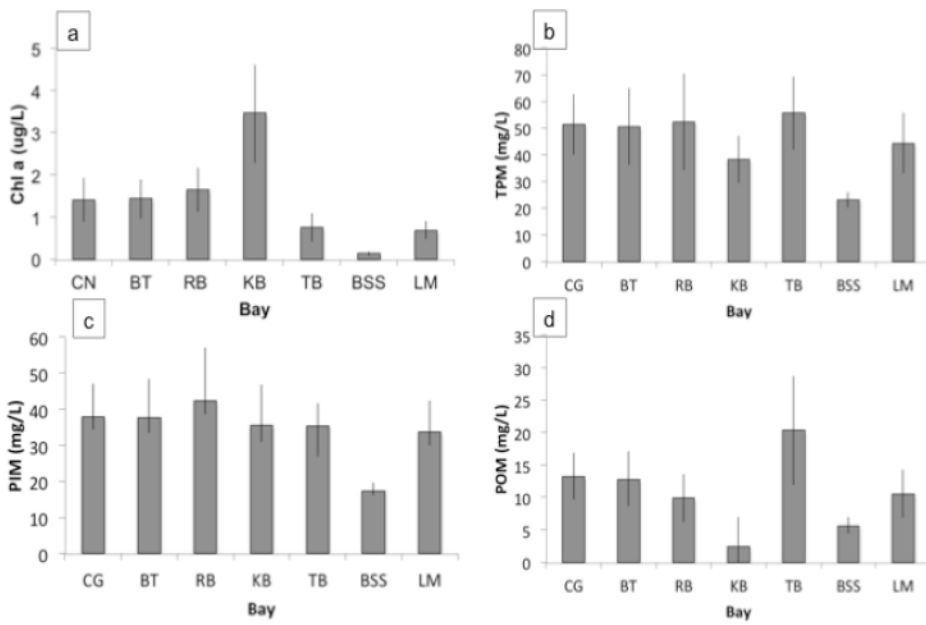

Figure 4 Average (±SE) measurements of a) water column Chl*a,* and b) particulate matter (TPM) including c) its inorganic (PIM) and d) organic (POM) components taken in spring, summer and fall 2013 from sampling sites in 7 bays in eastern NB (n=9). Within site variation was large and measurements violated assumptions of ANOVA and PERMANOVA so we cannot confidently infer significant differences between sites.

Eelgrass tissue nutrient and isotope content exhibited the most striking differences between sites (Appendix 4: Tables 2,9-15). AG tissue N was significantly higher in CN and LM compared to RB, TB, and BSS (Figure 5, Appendix 4: Table 2,9). AG N tissue content in BT was intermediate, but more similar to RB and KB than to the elevated values seen in CN and LM. In contrast, despite a significant site effect, post-hoc tests could not detect differences in BG tissue N content among sites (Figure 5, Appendix 4: Table 2,10). Similarly, no strong differences in AG or BG C was found between sites

(Appendix 4: Figure 1) despite a significant site effect for AG C but not for BG C (Appendix 4: Table 2,11). At sites where tissue N content was highest (CN, LM, RB) and C:N ratios were lowest, some samples contain more N content than the threshold of 18 mg N per g dry weight noted by Duarte (1990,1992), above which plants are no longer N limited (Figure 6).

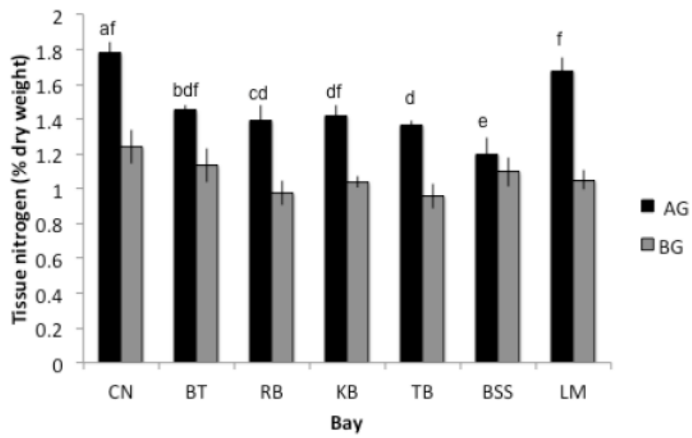

Figure 5. Average N content of AG and BG eelgrass tissue sampled in spring, summer and fall 2013 from 7 bays in eastern NB (± SE, n=12). Significant differences ($p<0.05$) in AG tissue N are indicated by letters; no significant differences in BG tissue N were detected between sites.

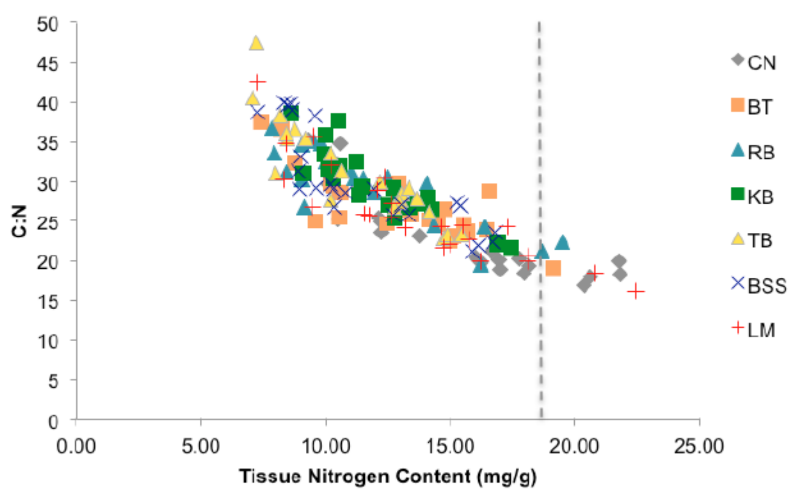

Figure 6. C:N ratio in AG and BG eelgrass (shoots, roots and rhizomes) versus N concentration (mg/g dry weight) between June–October 2013. The vertical dashed line indicates the threshold values for maximum growth of eelgrass based on the balance of external nitrogen supply and internal demand by plants. Tissue measurements to the right of the line represent ambient nitrogen saturation, above which growth does not increase with the addition of more nitrogen. As nutrient availability increases, uptake of nitrogen increases, reducing the CN ratio in tissue (Duarte 1990, 1992).

AG and BG N isotopes exhibited a similar trend between sites. AG $\delta^{15}N$ was significantly higher in LM tissue than all other sites (Figure 7, Appendix 4: Table 2,12). The lowest AG $\delta^{15}N$ values were collected from BSS and CN, while intermediate values were seen in BT, RB, KB and TB. Similarly, BG $\delta^{15}N$ was significantly higher in LM than all other sites, and the lowest values were in CN and BT (Figure 7, Appendix 4: Table 2,13).

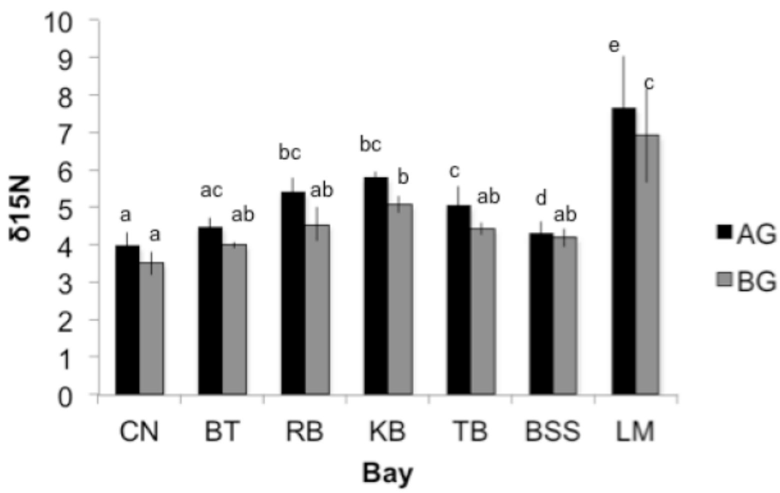

Figure 7. Average N isotope ($\delta^{15}N$) values in AG and BG eelgrass tissue sampled in spring summer and fall 2013 from 7 bays in eastern NB (± SE, n=12). Significant differences ($p<0.05$) in tissue isotopes are indicated by letters for AG and BG separately.

Carbon isotope values were similar in AG and BG components at all sites, though the differences between sites were more apparent in BG tissue. The lowest (most negative) AG $\delta^{13}C$ values were from KB, TB and BSS tissues and the highest in tissue from CN, BT and RB (Figure 8, Appendix 4: Table 2,14). Similarly, BG values were significantly lower in KB, TB and BSS, while tissues from CN, BT, RB and LM were similar (Figure 8, Appendix 4: Table 2,15).

Both summer AG and BG $\delta^{13}C$ were significantly positively related to both microphytobenthos Chl*a* concentration and to epiphytic algae% cover, but the relationships were more pronounced with BG tissue isotopes compared to AG isotopes (Figure 9). TB, KB and BSS had low levels of microphytobenthos and epiphytic algae % cover, as well as more negative $\delta^{13}C$ values in both AG and BG tissue. Conversely, CN, BT, RB and LM had the highest observations of both microphytobenthos and epiphytic algae % cover, and the highest AG and BG $\delta^{13}C$ values (Figure 9).

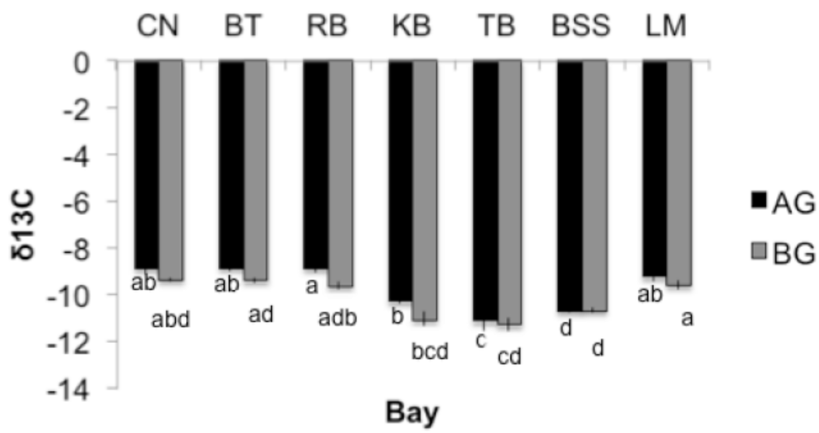

Figure 8. Average C isotope ($\delta^{13}C$), values in AG and BG eelgrass tissue sampled in spring, summer and fall 2013 from 7 bays in eastern NB (± SE, n=12). Significant differences in tissue isotopes are indicated by letters for AG and BG separately.

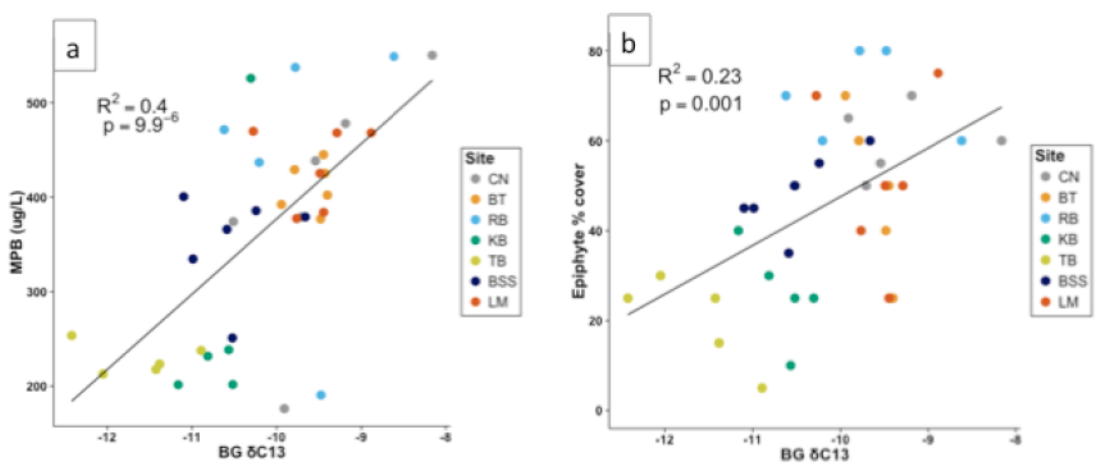

Figure 9. Linear relationships between BG $\delta^{13}C$ and microphytobenthos (a) and epiphytic algae % cover (b) in the six quadrats sampled in 7 bays in eastern NB in summer 2013. Similar, but weaker relationships were found with AG $\delta^{13}C$.

3.3.3 Linking Differences in Eelgrass Beds and Eutrophic Symptoms to Nutrient Loading, Flushing Time and Bivalve Aquaculture

a) BIO-ENV analysis

Application of the ENV-ENV analysis on different combinations of eutrophic/eelgrass variables showed a correlation between some estimates from the NLM, flushing time, and Δ-N as well as aquaculture area/density measurements (Table 2). Nitrogen loading rates (kgTDN ha bay^{-1} yr^{-1}, kgTDN ha watershed^{-1} yr^{-1}) were the variables that were most commonly and strongly correlated with the combination of both eelgrass structure and eutrophic symptom variables (excluding water column variables). The highest *Rho* statistic between site characteristics and eelgrass and eutrophic symptom variables (0.57) was produced when the following variables were assessed together: eelgrass shoot density and canopy height, epiphytic and benthic algae % cover, microphytobenthos, and summer AG and BG Tissue N, δ^{15}N, δ^{13}C (Table 2). We used this combination of eelgrass/ eutrophic variables in the remainder of the multivariate analysis (nMDS ordination, HCA, PERMANOVA).

Table 2. Results of BioEnv (EnvEnv) analysis comparing the similarity between site characteristics and multivariate objects of eutrophic/eelgrass variables (n=6 for all quadrat variables) in normalized Euclidean distance matrices. The site characteristics that best correlated with combinations of eutrophic symptoms/eelgrass bed parameters are shown and the *Rho* statistic indicates the strength of the rank correlation (-1 - +1). All objects contain observations collected during summer sampling in the 7 bays in eastern New Brunswick (August 5-12th, 2013), except seasonal water column variables which are assessed individually (n=9). The best combination of eelgrass structure and eutrophic variables and site characteristics is shown in bold font.

Eutrophic/eelgrass variables in multivariate distance matrix	Environmental variables	***Rho*** statistic
Eelgrass shoot density and canopy height, AG and BG biomass, epiphytic and benthic algae % cover, tissue N, δ^{15}N, δ^{13}C , microphytobenthos, sediment organic (%),	N loading rate (kg TDN ha bay^{-1} yr^{-1}), N loading rate (kg TDN ha watershed^{-1} yr^{-1})	0.501
Eelgrass shoot density and canopy height, epiphytic and benthic algae % cover, Tissue N, δ^{15}N, δ^{13}C , microphytobenthos	**N loading rate (kg TDN ha bay^{-1} yr^{-1}), N loading rate (kg TDN ha watershed^{-1} yr^{-1})**	**0.570**
Tissue (% N, %C, δ^{15}N, δ^{13}C) (summer)	N loading rate (kg TDN ha bay^{-1} yr^{-1}), N loading rate (kg TDN ha watershed^{-1} yr^{-1})	0.641*
Tissue (% N, %C, δ^{15}N, δ^{13}C) (n=12, all seasons)	N loading rate (kg TDN ha watershed^{-1} yr^{-1})	0.423
Sediment (microphytobenthos, Sediment organic)	N loading rate (kg TDN ha bay^{-1} yr^{-1}), Δ-N	0.222
Eelgrass structure (SH, canopy height, eelgrass % cover, AG and BG biomass)	Total N load (kgTDN yr^{-1}), Δ-N, sampling depth	0.307
Annual macroalgae (epiphytic and benthic algae % cover)	Flushing time (h), aquaculture density (bags ha bay^{-1}), (bags ha lease^{-1})	0.152
Primary Productivity (epiphytic and benthic algae %cover, microphytobenthos)	Δ-N, aquaculture active lease (ha), aquaculture total bag count, aquaculture density (bags ha lease^{-1})	0.174
Water column primary productivity (Chl*a*, PIM, POM) (n=9, all seasons)	Flushing time (h), aquaculture total bag count	0.118

* Although this Rho statistic is higher, the measured variables assessed only include eelgrass tissue C and N content and isotopes, and therefore does not represent multiple symptoms of eutrophication or measures of eelgrass bed structure.

We also assessed smaller combinations of related variables (sediment characteristics, eelgrass structure, primary production, summer eelgrass tissue characteristics, annual algae, and seasonal water column measurements). N loading rates (kgTDN ha bay^{-1} yr^{-1}, kgTDN ha watershed^{-1} yr^{-1}) produced a high *Rho* statistic (0.64) with the combination of eelgrass tissue N, C, δ^{15}N, and δ^{13}C (Table 2). N loading per unit bay area was also correlated with sediment variables. Δ-N, flushing time, and aquaculture lease area, total bag count, stocking density per unit bay area, and stocking density per ha lease were also correlated with the smaller combinations of variables representing annual primary productivity water column and sediment primary production, and annual algae (Table 2). These correlations were weaker than those where N loading rates were identified as the best site characteristics. Δ-N, sampling depth and total annual N load (kgTDN yr^{-1}) were the best correlated variables with eelgrass structure characteristics.

b) Ordination and cluster analysis

Hierarchical cluster analysis overlaid on the nMDS
ordination of eutrophic/eelgrass variables (the combination of variables that produced the highest *Rho* statistic in the Env-Env analysis - no water column variables are included) revealed patterns of objects clustering within and between sites (Figure 10-11). First, objects (i.e. n = 6 observations of eelgrass and eutrophic characteristics per site) from the sampling sites in CN, BT and RB are more similar to each other than objects from the other 4 sites (Figure 10). Objects from KB and TB constitute another cluster, and objects from BSS and LM are primarily clustered unto themselves; observations in one quadrat from BSS were more similar to those in CN, BT, RB. BSS and LM clusters are the most disparate in the ordination (Figure 10). This ordination also reveals there is some within site variation of observations, notably that there is greater distance from cluster centroids to objects for CN, KB and BSS. Generally, though, objects from a site are part of a single cluster, indicating that between site differences are greater than within site differences. The stress factor for the nMDS ordination (0.16) is below the recommended cut off value of 0.2 and the R^2 value for the relationship between distance of scores on the ordination and distance of objects in the Euclidean distance matrix was high (0.9). Therefore this ordination is maintaining the distance between objects in the original matrix, and

effectively represents the differences in eelgrass structure and eutrophic symptoms between some sites and the similarity between others.

The vectors fitted to the ordination of eelgrass/eutrophic objects were selected based on the results of the ENV-ENV and *envfit* analysis: N loading rate (kgTDN ha bay^{-1}yr^{-1}) was the most commonly correlated site characteristic, and flushing time, aquaculture stocking densities (bags ha lease^{-1}, bags ha bay^{-1}), and Δ-N were important when smaller groups of variables were assessed (Table 2, Figure 10). While the ENV-ENV finds the highest similarities between combinations of objects in two matrices, the R^2 and p-values produced using the *envfit()* function in "*vegan*" represent the strength and significance of the linear correlation between each site variable and the combination of eutrophic/eelgrass variables on the 2D ordination (Appendix 4: Table 19).

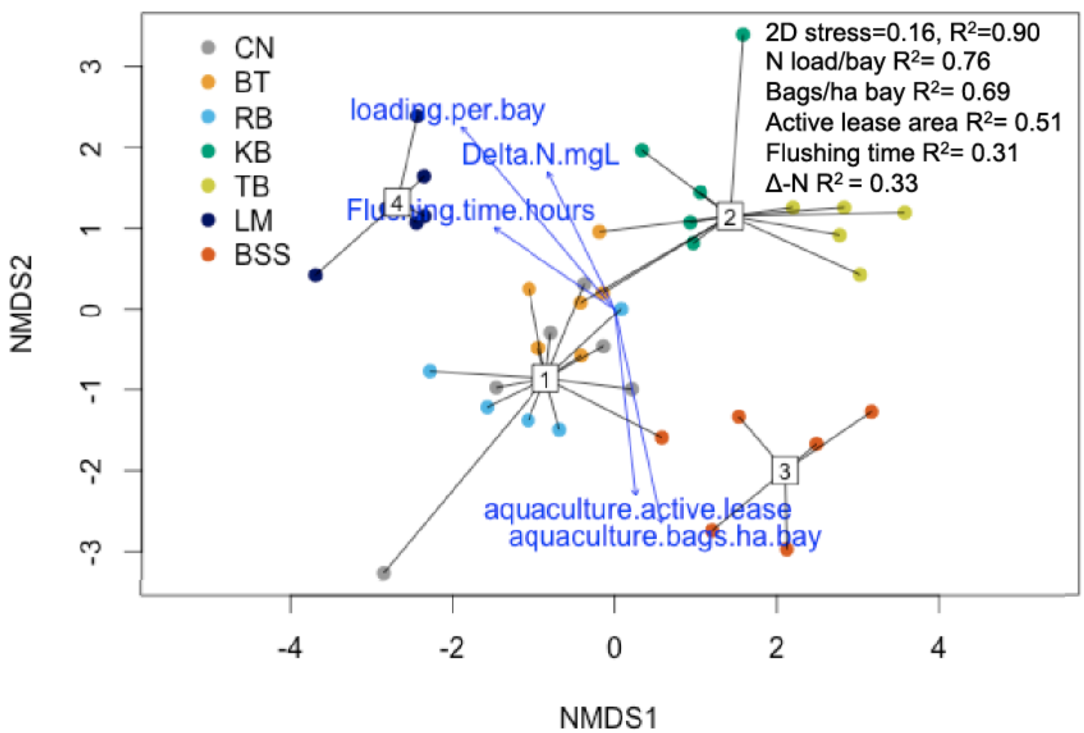

Figure 10. nMDS ordination of multivariate objects representing a combination of eelgrass structure and eutrophic indicator variables (eelgrass shoot density and canopy height, epiphytic and benthic algae % cover, microphytobenthos, AG and BG tissue %N, $\delta^{15}N$ and $\delta^{13}C$ isotopes) (n=6 objects per site). The R^2 value for the ordination is the agreement between object distance on the ordination and object distance within the Euclidean distance matrix. HCA clusters and distance from objects to centroids are overlaid, as are vectors of site characteristics that correlate with the ordination axis scores (Wickham 2009, Oksanan et al. 2013).

N loading per bay (kg TDN ha bay^{-1} yr^{-1}) was significantly and negatively correlated with the ordination axis scores (p=0.001) and had the highest R^2 (0.82) (Figure 10). Aquaculture stocking density per bay (bags ha bay^{-1}) area was significantly positivelycorrelated with axis scores (R^2 =0.69, p=0.001). Aquaculture active lease area (ha) was similarly correlated (p=0.001) although the R^2 value was comparatively reduced (0.57) (Figure 10). Flushing time and Δ-N were significantly correlated with objects in the ordination, however the R^2 values belie the weaker correlation compared to N loading per bay area or aquaculture bag density (Figure 10, Appendix 4: Table 19). The shorter length of the flushing time vector also indicates this, although directionally flushing time and Δ-N are correlated with the change in the ordination scores similar to loading per unit bay area.

The ordination of these eelgrass/eutrophic objects and their relationship with N loading rate per bay (kg TDN ha bay^{-1} yr^{-1}), aquaculture stocking density (bags ha bay^{-1}), aquaculture active lease area (ha), and flushing time is further represented in Figure 11. We show the same nMDS ordination of eelgrass and eutrophic variables, and size the ordination scores according to the relative N loading rate (Figure 11a), flushing time (11b), aquaculture bags ha bay^{-1} (11c), and active lease area (11d) at each site. We chose these variables as they were most commonly selected in the ENV-ENV procedure and significantly correlated with the 2D ordination scores (Figure 11, Appendix 4: Table 19).

Figure 11a illustrates that objects from LM, which has the highest estimated N loading rate, are less similar than those from sites with lower loading rates (BSS, TB). CN, BT, RB and KB have intermediate levels of loading rates and exhibit more similarity (less distance) to each other than to either BSS or LM on the lower and upper extremes of loading rates. Figure 11b does not reveal a distinct pattern between objects in clusters, and reflects the lower R^2 value. Figure 11c exhibits that objects with similar aquaculture stocking densities are more similar to each other (e.g. RB and BSS). Still, as the lower R^2 indicates, this correlation is not as strong as loading rate per unit bay area. Figure 11d, similar to flushing time, does not show as distinct a pattern between sites as loading rate and stocking density.

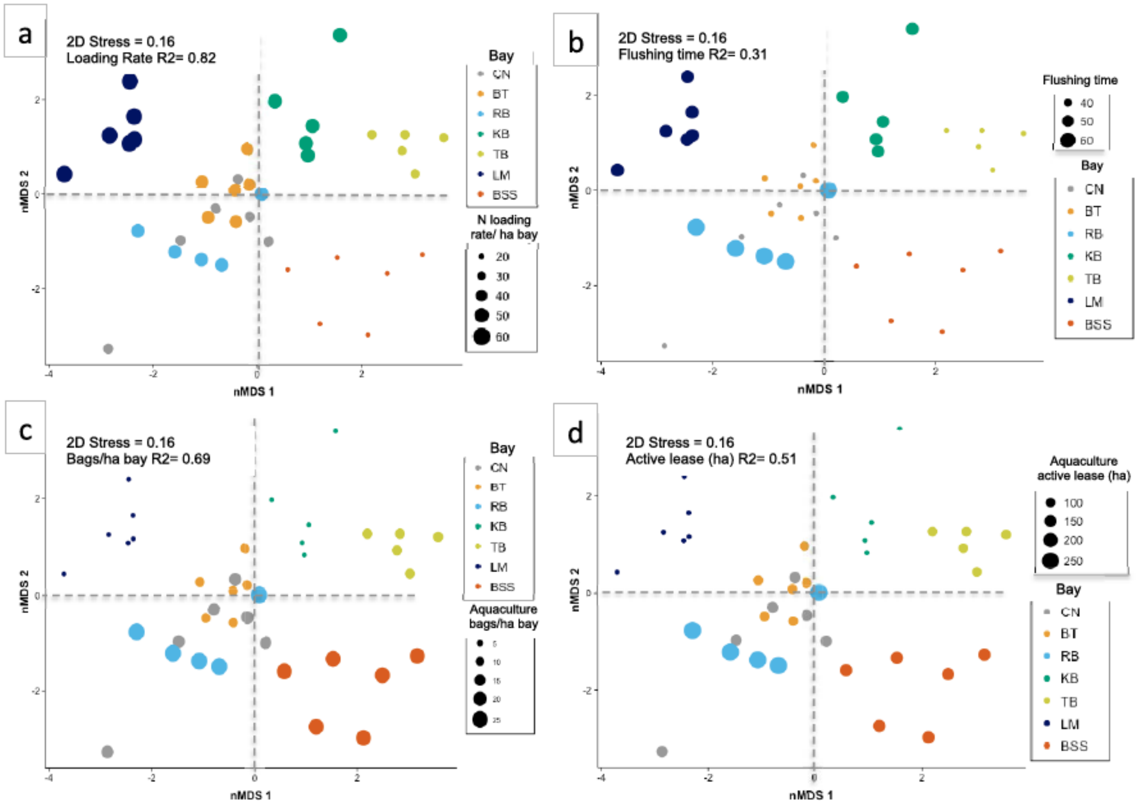

Figure 11. nMDS ordination of distances between multivariate samples in the matrix of eelgrass structure and eutrophication variables from habitats in 7 bays (Wickham 2009, Oksanan et al. 2013). The site variables that have the highest linear correlation with the nMDS ordination of the eelgrass and eutrophic variables identified previously are overlaid by size: (a) N loading rate (kg TDN ha bay^{-1} yr^{-1}), (b) Flushing time (h) (c) Aquaculture stocking density (bags ha bay^{-1}) (d) Aquaculture active lease area (ha).

c) PERMANOVA analysis

Lastly we performed the multivariate PERMANOVA on the objects of combined eutrophic/eelgrass variables from sites with N loading rate (kg TDN ha bay^{-1} yr^{-1}) and aquaculture stocking density (bags ha bay^{-1}) area as factors, chosen because they showed up most consistently in the ENV-ENV analysis and were highly correlated with the nMDS ordination. Both these factors and their interaction explained a significant amount of the variation between the objects of combined eutrophic/eelgrass variables from all sites (Table 3). N loading rate is a more important factor than aquaculture stocking density. The interaction of these factors also explains a significant amount of the variance, more so than stocking density, but less than solely loading rate. N loading rate and the interaction of loading rate and stocking density per unit bay area explained a significant amount of the variation between objects containing fewer but related variables (tissue characteristics, sediment characteristics, primary production, and eelgrass structure). Stocking density explained a significant amount of the variation in primary production (microphytobenthos, annual algae and water column Chl*a* and particulates), while N loading rate explained the most variation in summer eelgrass tissue characteristics and sediment characteristics (Table 3). The assessment of watercolumn primary production variables violated the assumptions of multivariate homogeneity of group dispersions (variances), so significant results are not reliable (Table 3) (Anderson 2001, Anderson and Walsh 2013). Similarly, when we assessed eelgrass tissue from all seasons, assumptions of homogeneity were also violated, so we continue to use solely summer measurements.

Table 3. Multivariate PERMANOVA results of the effect of N loading rate (kg TDN ha bay^{-1} yr^{-1}) and aquaculture stocking density (bags ha bay^{-1}) on combinations of the following variables[1,2] (n=6): above (AG) and below ground (BG) eelgrass tissue N content, $\delta^{13}C$, $\delta^{15}N$ ratios, Microphytobenthos, epiphytic and benthic algae % cover, sediment organic content (%), eelgrass shoot density, canopy height, % cover, AG and BG biomass. Water column variables (n=9) from all seasons were assessed separate from other quadrat variables. Univariate results for each eelgrass/eutrophic variable are also shown (n=6 for all quadrat data, n=9 for water column variables). Significance levels: 0.05=*, 0.01=**, ≤0.001=***. √V are estimates of the components of variance for each of the factors in the model. Negative V indicates there is no evidence against the null hypothesis. Variables that violated the assumptions of homogeneity of variance are shown in italics: the significance of these results is not expressed with confidence as a result of breaking the assumptions.

PERMANOVA	Source (df)			
Multivariate PERMANOVA	N loading rate (kgTDN ha bay^{-1}yr^{-1}) (1)	Aquaculture active lease area (ha) (1)	Loading x active lease area (1)	Residuals (35)
shoot density, canopy height, AG and BG biomass, epiphytic and benthic algae % Cover, microphytobenthos, AG and BG N, AG and BG $\delta^{13}C$, $\delta^{15}N$, Sediment organic (%)				
Pseudo ***F***	7.52***	4.09***	4.32***	
√V	9.62	7.10	7.29	3.51
shoot density, canopy height, epiphytic and benthic algae % Cover, microphytobenthos, AG and BG N, $\delta^{13}C$, $\delta^{15}N$				
Pseudo ***F***	12.55***	6,35***	7.18***	
√V	9.27	6.59	7.01	2.62
Tissue (AG and BG % C, % N, $\delta^{13}C$, $\delta^{15}N$) n=6 (summer only)				
Pseudo ***F***	19.96***	5.06**	6.52**	
√V	8.27	4.16	4.72	1.85
Sediment (microphytobenthos and Sediment Organic content)				
Pseudo ***F***	6.14**	5.29*	4.72*	
√V	3.02	2.80	2.65	1.22
Eelgrass structure (shoot density, canopy height, % cover, AG and BG biomass)				
Pseudo ***F***	3.00*	2.00	5.85***	
√V	3.51	2.86	4.93	2.04

Annual macroalgae (epiphytic, benthic algae %cover)				
Pseudo *F*	3.00	12.83***	3.38*	
$\sqrt{V}$	2.05	4.24	2.17	1.18
Non-water column primary production (epiphytic, benthic algae %cover, microphytobenthos)				
Pseudo *F*	2.90*	10.75***	3.90*	
$\sqrt{V}$	2.51	4.83	2.91	1.47
Water column primary productivity (Chl*a*, PIM, POM) n=9 (all seasons)				
Pseudo *F*	53.45***	3.34*	3.72*	
$\sqrt{V}$	8.85	2.18	2.33	1.21

Univariate PERMANOVA[3]	N loading rate (1)	Aquaculture active lease area (ha) (1)	Loading x active lease area (1)	Residuals (35)
AG % Tissue N				
Pseudo *F*	38.43***	6.96*	22.98***	
$\sqrt{V}$	5.49	2.34	4.24	0.88
BG % Tissue N				
Pseudo *F*	0.002	0.38	0.06	
$\sqrt{V}$	0.01	0.15	0.09	0.33
AG $\delta^{15}N$				
Pseudo *F*	94.86***	7.58**	9.95**	
$\sqrt{V}$	8.13	2.30	2.63	0.83
BG $\delta^{15}N$				
Pseudo *F*	38.34***	6.93*	22.98***	
$\sqrt{V}$	5.49	2.34	4.25	0.88
AG $\delta^{13}C$				
Pseudo *F*	7.29*	6.36*	4.95*	
$\sqrt{V}$	2.05	1.91	1.69	0.76
BG $\delta^{13}C$				
Pseudo *F*	43.44***	20.80***	11.93***	
$\sqrt{V}$	4.17	2.88	2.18	0.63
microphytobenthos				
Pseudo *F*	2.70	6.93*	4.83*	
$\sqrt{V}$	161.08	257.54	215.08	97.83
Sediment organic (%)				
Pseudo *F*	9.81**	3.53	4.59*	
$\sqrt{V}$	0.05	0.03	0.04	0.02
benthic algae % cover				
Pseudo *F*	4.30	2.39	0.86	
$\sqrt{V}$	0.05	0.04	0.02	0.02
epiphytic % cover				
Pseudo *F*	0.74	31.18***	7.81*	
$\sqrt{V}$	0.14	0.91	0.46	0.17
Shoot density				
Pseudo *F*	17.18***	1.21	29.77***	
$\sqrt{V}$	164.62	43.61	216.72	39.71
Canopy height				
Pseudo *F*	4.18*	2.82	5.99*	
$\sqrt{V}$	23.02	18.90	27.56	11.26
AG biomass (gm^{2-1})				
Pseudo *F*	1.11	1.34	0.12	
$\sqrt{V}$	349.33	393.11	272.65	372.92
BG biomass (gm^{2-1})				
Pseudo *F*	0.05	4.15	0.23	
$\sqrt{V}$	52.87	1666.36	274.29	734.95
Chl*a* (μgL^{-1})				
Pseudo *F*	1.46	3.40	1.01	

√V	*1.11*	*1.70*	0.93	0.92
POM (mgL^{-1})				
Pseudo *F*	0.56	0.12	3.30	
√V	4.25	1.36	2.14	0.78
PIM (mgL^{-1})				
Pseudo *F*	*29.28****	*3.01*	7.46*	
√V	*122.89*	*39.40*	62.02	22.72

[1] Water column variables (Chl*a*, PIM, POM) were not sampled within quadrats so we did not include them with other multivariate measures, all sampled in the 6 quadrats cores were taken.
[2] Percent cover of eelgrass is not included in the combination of variables as there is a measure of percent cover of benthic algae, which resulted in autocorrelation of these two variables in the distance matrix.
[3] Dependent variables are not scaled for univariate analysis so variances reflect actual units of measurement

Univariate PERMANOVA with N loading rate (kg TDN ha bay^{-1} yr^{-1}) and stocking density per unit bay area as factors revealed similar trends to the multivariate PERMANOVA (Table 3). N loading rate explained a significant amount of the variation in AG tissue N content, AG and BG $\delta^{13}C$ and $\delta^{15}N$, eelgrass Shoot density, canopy height and sediment organic content. Stocking density explained a significant amount of the variation for BG $\delta^{13}C$ and $\delta^{15}N$, AG $\delta^{15}N$, microphytobenthos, and epiphytic %. The assumption of homogeneity of variance was not met for eelgrass tissue N content or isotope measurements and their relationship with stocking density, so we cannot comment on the reliability of these results, nor the significant interaction between N loading rate and stocking density in relation to these variables. Still we note that the interaction between N loading rate and stocking density explained a significant amount of the variation in microphytobenthos, sediment organic content, epiphytic % cover and eelgrass shoot density and canopy height (Table 3). The assumption of homogeneity of variance was also violated for all univariate analysis of water column characteristics (Chl*a*, TPM, PIM, POM, Table 3).

3.4 Discussion

Our extensive field survey in the summer of 2013 revealed differences in eutrophication symptoms and eelgrass bed structure across seven bays in eastern NB. When assessed in multivariate combinations we found similarities between certain sites (e.g. RB, BT, CN as well as KB, TB), while others were more disparate (LM, BSS). Estimates of N loading rate (kgTDN ha $bay^{-1}yr^{-1}$) were highly positively correlated with the combined variables of eelgrass bed structure and eutrophication, while flushing time and aquaculture area and density showing strong negative correlations with these habitat variables. Moreover, N loading rate and aquaculture stocking density (bags ha bay^{-1}) explained significant amounts of variation in the combined and individual sets of eelgrass and eutrophic variables, including eelgrass bed structure, tissue contents, sediment characteristics and annual algae. Finally, because primary producers (eelgrass and annual algae) can selectively uptake ^{12}C over ^{13}C isotopes, carbon isotope ratios ($\delta^{13}C$) may be an effective indicator of annual primary production in eelgrass habitats as evidenced by its positive linear relationships with microphytobenthos and epiphytic annual algae cover. Our results have strong implications for effective monitoring and management of eutrophication symptoms in coastal waters.

3.4.1 Eutrophication Symptoms

Consistent with the effects of N enrichment in coastal water bodies we saw a concurrent increase in multiple eutrophic symptoms across the sites we assessed. The indicators of eutrophication that were most differentiated between sites were % cover of epiphytic annual algae on eelgrass blades and microphytobenthos (Chl*a* concentration in sediments). These symptoms were present at a higher degree in CN, BT, RB and LM compared to KB, TB and BSS. Compared to epiphytic percent cover of benthic algae was very low across our sites, consistent with previous findings where benthic algae was much more pronounced in PEI than NB (Schmidt et al. 2012). Furthermore, the tissue N content in eelgrass tissues was also heightened, especially in CN and LM. Increased concentration in eelgrass tissue N has been linked to heightened concentrations of ambient N in the environment and the presence of additional eutrophic symptoms, both in this region (Schmidt et al., 2012) and further afield (e.g. Nixon and Pilson 1983, Bricker et al. 2003). Tissue samples from CN, LM and RB fall above the threshold identified for N limitation (>18 mg/g dry weight or 1.8% N content in shoots, Figure 6, Duarte et al. 1990), indicating that N in these bays is sufficient for or exceeding the internal demand of eelgrass. The surplus N is also available to annual algae and phytoplankton, as evidenced by the higher epiphyte load and microphytobenthos concentrations in LM, CN and RB. Sediment organic content did not reflect any patterns between sites that would be indicative of a gradient of eutrophication. Instead, we suggest the relatively high values from TB may be related to a harbor fire that happened approximately 4 months before sampling only 200m from the sampling site. Interestingly, water column particulate matter, especially POM was elevated in this bay across seasons (Figure 4) and benthic algae, sponges and detrital matter were more prevalent here than in other sites (Figure 3, pers. observation), and more notable than in 2003 and 2007 (Lotze et al. 2003, Schmidt et al. 2012). We cannot comment on how this would directly contribute to the high organic content of sediments or increased particulate matter, but potentially deposition of ash and particulates from the fire impacted the organic materials in the eelgrass bed, altering the capacity for breakdown of organic matter, or simply adding more organic detritus.

Isotopic characteristics of eelgrass tissue may also be a useful marker of eutrophication. As a C3 plant, eelgrass has some capability for selective uptake of the light carbon isotope ^{12}C from CO_2 (and HCO_3^-) sources. Therefore, the carbon isotope ratio of *Z. marina* is slightly diminished (-11 to -10) compared to atmospheric carbon ratio (≈-7.8), and ambient content common in bays and estuaries (≈-9) (Hemminga and Duarte 2000). Seagrasses (and other C3) plants, however, lose the capacity for selectiveness when the supply of the lighter isotope becomes limiting. This could arise when there is a rapid uptake of C by phytoplankton and macroalgae, which will also selectively take up the ^{12}C isotope. Therefore, in areas where primary production is high, for example where the concentration of photosynthetic organisms in the water column (phytoplankton, epiphytes) or sediment (microphytobenthos) are elevated, seagrasses may be enriched in ^{13}C relative to plants from sites where they can be selective in the uptake of light vs. heavy carbon isotopes (Hemminga and Mateo 1996, Hemminga and Duarte 2000). Regressions of AG and BG $\delta^{13}C$ values against microphytobenthos and epiphyte cover both exhibit strong positive relationships (Figure 9). Especially KB and TB, and to some degree BSS are bays where N loading rates and the human footprint in the watersheds is comparatively low (Table 1, Chapter 2). These bays exhibit lower microphytobenthos concentrations, lower epiphyte cover, and lower BG $\delta^{13}C$ which indicates these bays are symptomatically lower on the eutrophic spectrum than CN, BT, RB and LM. Chapter 2 reviews the effectiveness of N isotopes in eelgrass tissue in corroborating N loading estimates where wastewater is the primary N contribution. For instance, the high $\delta^{15}N$ values in LM (7.3± 1.3) are significantly higher than tissue N isotopes in any other site. This reflects the high proportion of wastewater N entering that bay from seafood processing and MWWT, as LM is the only site where wastewater, not atmospheric deposition, is the largest estimated source of N in the watershed and bay (Figure 1b). This illustrates that eelgrass tissue is an effective tool for identifying the impact of wastewater N on primary producers in this region, analogous to findings in New England and Baltic Sea (e.g. Valiela et al. 1997b, McClelland and Valiela 1998, Voss et al. 2000).

Water column parameters, including Chl*a* and TPM did not prove effective indicators of eutrophication in this study. Although we aimed to sample across three seasons, high variability within and across seasons and sites, as well as intermittent rainfall and storm events (e.g. leading to very high values in KB) prevented us from gaining significant differences in water column primary productivity and particulate matter across our sampling sites. Still, the merits of water column Chl*a* levels as an indicator of eutrophication and predictor of negative effects on eelgrass structure and extent have been shown many times over (see Nixon and Pilson 1983, Bricker et al. 2003, Latimer and Rego 2010), including in eastern NB (Schmidt et al. 2012). These measures, however, require more frequent and less weather-disturbed sampling. Our findings suggest that microphytobenthos and annual algae cover (epiphytes) may be more robust indicators of eutrophication when a site can only be monitored at a single time point in a season. Daily water movements from wind or tides, and precipitation events may have less direct impacts on these variables than the distribution and concentration of phytoplankton and particulate matter in the water column.

3.4.2 Eelgrass Structure

Increased primary production in coastal systems has the potential to negatively impact eelgrass due to increased light attenuation, decreased eelgrass production, and increased oxygen consumption upon the decomposition of phytoplankton and annual algae at the sediment water interface (see Nixon and Pilson 1983, Bowen and Valiela 2001a, Bricker et al. 2008). Decreases in shoot density, % cover and biomass have previously been identified at sites with elevated eutrophication symptoms (water column Chl*a*, annual epiphytic and benthic algae) in the Southern Gulf region. This was quite variable and more pronounced in PEI than NB (Lotze et al. 2003, Schmidt et al. 2012). Similar to the 2003 and 2007 measurements at our sites in eastern NB, eelgrass bed structure was also quite variable in the 2013 sampling. The anomaly in canopy height between all sites was

KB, where the canopy was on average 20cm taller than in other bays, which was also the case in 2003 (Lotze et al. 2003). Although an increase in canopy height can be associated with increases in opportunistic algae and light attenuation, the tall canopy height in KB is likely a result of the high tannin content (result of high dissolved organic carbon content) in the water column reducing the light penetration (Bukaveckas & Robbins-Forbes 2000). The KB watershed is characterized by extensive wetland, natural peat land and coastal saltmarshes that have a high capacity for transporting dissolved organic carbon to the receiving water body (Thibault et al. 2000, Clair and Ehrman 2013). Shoot density was highest in BSS and LM, concurrent with lowest canopy height, resulting in similar AG biomass across all sites except KB: the higher AG biomass in KB is a result of a tall canopy and intermediate shoot density relative to the other sites. There were no significant differences in BG eelgrass biomass and percent cover across sites; however, percent cover of eelgrass was highest in KB and BSS. Overall, these results suggest higher cover and biomass at some less impacted sites (e.g. KB, BSS), but at this time the effects of eutrophication are not having strong secondary effects such as a significant decline or loss of eelgrass beds in these bays. Yet the increased primary production (epiphytic algae, microphytobenthos) and tissue N content at CN, BT, RB and LM indicate that impacts of excessive N loading are apparent and affecting these habitats, which is consistent with previous findings in NB (Lotze et al. 2003, Schmidt et al. 2012).

3.4.3 Integration of Field Survey and Site Characteristics

Despite variability in individual parameters, our multivariate analyses indicate that the combination of multiple eutrophic and eelgrass variables produces 4 distinct clusters of sites (Figure 10,11) and is well correlated with N loading rates, bivalve aquaculture lease area and density, and to some extent flushing time and Δ-N. Thereby, N loading rate (kgTDN ha bay^{-1}yr^{-1}) explained more of the variance among sites than aquaculture bag density per unit bay area or active lease area. Chapter 2 showed how both the magnitude and sources of N loading are represented in eelgrass tissue N content and N isotope values, respectively, and results of our field survey illustrate how N loading rate also impacts eutrophic symptoms (e.g. epiphytic algae, microphytobenthos) and partly eelgrass bed structure: differences in eelgrass structure were not as strong between sites, but biomass, an integration of canopy height and shoot density (Skinner et al. 2013), may be driving some of these trends. This is consistent with previous research in this region and the northeastern U.S. (Valiela et al. 1997b, Schmidt et al. 2012).

Although cultured oysters are not likely impacting eelgrass at a bay-wide scale in our study region, perhaps as a result of lower stocking density with the transition to suspended aquaculture (Comeau 2013, Vance 2013), increased filtration and removal of phytoplankton may impact water clarity and macrophytes in close proximity (near-field effects) to the aquaculture leases. This effect lessens with increasing distance from a lease (Skinner et al. 2013, Vance 2013). On the other hand, the effect of suspended bivalve leases on eelgrass under and in close proximity to leases may directly negatively impact eelgrass through increased organic enrichment of the bay floor (Grant 2005), and increased nutrient release at the sediment water interface (e.g. Hatcher et al. 1994, Skinner et al. 2013). Our sampling site in BSS offers an interesting intersection between benthic-pelagic coupling of cultured bivalve impacts. Our sampling site was located approximately 30m from an active aquaculture lease, while all other sites were at least 200m from active leases. BSS may have been more affected by the filtration and removal of organic matter from the water column, and in combination with very low N loading from the watershed and a faster flushing time relative to our other bays, this may have resulted in the lowest concentration of water column Chl*a* and TPM here across all sites and seasons (Figure 4). Yet, compared to KB and TB, BSS exhibited elevated microphytobenthos and epiphytic algae levels (although they were reduced compared to CN, BT, RB and LM). This may indicate the influence of increased organic matter deposition, nitrogen release, and mineralization at the sediment water interface, increasing the amount of N available to annual algae in the benthic portion of the ecosystem (Hatcher et al. 1994, McKindsey et al. 2006, Skinner et al. 2013). Because N loading rates per unit bay area are so low in BSS, we suggest that the slightly higher expression of eutrophic symptoms in BSS compared to KB and TB, leading to it clustering independently, may be a result of the near-field nutrient enrichment effects of the high density (bags ha bay^{-1}) suspended aquaculture and not terrestrial or atmospheric loading (Table 1, Figure 10, 11). In eelgrass beds with increasing distance from leases in BSS we would expect to see a reduction in epiphyte cover and microphytobenthos, reflecting the low N loading rate per unit bay area and relatively quick flushing of the system at the bay-scale. Acuaculture may be having an impact at our sampling site in BSS, which is driving the envfit correlation seen in Figure 10, yet overall it appears that oysters do not reduce or have any measureable effect on eutrophication symptoms in eelgrass habitats in our sampling sites under our sampling design. We do not quantify N loading from bivalve aquaculture within the NLM (Chapter 2), however it is a source that deserves attention, especially for eelgrass habitats near-field to leases.

In contrast to BSS, LM exhibits some of the highest concentrations of microphytobenthos and epiphyte cover, and has no additional impact of aquaculture. Therefore, these symptoms are a result of the high N loading rate and additionally long flushing time relative to BSS, CN, and TB (Table 1, Figure 10, 11). Flushing time was less highly correlated with eelgrass and eutrophic symptoms than N loading rate, however, again indicating the N loading is the principal driver of eutrophication here.

KB and TB exhibit similarly low expression of eutrophication symptoms and similar eelgrass structure, as evidenced by their close proximity in the nMDS and HCA results. Overall, KB and TB have lower N loading rates per unit watershed area than CN, BT, RB and LM, and reduced eutrophication symptoms with the lowest amounts of microphytobenthos and epiphyte loads. Lastly, both KB and TB have either low or no aquaculture: TB has a small lease area relative to bay size (~2.6%) >500m from our sampling site (Table 1), and KB has no active lease area for cultured bivalves, so we expect the eutrophic conditions to primarily reflect N loading from terrestrial and atmospheric sources. Still we note that the source of N loading may be an important factor influencing eutrophication symptoms in these bays: TB and KB are the only sites with no point sources of wastewater (Chapter 2), and although N loading rate per unit bay area in KB is actually higher than CN, BT, TB and BSS, eutrophic symptoms are minimal here. Therefore, the relative proportion of DIN and DON may influence the type and magnitude of primary producers that can utilize the ambient N (Bricker et al. 2003). Moreover, both sites fall within protected natural areas, KB within the Kouchibouguac National Park and TB as a RAMSAR protected wetland site, which may explain the lower human footprint and lower eutrophication symptoms there.

In comparison, CN, RB and BT are all large, forested watersheds, but have higher N loading rates (per ha watershed and per ha bay) respective of the higher population densities and point sources of N (see Chapter 2). These sites may be grouped by virtue of similar bathymetry and location in the study region (Figure 1), but eelgrass bed structure and eutrophic symptoms are also similar among these three sites, explaining their tight clustering in the nMDS (Figure 10, 11). Additionally they receive similar N loading per ha watershed and bay and aquaculture covers a similar proportion of the bay area (2-5%, Table 1), but is also similarly distant (>200m) from sampling sites, likely outside the zone of influence of shading, filtration, and biodeposition on benthic and water column characteristics (Skinner et al. 2013, Vance 2013). Therefore, the intermediate symptoms of eutrophication in CN, BT and RB relative to the higher values in LM and lower in KB, TB, and BSS, correspond to N loading rates per ha bay and watershed (Table 2, Figure 10,11).

Overall, our multivariate approach of linking site characteristics and combinations of eutrophic/eelgrass variables provided very clear and plausible results. Based on the revealed patterns, we suggest that bays with high N loading rates (per ha bay and per ha watershed) and low flushing times (e.g. LM, RB) are more susceptible to eutrophication and potential secondary impacts on eelgrass structure and biomass in the future. Bivalve aquaculture may play a mediating role in the water column in close proximity (near field) to the active lease area (Vance 2013), as potentially seen in BSS. In eutrophic systems this may have a positive effect and reduce the water column organic load in the near-field zone around aquaculture. Overall, however, aquaculture may not have a measureable effect on eelgrass structure or primary eutrophication symptoms at the bay-wide scale. Conversely, shading also causes the loss of eelgrass beds within an active lease area underneath the bivalve bags/cages (Skinner et al. 2013) and may increase organic deposition and eutrophic symptoms in benthic portions of the near-field habitat, while simultaneously reducing primary production in the water column (e.g. Hatcher 1994, Newell 2004, McKindsey et al. 2006). Lastly, the source of N loading may play a role in the expression of eutrophic symptoms, and point sources of wastewater N may result in the higher expression of eutrophic symptoms in these bays (CN, BT, RB, LM).

3.4.4 Broader Context and Conclusions

Our model estimates from the application of the Waquoit Bay NLM (Chapter 2) are highly associated with patterns of eutrophication in eelgrass habitats in seven bays across eastern NB. These bays can be compared to those on PEI that receive significantly elevated levels of nitrate from agricultural practices (Grizard 2013). Estimated nitrate (NO_3^-) loadings per unit watershed area in the majority of estuaries on PEI exceed our estimates of Total Nitrogen (DON, NO_x, NH_3, NH_4^+) for all estuaries except BSS and LM. As discussed previously, BSS has a small watershed:bay area ratios and low N loading per unit bay area, indicating high dilution potential within this system. Although LM has high TDN loading relative to other sites in NB, and in concert does exhibit a heightened eutrophic response compared to the sites with lower N loading rates, our field survey did not show that significant shifts in eelgrass structure were occurring relative to our other sites, and the large mats of *Ulva* that characterize eutrophic systems in PEI were absent (Schmidt et al. 2012). Therefore, the impact of N loading in NB is currently not as severe as in PEI, but the heightened eutrophic response in sites with higher N loading (CN, BT, RB and LM) clearly illustrate that effects are not benign.

The effects of eutrophication can be defined in two stages: primary and secondary. The primary stage includes the presence of increased primary productivity, for instance the increased presence of annual macroalgae and Chl*a* concentration in the water column (or in our case sediment surface microphytobenthos). Below we briefly compare the primary symptoms of eutrophication in our bays using the National Estuaries Eutrophication Assessment (NEEA, http://ian.umces.edu/neea/, Bricker et al. 2003, 2008) from the United States, which integrates the assessment of eutrophic symptoms in estuaries and influencing factors such as N loading and flushing time. Secondary and severe symptoms of eutrophication include changes to the structure of the eelgrass beds, such as reductions in shoot density, cover and eelgrass biomass (Short et al. 2006b, Bricker et al. 2008). Below we compare the structure of eelgrass beds in our study areas using the global seagrass monitoring framework SeagrassNet (www.seagrassnet.org/, Short et al. 2006ab).

The heightened epiphyte cover in CN, BT, RB and LM is similar to habitats exhibiting low-medium responses to primary symptoms under the NEEA framework, in that they are not incapacitating the functionality of the eelgrass habitats, but are consistent throughout the habitat and are in systems with point sources of wastewater (no standard quantification measure, Bricker et al. 2003). Primary eutrophic symptoms at these sites are comparable to Hampton Harbor, New Hampshire (Bricker et al. 2007). KB, TB and BSS exhibit epiphyte cover analogous to bays with low primary symptoms like Damariscotta River, Maine (Bricker et al. 2007). Our measurements of water column Chl*a* are in the low category (0-5μgL^{-1}) in all bays except KB, where some samples were within the medium (5-20μgL^{-1}) category, but these were measured after heavy rainfall and storm event that may have stirred up the system creating a bloom. Currently, benthic Chl*a* concentrations are not a part of the NEEA monitoring framework, but as we show microphytobenthos may be an effective integrative measure of primary production, and perhaps represent the primary production levels at our sites better than our water column Chl*a* samples. Currently, eelgrass structure in our sites does not reflect secondary symptoms of eutrophication (this study, Schmidt et al. 2012). Percent cover of eelgrass at all sites was >70%, and shoot density was between 72-190 shoots per m^2. This structure is comparable to the previously healthy seagrass monitoring site in Chincoteague Bay, Maryland prior to loss of habitat there as a result of steadily increased N and P concentrations (Short 2006a).

In many cases, primary symptoms of eutrophication eventually lead to secondary symptoms, for example the significant reduction in eelgrass cover and shoot density in Waquoit Bay as a result of increased urbanization (Bowen and Valiela 2001a), and the rapid deterioration of eelgrass habitat in Placentia, Belize with the removal of coastal mangroves that had provided buffering and N sequestration (Short et al. 2006a). Therefore, if primary symptoms have been identified then management and mitigation of N loading, such as more stringent point source effluent treatment, can be effective before structural and biomass changes in eelgrass habitats occur (Plante and Courtenay 2008, Bricker et al. 2003, 2008)

Of our seven bays, LM may be at an increased risk of eutrophication and development of secondary symptoms as it has a moderate-high N loading rate (63 kgTDN ha $bay^{-1}yr^{-1}$) in comparison to similar sized systems in the U.S., such as Casco Bay, Maine (Latimer and Charpentier 2010), and slower flushing times than the other six bays we assessed. In contrast, our sites in KB, TB and BSS are likely in a lower risk category for the development of more severe eutrophic symptoms due to their lower N loading rate per unit bay area (20-55 kgTDN ha $bay^{-1}yr^{-1}$) or ha watershed (1.5-7.3 kgTDN ha watershed^{-}

$^{1}yr^{-1}$) and quicker flushing time (30-52h). Currently low eutrophication symptoms, healthy eelgrass habitats, and low human impact in these watersheds make them comparable to Blue Hill Bay, Maine, rated as having low overall susceptibility to eutrophication (Bricker et al. 2003, 2007). Aquaculture leases cover approximately 18% of the bay surface area in BSS (Table 1), and direct shading or overwintering of cages and gear can completely remove eelgrass from underneath these leases. Therefore, in BSS the direct removal of eelgrass due to shading and detrital smothering may be a larger concern for eelgrass bed health than N loading (McKindsey et al. 2006, Dumbauld et al. 2009).

This research fills an important knowledge gap in linking quantitative estimates of anthropogenic nutrient loading to eutrophication symptoms in eelgrass beds in receiving coastal water bodies in this region. We also provide information on the importance of other influencing factors in the expression of eutrophication symptoms, including flushing time and bivalve aquaculture. Importantly, we show that individual measures of eutrophic symptoms and eelgrass structure can be highly variable, yet multivariate combinations can reveal very clear patterns among sites. Thus, monitoring programs should focus on a set of multiple variables in order to gain meaningful results. Our results allow for the distinction between high- and low-impacted sites and their eutrophic and eelgrass bed characteristics. Moreover, we have identified bays being at higher risk of eutrophication due to cumulative site characteristics (e.g. N loading, flushing time, aquaculture) causing changes to eelgrass habitats.

Chapter 4: Conclusion

4.1. Framework of Research

The goals of this research project were two fold: First, we aimed to quantify nitrogen (N) loading to 7 bays along the eastern coast of New Brunswick (NB), and identify the principal sources of N contributing to the estimated loading (Chapter 2). Secondly we linked estimates of the Nitrogen Loading Model (NLM) with measured parameters of eelgrass health and eutrophic symptoms in each bay (Chapter 3). These parameters are consistent with those measured in international frameworks monitoring eelgrass health and eutrophication in coastal ecosystems, such as SeagrassNet (seagrass habitat changes, Short et al. 2006b, www.seagrassnet.org) and the National Estuarine Eutrophication Assessment (NEEA, eutrophication classification, Bricker et al. 2003, 2007, 2008, http://ian.umces.edu/neea/). Therefore, we can compare and classify our study sites in accordance with these international frameworks (see Section 4.3 below). This research is relevant at this time because: a) The shallow bays along eastern NB are dominated by eelgrass, a designated ecologically significant species (ESS, DFO 2009, 2011) which provides important three-dimensional habitat for vertebrate and invertebrate species in addition to providing numerous other valuable ecosystem services; b) Previous research has illustrated that several of the bays we include in our assessment exhibit changes in eelgrass structure with increasing levels of eutrophic symptoms (Lotze et al. 2003, Schmidt et al. 2012 – include refs for Richibucto), and community watershed groups in the region have also identified negative changes in eelgrass habitats in response to perceived excessive N loading (pers.comm. Broken River Watershed Group, T. MacNitch, J Buck, I Milewski); c) Despite the identification of eutrophication problems and negative effects on eelgrass beds in some of the bays there does not yet exist any quantification of N loading for the eastern NB coast spanning from the Miscou Island to the southern NB.

4.2. Summary of Results

The results of the NLM (Chapter 2) reveal that atmospheric deposition to the watershed and bay surfaces is the dominant source of N in eastern NB, contributing between 72-94% of total annual N load to 6 of the bays we assessed. The only exception was LM, where atmospheric deposition contributes only approximately 23% of total loading, while wastewater comprises 72% of the estimated annual N loading. Seasonal measurements of eelgrass tissue N isotopes were successfully used to distinguish the high proportion of wastewater loading in LM compared to all other sites, indicating that they are a useful tool effectively integrating the signature of wastewater N loading in these bays during the summer growing season. Because the bays we assessed have variable watershed:bay size ratio, we found the best agreement between N loading rates per area of bay and characteristics of eelgrass tissue N content and N isotopes. Bay size and volume are important in terms of flushing time, with larger bays having longer estimated flushing times, and therefore a reduced capacity to quickly remove N entering the bay from freshwater sources (including groundwater). Multiple regression analysis illustrated that both N loading rate per unit bay area and flushing time were significantly positively correlated with increased eelgrass tissue N isotopes and N content. N loading rate was a more important variable and was more highly correlated to these eelgrass tissue variables than flushing time, but the interaction between these two factors was also significant, indicating that both these factors should be considered when assessing the impacts of nutrient loading in eelgrass habitats in this region.

The integration of NLM estimates with the field survey results (Chapter 3) reinforce that N loading rate per unit bay area is an important measure to consider when monitoring eelgrass health and eutrophication. N loading rate per unit bay area and aquaculture density (bags/ha bay) were the most highly correlated site characteristics with the nMDS scores of eelgrass and eutrophic variables that revealed four distinct clusters of study sites along an impact gradient. Again, N loading rate per unit bay area exhibited the best relationship with the ordination scores, and we do not suspect aquaculture exerted a measurable effect on our sites except in BSS, which is located within 30m of an active suspended lease. Interestingly, flushing time was not as highly correlated as measures of aquaculture lease area and bag density. It was, however, an important factor in the ENV-ENV analysis for the subgroup of primary production variables including annual algae and watercolumn productivity. Overall, an increase in epiphyte cover and microphytobenthos concentration were the most significant symptoms of eutrophication in the more impacted bays, as well as a shift in eelgrass shoot density and canopy height.

In contrast, eelgrass biomass decreased the strength of the multivariate ENV-ENV and PERMANOVA results, and water column variables were assessed separately due to unequal sample size and large variability: Too few water samples in combination with intermittent storm and rainfall events likely prevented us from gaining a clear picture of primary production and particulate matter in the water column across study sites. Future monitoring that incorporates more replicates and regular sampling of water parameters would be beneficial, as this is a comparable symptom of eutrophication that is used in other monitoring frameworks such as the NEEA.

4.3 Comparisons and Classifications of Study Sites

The structure of eelgrass beds in our sampling areas was variable between all our sites, consistent with previous research in 2003 and 2007 (Lotze et al. 2003, Schmidt et al. 2012). Percent cover was between 73-100% on average at each site, with no significant differences between sites, and shoot density ranged between 72-190 shoots per m^2. Synopses of SeagrassNet observations in low-impacted eelgrass habitats spanning New England to Brazil are not dissimilar to our measurements (Short 2006a).Yet measurements of shoot density and percent cover at SeagrassNet sites have been reduced by 20-80% in beds experiencing eutrophication as a result of improperly managed anthropogenic N loading (Short et al. 2006a). The integrity of the eelgrass bed structure at our sites indicates that we did not yet observe a transition from primary to secondary symptoms of eutrophication (Bricker et al. 2003). Yet if the primary eutrophic symptoms already present in CN, BT, RB, and LM increase further, the potential for increased light attenuation in the water column could begin to cause declines in eelgrass density and cover at these sites (e.g. Nixon and Pilson 1983, Bowen and Valiela 2001a, Bricker et al. 2008). This change could have impacts throughout these coastal bays that include loss of habitat for vertebrate and invertebrate species of importance, the loss of sediment stabilization and wave dampening, and the loss of nutrient and carbon storage and cycling within these coastal ecosystems (Orth et al. 2006, Short and Burdick 2007, Waycott et al. 2009, Short et al. 2011). Numerous examples of the shift from high shoot density and comprehensive cover of eelgrass to dramatically reduced cover as a result of eutrophication have been observed in North and South America under the SeagrassNet monitoring framework (e.g. Short et al. 2006a). These rapid changes have occurred over only 2 years in some cases, highlighting the importance of proactive management of N loading upon identification of primary symptoms. In many watersheds on Prince Edward Island (PEI), also in the Southern Gulf of St. Lawrence region, loading of N significantly exceeds those in New Brunswick (Grizard 2013). A previous regional field survey by Schmidt et al. (2012) identified secondary changes to eelgrass structure, including a decrease in shoot density and above-ground biomass, at sites with high compared to low nutrient impacts on PEI, whereas such changes were less obvious in NB. Many highly impacted PEI estuaries also exhibit a shift from eelgrass dominated communities to benthic algae, principally *Ulva lactuca* (Bugden et al. 2014). Knowing that changes to eelgrass bed structure and significant losses of eelgrass cover can result from higher N loading in this region, it is imperative to manage N loading in eastern NB before primary symptoms of eutrophication translate into negative changes in eelgrass cover and function.

Other research utilizing this NLM framework, or calibrated comparative frameworks, have presented thresholds for N loading per unit watershed area that correspond to significant reductions in eelgrass cover. In portions of Waquoit Bay, N loading rates greater than 20kgTDN ha watershed^{-1}yr^{-1} are associated with secondary eutrophic symptoms including a loss of eelgrass cover at the bay-wide scale (Bowen and Valiela 2001a). Based on our results, which estimate the highest N loading rate in LM as 21 kgTDN ha watershed^{-1}yr^{-1}, this threshold may be too conservative for the region since eelgrass beds are still being relatively intact. This is perhaps a result of these bays being relatively small and directly proximate to oceanic tidal influence (e.g. Patriquin 1976, Turcotte-Lanteigne and Ferguson 2013). 50 and 100 kgTDN ha watershed^{-1}yr^{-1} have also been proposed as threshold values at which eelgrass decline can be expected, based on a large-scale survey of 74 bays along the eastern coast of the U.S. (Latimer and Rego 2010). Because none of our estimated N loading rates are ≥50-100 kgTDN ha watershed^{-1}yr^{-1} these threshold values perhaps do not provide the best guidelines for the management of N loading in this region. Instead, we found N loading rates per unit bay area area to be a better determinant of site characteristics in terms of eelgrass and eutrophic symptoms. This may reflect the variation in watershed: bay size ratio between the 7 sites included in our analysis, as this loading rate estimate takes bay size into account. It is worthwhile to point out that while KB is more similar to TB and BSS in terms of the magnitude of primary symptoms, it has an N loading rate per ha of bay that is second only to LM. Therefore, we also address the issue of N sources within the watersheds. Interestingly, all bays except KB and TB have a point source of wastewater (seafood processing or MWWT) discharging into the bay. Epiphytic cover in these 5 sites is significantly elevated above epiphyte percent cover levels in KB and TB. microphytobenthos levels are also significantly elevated in these 5 sites (although not as remarkably in BSS) compared to KB and TB without wastewater loading. The relative amounts of DIN to DON entering each bay from different sources may influence the type of primary producers that are able to grow there (Bricker et al. 2003). Because we do not directly measure different forms of N from each source assessed we cannot comment on this definitively. Still, N isotope contents do indicate that wastewater N is being taken up by eelgrass in each bay, in proportions representative of the magnitude of wastewater loading (see also McClelland and Valiela 1998, Cole et al. 2006, Schubert, et al. 2013). This suggests that attention of management of N loading should consider both the quantity (kgTDN ha bay^{-1}yr^{-1}) and source of N entering these systems, both of which are provided by the NLM.

Although aquaculture bag density and active lease were correlated with the combination of eelgrass and eutrophic characteristics in these bays, we do not include it in our overall final classification of these bays for 3 reasons: a) The only site at which we perhaps saw a measureable effect was BSS, where our sample site was located approximately 30m from an active suspended lease; b) Our other sampling sites are located >200m from active leases, and the impact of aquaculture on nutrient and primary production dynamics is variable and perhaps negligible at this distance based on stocking densities of suspended aquaculture (see Comeau 2013, Vance 2013, Skinner et al. 2013); c) Recent research in eastern NB has indicated that at current stocking densities in most bays cultured bivalves do not exert bay-wide top-down influence on the organic load in the water column (Comeau 2013).

There are bays in the region where suspended aquaculture has a documented effect on watercolumn organic load (e.g. Tracadie (PEI) , St. Peters Bay (PEI), Baie du Village (NB); Grant et al. 2005, Comeau 2013, Guyondet et al 2013). In the bays included in this research, however, there is likely too little bivalve biomass to exert baywide effects on watercolumn primary production and consequent far-field effects on eelgrass structure (Grant et al. 2005, Comeau 2013). Our research and the contrast in distance from active leases between our bays reinforces that the impacts of bivalve aquaculture on primary and secondary symptoms of eutrophication in eelgrass habitats are scale dependent (McKindsey et al. 2006, Skinner et al. 2013, Vance 2013). Therefore, we note the potential influence of aquaculture where relevant (BSS), but do not include it in our overall assessment of bay eutrophication susceptibility.

Combining our results from chapters 2 and 3, we can classify our study sites in eastern NB relative to one another, and to other bays in the continental U.S. in terms of overall eutrophication susceptibility and potential impacts to eelgrass habitats (Figure 1). For this classification, we can use a framework adopted by the NEEA, reviewed in detail by Bricker et al. (2003, 2008). This framework incorporates nutrient influencing factors (N loading and flushing time), overall eutrophic conditions, and future outlook of nutrient impacts in each bay, which are then combined into an overall rating.

Cumulatively, we propose that BSS and TB are at low risk for developing further eutrophication symptoms if populations in these watersheds remain stable and the maintenance of naturally covered riparian zones is upheld (Figure 1). Both these watersheds currently have low N loading rates per area of bay, quick flushing times, and are cumulatively less symptomatic of eutrophication than the other five sites. Additionally, future outlook does not appear imminently different at either site: the RAMSAR conservation area borders the northern edge of the TB bay, while the rest is primarily natural land cover. BSS is a small watershed that is largely wetland, forest and cultured peat land. The watershed:bay ratio in BSS is very small, therefore significant additional point sources of N (e.g. seafood processing plants) would need to be present in order to increase loading at the bay scale to be comparable to bays like CN, BT, RB and LM. The slightly higher microphytobenthos and epiphyte cover at our sampling site in BSS may reflect near-field effects of bivalve aquaculture such as increased organic matter and nitrogen regeneration at the sediment-water interface (Hatcher et al. 1994, Grant 2005). Yet simultaneously the high clearance rate oysters are capable of may be depleting phytoplankton concentrations in the water column, evident in the consistently low water column Chl*a* in BSS (Newell 2004). We see this interaction only in BSS where our sampling site is located close (30m) to an active aquaculture lease. With increasing distance from the active lease in this bay we expect eelgrass habitats to reflect the low terrestrial and atmospheric N loading rates with magnitudes of epiphyte cover and microphytobenthos more similar to KB and TB.

We classify KB as moderate-low in this region (Figure 1). KB has a large watershed:bay ratio, and consequently has the second highest N loading rate per unit bay area, despite the lowest loading per unit watershed area. Additionally, KB has a flushing time similar to LM: longer than those estimated for CN, BT, TB and BSS. Perhaps in part due to the relative lack of wastewater N entering this system compared to the 5 sites mentioned previously, eelgrass beds in KB exhibit low eutrophication symptoms in addition to high shoot density and average eelgrass coverage of 98% in the sampling area. Moreover, the majority of the KB watershed is a National Park, and increases in N loading above contributions from atmospheric deposition are not anticipated. Therefore, because the eutrophication influencing factors in KB are larger than in BSS and TB, yet eutrophication symptoms are low and future outlook is positive, we group KB as moderate-low susceptibility to N loading.

CN and BT are similar watersheds in that they are largely forested, have larger centers of settlement compared to KB, TB and BSS, and have point sources of N including MWWT and seafood processing plants. We rate these bays as moderately susceptible due to their intermediate loading rates per unit bay area, intermediate flushing times, and the exhibition of higher primary symptoms of eutrophication relative to KB, TB and BSS (Figure 1). No significant differences in eelgrass structure or secondary symptoms were identified, however. Future N loading of these watersheds may be negatively affected by new forestry harvesting policies in NB: in 2014 the Government of New Brunswick decreased the area of crown land that is off limits to forestry from 28% (set in 2012) to 23% (DNR). An increase in the harvest of forest stands in these watersheds (CN, BT, RB, KB, TB) will periodically reduce the amount of forest cover, thereby reducing the sequestration potential of atmospherically deposited N to the watershed surface area.

Lastly, we classify RB and LM as being at moderate-high risk of eutrophication (Figure 1). Although eelgrass habitat was more similar to those in CN and BT, RB and

LM have higher N loading rates per unit bay area relative to CN, BT, TB, and BSS. Additionally, these bays have N loading rates per unit bay area which are in the upper 50th percentile of loading rates estimates by Latimer and Rego (2010) in their large-scale analysis of 74 eastern U.S. bays. The N loading rates here are greater than those in Buzzards Bay, however, are more than 3x smaller than loading rates to bays with very high human density such as Chesapeake Bay and Pamlico Sound (Latimer and Rego 2010). Within our region these watersheds have the longest flushing times, making them more susceptible to the maintenance of elevated ambient N availability. N loading in RB may also be affected by the changes to the NB Crown Forestry Act (DNR) described above, which may worsen the future condition of the bay. On the other hand, N loading from the seafood processing plant in LM is unlikely to worsen, as many improvements have been made to reduce the N contribution to this bay since 2003 (Plante and Courtenay 2008). Therefore, we feel eelgrass habitats in these two sites are similar in their overall eutrophic condition, susceptibility and outlook.

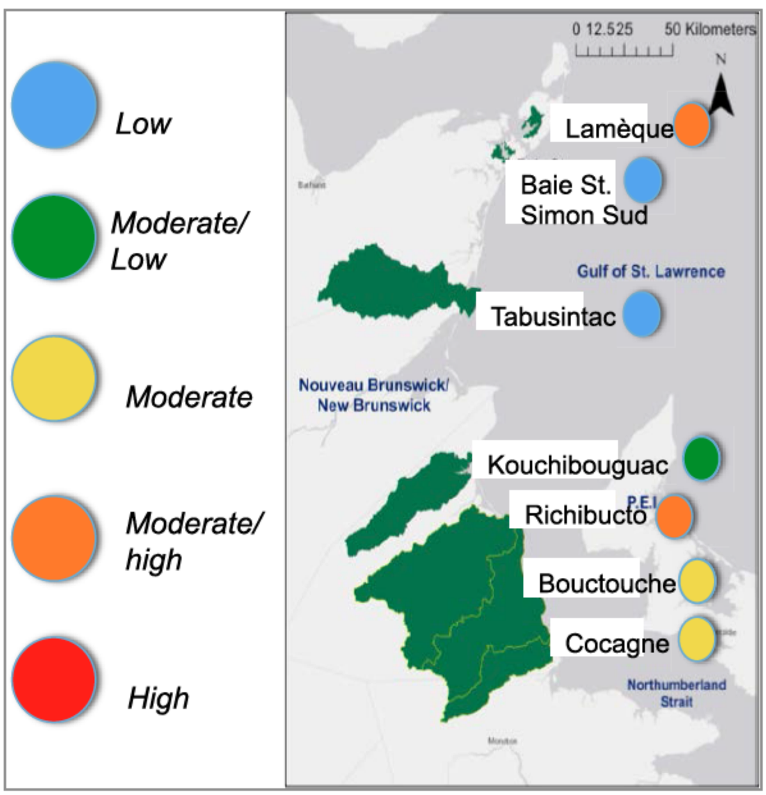

Figure 1. Overall rating of succeptibility to eutrophication for each bay assessed in this project. The rating system corresponds to the National Estuarine Eutrophication Assessment framework (Bricker 2003, 2007, 2008), which incorporates influencing factors (e.g. N loading and flushing time), eutrophic condition (primary and secondary symptoms), and future outlook of each system.

Overall, this research reveals that this region of New Brunswick may contain bays that as of yet reveal little negative human impact relative to most sites in the Northeastern United States and many in PEI that have exhibited marked declines in eelgrass health and distribution. This research is therefore important at filling in the gap at the lower end of the nutrient loading and eutrophication gradient in coastal systems dominated by eelgrass. This does not mean, however, that these bays can or should be subjected to increased N loads, but instead emphasizes that at current loadings these bays are still able to sustain the unique and essential eelgrass habitats that provide significant ecological, social and economic services for the region.

4.4 Future Outlook and Management Implications

Although I identify nutrient loading as a threat to the eelgrass habitats in this region, there are numerous other human impacts that may increasingly negatively contribute to the cumulative effects in these habitats. These potential effects include aquaculture expansion direct habitat destruction (e.g. construction of marinas, dredging), chemical pollution (e.g. pesticides and herbicides), and climate change. The direct destruction of these habitats may be more easily mitigated climate change, which may impact eelgrass habitats in numerous and variable ways. Climate change may, non-monotonically, impact seagrass habitats in eastern NB through these following mechanisms (and potentially numerous others): i) Sea level rise will not only increase the depth of seawater in our study bays, increasing the potential for light attenuation in the water column prior to interception by seagrasses, but also increasing the nutrient dilution potential with increased bay volume (Hemminga and Duarte 2000). Sea level rise may also impact the sand barrier islands that separate these protected bays from the Northumberland Strait (Hemminga and Duarte 2000). Breaches in these barriers change hydrodynamic and circulation patterns and the distribution of eelgrass in response to the shift of tidal channels, as shown in RB and TB (Turcotte-Lanteigne and Ferguson 2013); ii) Sea surface temperatures (SST) will rise in response to climate change, and in eastern NB summer SSTs are already normally between 20-25°C. The proposed upper temperature limit for *Z. marina* in the northern hemisphere is 28°C. Therefore, prolonged (even more than 6 days) temperatures of greater than 28°C may decrease the productivity and survival potential of eelgrass in this region (Abe et al. 2008); iii) Increased CO_2 concentrations in the water column may actually increase the photosynthetic rate of *Z. marina,* especially in light-limited environments. This is potentially beneficial to eelgrass habitats, which as a result of increased water depth, and confounding factors like high phytoplankton and epiphytic algae cover resulting from nutrient enrichment, may be increasingly light limited (Palacios and Zimmerman 2007). Lastly, other potential effects of climate change that may impact and alter the functionality of eelgrass habitat in this region include higher storm frequency and severity, less sea-ice cover in winter, and an increased potential for invasive species that could inhabit this area as it warms and the growing season increases.

These multiple human impacts must all be considered in coastal- ecosystem wide management. The research that I present here can contribute to ecosystem-wide management of this region that is inclusive of nutrient loading monitoring and mitigation. Because atmospheric loading is a ubiquitous and dominant source of N throughout the region, management actions may be effectively targeted towards the maintenance of natural land cover and wetlands, which have an increased capacity to sequester N (Hill 1996, Driscoll et al. 2003). This is especially pertinent in riparian areas bordering freshwater and coastal shores, therefore the continued adherence to maintaining a 30m buffer zone in accord with the NB Clean Water Act is essential (GNB 2003). We recommend special management attention should be focused on reducing N loading per unit bay area to coastal bays in this region that are at a moderate-high risk (RB and LM). In these watersheds, higher standards and treatment of effluent may help to reduce annual N loading rates, preventing further deterioration of the eutrophic condition in eelgrass habitats. Management of N loading per unit bay area is increasingly relevant considering the cumulative and variable impacts that will result from climate change: the management and mitigation of N loading in this region will aid in the resilience of eelgrass habitats, which provide essential ecosystem functions and services to coastal ecosystems and human communities of this region.

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Appendix 1. Supplementary Information (Land Use, Buffer Zone)

Table 1. Overview of watershed land uses in the 7 watersheds assessed for the NLM application in eastern NB. Digital data provided by the NB Department of Natural Resources and Service New Brunswick allowed us to quantify the amount of watershed surface area under each type of land use (NB DNR 2012, SNB 2012). Area of agricultural land is from the 2011 Census of Agriculture (Statistics Canada 2012). The most recent land cover estimates and civic address data are from 2012, however in some watersheds data has not been updated since 2002.

Site [abbreviation]	Forest/ Scrubland (ha)	Wetland (ha)	Peat harvest land (ha)	Agriculture land (ha)	Industrial land (ha)	Infra-structure land (ha)	Turf/ Settlement land (ha)
Cocagne (CN)	29,053	1,546	202	2,150	140	422	1,639
Bouctouche (BT)	63,857	3,018	502	7,885	303	11,578	3,547
Richibucto (RB)	113,941	8,591	42	5,972	388	1,539	4,834
Kouchibouguac (KB)	44,670	11,610	2,700	1,091	40	223	321
Tabusintac (TB)	66,694	3,629	797	1,021	42	349	1,338
Baie St. Simon Sud (BSS)	1,412	320	1,456	1.2	23	10	1,460
Lamèque (LM)	1,609	208	741	302	25	57	1,197

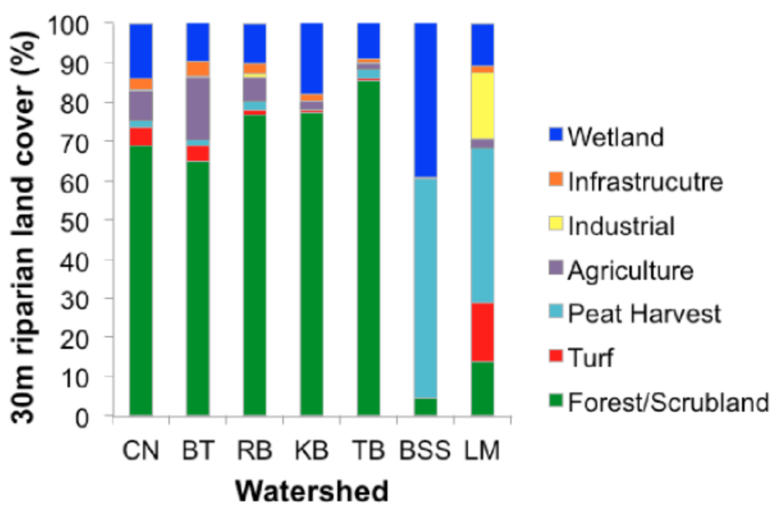


Figure 1. Land use within 30m of freshwater or coastal waterways in 7 watersheds in eastern NB. The riparian zone land cover within each watershed is similar in proportion to land use within the entire watershed.

Appendix 2: Background data used for NLM estimate

Point Sources of Nitrogen Loading

Table 1. Municipal wastewater treatment (MWWT) information on facilities, effluent discharge rate, and N concentration in effluent for watersheds assessed in NB. All data was sourced from the NB Department of Environment and Local Government (Pers. comm F. LeBlanc) (See main text Table 1: **Eq. 1**).

Watershed [abbreviation]	Discharge Location	Type of Treatment	Design Flow Rate (L/day)	Actual Flow Rate (L/day)	Days/year in operation	Dates of observations
Cocagne [CN]	Plage Acadie Beach Inc - Subdivision (15 cottages at this time and capable of having a maximum of 18 cottages)- discharges into Cocagne Harbour	OSI Advantex AX-100tetile-based packed bed filter	15,000 (or 375 L/bed/day)	5,625	365	April-October 2011 (4 dates)
	Foyer Arsenault - Special care home for people with mental and physical disabilities (15 beds in 2011 and capable of having a maximum of 40 beds)- discharges into Cocagne Harbour	Rotating Biological Contactor (RBC) and engineered wetland	68,000 and 20,000 respectively	16,000	Varies (~183)	June and October, 2011
Bouctouche [BT]			2,364,000 L/day (5,200 people)	796,483	365	2009 September
	St. Antoine #1 McKee Mills- Discharges into Little Bouctouche River			500,000	365	July 2006-Sept 2009
	St. Antoine #2 Rue Henrie-Discharge into Epirion River (Smelt Brook- feeds also into Little Bouctouche River)			173,000	365	May 2006-Sept 2009
Richibucto [RB]	Richibucto- Mooney Creek;	Aerated Lagoon with a polishing pond and UV disinfection;		795,000	365	2011-13
	Rexton - Beatties Creek;	Single-celled Facultative Lagoon		684,000	365	2011-13
Kouchibouguac [KB]	N/A	-	-	-	-	-
Tabusintac [TB]	N/A	-	-	-	-	-
Baie St. Simon Sud [BSS]	N/A	-	-	-	-	-
Lamèque [LM]	The town of Lamèque discharges their lagoon effluent to the Ruisseau Jean-Marie, which results in indirect discharge to the Baie de Lamèque	Aerated Lagoon	Unknown	785,000	365	Dec 2010- Dec 2011

Continued on next page

Table 1 Continued: Nutrient Concentration measurements in MWWT effluent (post treatment) (Pers. comm F. LeBlanc, NB DELG)

Watershed [abbreviation]	Mean Ammonia in effluent (NH_3) (mg/L)***	Stdev (+/-)	# measurements	Mean Nitrate in effluent (NO_3^-) (mg/L)****	Stdev (+/-)	# measurements
[CN]	1.08	1.09	2	26.67	4.51	3
	1.65	1.78	2	17.60	5.24	2
[BT]	11.30		1	0.06		1
	3.28	1.65	9	0.17	0.14	9
	4.01	2.93	8	0.16	0.10	8
[RB]	4.76	3.45	11	0.59	0.19	7 (NOx)
	6.51	3.30	15	0.09	0.02	4 (NOx)
[KB]	-	-	-	-	-	-
[TB]	-	-	-	-	-	-
[BSS]	-	-	-	-	-	-
[LM]	18.96	7.68	12	-	-	-

*** Values for Ammonia that are = 0.09 are those that are below limit of quantification (LOQ) (1.0)
**** TKN is Total

Table 1 Continued: Nutrient Concentration measurements in MWWT effluent (post treatment) (Pers. comm F. LeBlanc, NB DELG)

Watershed [abbreviation]	Mean Nitrite (NO_2) (mg/L)	Stdev (+/-)	# measurements	Mean NOx loading (Kg/yr)	Mean NH_3 loading (Kg/yr)	DON loading (Kg/yr)	Total Nitrogen loading (kg TN/yr)
[CN]	0.31	0.11	3	57.6	2.2	58.8	120
	0.26	0.12	2	56.8	4.8	60.9	
[BT]	0.14		1	3,343.2	3,285.1	3,372.3	4,988
	0.15	0.09	9	656.0	598.6	1,190.8	
	0.09	0.02	8	268.7	253.2	425.3	
[RB]	NOx			1,553.4	1,369.9	2,116.3	4,316
	NOx			1,648.4	1,611.9	2,200.1	
[KB]	-	-	-	-	-	-	-
[TB]	-	-	-	-	-	-	-
[BSS]	-	-	-	-	-	-	-
[LM]	-	-	-		5,432.5	6,632.1	6,632

****Values for Nitrates <0.05 are below LOQ (0.05)

Table 2. Seafood processing facility information, nutrient concentrations in effluent, and nitrogen loading estimates for Total Kjeldahl nitrogen (TKN) and Ammonia. TKN was used in the estimate of nitrogen loading to each bay, and is the commonly measured N metric in wastewater combining organically bound N and Ammonia in wastewater. Results of all effluent measurements are an average of 2008-2012. (See main text, Table 1: **Eq. 2**). All data was sourced from the NB Department of Environment and Local Government (Pers. comm F. LeBlanc)

Watershed [Abbreviation]	Plant name	Species/quantities (million lbs/year)*	Flow (m3/day)	Flow (L/day)	Production days/ yr	Production hrs/ day
[CN]	Suncoast Seafood Inc (formerly Seadeli (1996) Ltd	lobster/0.8, crab/0.075	20.0	20,000	110	8.0
	Cocagne Seafood (1995) Ltd	lobster, crab, mussels, cod, scallops, shrimp, 300 000 smelts	24.5	24,500	143	14.0
[BT]	St. Thomas Fish Market Inc.	lobster/ 0.1, mackerel/0.01, smelt, bar clams	49.0	49,000	100	7.0 (some 3.5 hour days)
	Mills Sea Food Ltd	ocean quahog/0.25-3, razor clam/0.022, arctic wedge clam/ 0.022, snow crab/0.25, king crab/0.5	Unknown		unknown	unknown
	B.A. Richard Ltd	lobster/4	508.0	508,000	118	8.0
[RB]	Captain Dan's Inc	lobster/3, crab/1	255.0	255,000	212	9.0
	Richibucto Village Fisherman's Co-operative limited	lobster/0.65, crab/ 0.9	140.0	140,000	98	8.0
	Village Bay Sea Products Ltd.	lobster/ 4, crab/ 0.5, 2 million oysters	52.0	52,000	108	10.0
	Reidpath Cold Storage Ltd.	mackerel/ 5, herring/ 5*	60.0	60,000	25	7.0
[KB]	N/A					
[TB]	N/A					
[BSS]	Pecheries G.E.M. LTEE- G.E.M. Fisheries Ltd.	sea cucumber/ 2, lobster/ 3.5	388.0	388,000	150 (30 days sea cucumber, 120 days lobster)	8.5
[LM]	Association Coopérative des Pêcheurs de l'Ile Ltee	snow crab/ 3 - 4, shrimp / 14-15, rock crab/ 3-4, fish meal (not available)*	1346.4	1,346,400	212	14.5

* Used literature values of effluent nitrogen characteristics for the species processed (AMEC 2004, Garron and Rutherford, 2004).

Continued on next page

Table 2. Continued. Nutrient concentration in seafood processing plant effluent (post treatment) (Pers. comm F. LeBlanc, NB DELG)

Watershed [Abbreviation]	TKN in effluent (mg/L)	Phosphorus (mg/L) **	Ammonia (mg/L)	TKN loading (kg/year)	Ammonia loading (kg/year)	Total TKN/ watershed (kg/yr)	Total Ammonia/ watershed (kg/yr)
[CN]	81.9		5.1	180	15	1,249	50
	305.0	47.0	10.0	1,069	35		
[BT]	78.8*		6.9*	385**	34	5,780	154
	40.5*		6.9*	N/A	N/A		
	90.0	9.0	2.0	5,395	120		
[RB]	32.0	3.0	unknown	1,730	N/A	3215	439
	66.0	10.0	15.0	906	315		
	72.0	6.0	22.0	404	124		
	117.0*			176*	0		
[KB]				0	0	0	0
[TB]				0	0	0	0
[BSS]	10.5	2.9	1.7	611	98	611	98
[LM]	137.0	18.5	11.6	39,105	31	39,105	3,311

* Used literature values of effluent nitrogen characteristics for the species processed (AMEC 2004, Garron and Rutherford, 2004).
** Phosphorus levels are elevated as well- although nitrogen is considered the limiting nutrient in estuarine and coastal ecosystems worldwide, phosphorus in rivers flowing may contribute to increased primary production and eutrophication there.

Table 3. Data used to estimate nutrient loading in surface runoff from peat harvesting. Peat harvesting is treated as a point source as drainage ditches are built to direct surface runoff into sedimentation ponds, after which the water from sedimentation ponds is directed into streams that drain into each bay (See main text Table 1, Eq. 3). (See footnotes for data sources)

Watershed [abbreviation]	Mean runoff coefficient*	Min. runoff coefficient*	Max runoff coefficient*	Precipitation (m)	Average annual runoff (m)	Min annual runoff (m)	Max annual runoff (m)	[TDN] in runoff from mining (mg/L)**	Peat mining area (ha)
[CN]	0.36	0.25	0.48	1.13	0.41	0.26	0.66	0.37	201.87
[BT]	0.36	0.25	0.48	1.13	0.41	0.26	0.61	0.37	501.94
[RB]	0.36	0.25	0.48	1.13	0.42	0.20	0.66	0.37	41.71
[KB]	0.36	0.25	0.48	1.16	0.42	0.20	0.66	0.37	2,700.00
[TB]	0.36	0.25	0.48	1.09	0.38	0.22	0.59	0.37	797.40
[BSS]	0.36	0.25	0.48	1.05	0.38	0.22	0.59	0.37	1,455.86
[LM]	0.36	0.25	0.48	1.05	0.38	0.22	0.59	0.37	741.37

Table 3 continued: Nutrient loading from peat harvest area.

Watershed [abbreviation]	Total volume of surface runoff (m^3)	Min volume of surface runoff (m^3)***	Max volume of surface runoff (m^3)***	Total Nitrogen Load (kg TDN/ year)	Min TDN loading (kg TDN/yr)	Max TDN loading (kg TDN/yr)
[CN]	830,805	522,832	1,326,726	308.73	108.75	811.96
[BT]	2,065,759	1,299,997	3,044,162	767.64	270.40	1,863.03
[RB]	176,280	84,684	273,541	65.51	17.61	167.41
[KB]	11,412,002	5,482,272	17,708,530	4,240.70	1,140.31	10,837.62
[TB]	3,050,115	1,790,389	4,729,818	1,133.42	372.40	2,894.65
[BSS]	5,568,788	3,268,820	8,635,512	2,069.36	679.91	5,284.93
[LM]	2,835,786	1,664,576	4,397,449	1,053.78	346.23	2,691.24

* Estimated from measured Canadian mining runoff coefficients with similar drainage networks and standards for settling ponds and buffer zones (Klove 2001, St Hilaire et al. 2004, Swystun 2013)

** From St. Hilaire et al.(2004), nitrate and TN measurements from three outflow streams draining the St. Charles Peat Bogs measured through 1996-2001. Range was between 0.21-0.61 mg/L, with the mean [TDN] between all sites being 0.372.

*** Based on standard deviation of runoff coefficients from other comparable operations and precipitation amounts between 1995-2005 (Appendix 2: Table 1).

Table 4. Total annual precipitation at 4 monitoring stations in Eastern New Brunswick between 1995-2005. Two of these stations were decommissioned after 2005 (Environment Canada, 2012).

Precipitation Measurements	Year	Environment Canada monitoring station [watersheds corresponding to NLM]			
		Haut Shippegan [BSS and LM]	Miramichi [TB]	Kouchibouguac [KB]	Bouctouche [CN, BT, RB]
Annual precipitation (mm yr^{-1})	1995	1161.2	1009.5	1278.0	1055.6
	1996	901.0	825.1	1040.4	1256.0
	1997	1026.0	1103.0	1368.9	1039.3
	1998	918.0	1063.0	1160.7	1133.4
	1999	954.0	977.0	814.8	1193.9
	2000	969.0	1189.9	1219.4	1058.5
	2001	1238.0	1245.2	1170.6	1118.0
	2002	1237.0	1265.8	1293.5	1371.7
	2003	904.0	1037.4	1002.6	1053.4
	2004	1103.2	1186.6	1257.7	1073.5
	2005	1142.8	1109.5	905.7	1078.2
Average precipitation (mm yr^{-1})		1050.4	1092.0	1160.7	1130.1
Min (mm yr^{-1})		901	825.1	814.8	1039.3
Max (mm yr^{-1})		1238	1265.8	1368.9	1371.7
SD		130.9	129.5	174.9	104.5

Table 5. Information to calculate nitrogen loading from septic systems using civic addresses, number of person per house, and standard nitrogen production per person per year (See main text Table 1, **Eq. 4**) (See footnotes for data sources)

Watershed [abbreviation]	Civic Addresses >200m*	Civic Addresses <200m*	Average # people/ house**	Total population	Average nitrogen loading/ person (kg N/person/yr)***	Loading >200m (kg TDN/yr)****	Loading <200m (kg TDN/yr)****	Total Loading (kg TDN/yr)
[CN]	3,362	2,071	2.33	12,041	4.19	7,635	7,125	14,759
[BT]	7,498	2,564	2.34	25,868	4.19	17,977	9,457	27,434
[RB]	6,497	2,659	2.26	20,693	4.19	15,044	9,472	24,517
[KB]	1,238	259	2.25	3,368	4.19	2,854	919	3,773
[TB]	1,623	616	2.24	5,015	4.19	3,725	2,175	5,900
[BSS]	214	109	2.30	743	4.19	504	395	900
[LM]	621	385	2.26	2,274	4.19	1,438	1,372	2,810

* Assumes 10% of these are seasonal and only occupied for half a year
** Statistics Canada 2001-11
*** USEPA (Klove 2001, U.S. Environmental Protection Agency 2002, St Hilaire et al. 2004, Swystun 2013)
**** According to loss equations in NLM (Valiela et al. 1997a)

Non-Point Sources of Nitrogen

Table 6. Nitrogen application and loading estimates from synthetic fertilizers and manure to each bay considered in this NLM application (See main text Table 1, Eq. 5).

Watershed [abbreviation]	Area of synthetic fertilizer application (ha)*	Area of manure application/ livestock fertilization (ha)*	Synthetic fertilizer N applied in watershed (kg N/ yr)**	Manure fertilizer applied (kg N/ yr)***	Synthetic fertilizer N loading rate (kg TDN/ yr)****	Manure N loading rate (kg TDN/yr)*****	Total N loading (kg TDN/yr)
[CN]	172.0	381	173,899	11,429	1,640	1,767	3,408
[BT]	510.0	308	51,561	9,239	4,864	1,428	6,292
[RB]	440.0	233	44,484	6,989	4,196	1,081	5,277
[KB]	71.4	35	7,219	1,050	681	162	843
[TB]	340.0	102	34,374	1,144	3,242	177	3,419
[BSS]	1.9	0	187	0	18	0	18
[LM]	180.8	0	18,279	0	1,724	0	1,724

* From Statistics Canada 2001-2011, and digital data from SNB (Statistics Canada 2012, GeoNB 2012)
** Based off average recommended rate of nitrogen fertilizer application for crops in New Brunswick of 101.1 kg N/ha/yr (NB LDB, 2001). Here we make the assumption that farmers in New Brunswick follow provincial guidelines. See Appendix 1, Table 7 for crop specific fertilizer guidelines.
*** Amount is nitrogen remaining after losses during storage and spreading (volatilization) of total manure produced in watershed (Yang 2006, Huffman et al. 2008, Yang et al. 2011)
**** Loading after losses in vegetative crop layer, vadose zone, and aquifer, according to equations in NLM (Valiela et al. 1997a, Latimer & Charpentier 2010)

Table 7. Recommended rate of nitrogen fertilizer application for crops in New Brunswick (NB LDB, 2001).

Crop	Fertilizer application recommendation (kg N/ ha crop/ yr)
Pasture, grass forage, hay and silage, ryegrass, timothy, triple mix, mixed forage	96
Potatoes (average of all types)	135
Raspberries and Strawberries	102
Mixed Vegetables	120
Hay or Barley	45
Oats	70
Corn	140
Average of all crops	101

Table 8. Nitrogen application and loading to bays in Eastern New Brunswick from organic and synthetic turf and garden fertilizer (See main text Table 1, **Eq. 6**).

Watershed [abbreviation]	Approximate area for turf fertilization (ha)*	Concentration of fertilizer applied (kg TN/ha/yr) **	Proportion of properties using synthetic and or organic fertilizers ***	Fertilizer N applied (kg TN/ha/yr)	N loading from fertilizer (kg TN/yr)****
[CN]	425	150	0.38	23,728	2,238
[BT]	879	150	0.38	49,118	4,633
[RB]	637	150	0.38	35,570	3,355
[KB]	77	150	0.38	4,274	403
[TB]	162	150	0.38	9,054	854
[BSS]	1.3	150	0.38	71	7
[LM]	137	150	0.38	7,642	721

* From SNB digital data and google earth survey: average of 30% of settlement area in region is lawn/garden
** From (NB DA 1989)
*** Statistics Canada 2005-12 (Statistics Canada, 2012)
**** losses according to NLM equations (inc volatilization upon application) (Valiela et al. 1997a)

Table 9. Atmospheric Nitrogen deposition and loading to each watershed. Deposition was calculated for inorganic and organic nitrogen (summed to produce total nitrogen estimated of loading). Nitrogen loadings estimates were calculated for wet and dry indirect loading, as well as wet and dry direct loading. We did not include estimates of DON in dry deposition as there was insufficient research available for this region regarding the potential proportion of DON in dry deposition (See main text Table 1, **Eq. 8,9**).

Watershed [Abbreviation]	Deposition*	Atmospheric Deposition (kg/ ha/ yr)			Loading (kg/ yr)*****	Total direct loading (kg/ ha/ yr)	Total Indirect loading (kg/ yr)
		DIN (NH4+, NO3-)**	DON***	TDN****	TDN	TDN	TDN
[CN]	Wet Indirect	5.52	2.37	7.89	24 158	28 647	41 068
	Wet Direct	5.52	2.37	7.89	19 226		
	Dry Indirect	5.52	-	-	16 911		
	Dry Direct	3.86	-	-	9 421		
[BT]	Wet Indirect	5.52	2.37	7.89	55 910	44 809	95 047
	Wet Direct	5.52	2.37	7.89	30 073		
	Dry Indirect	5.52	-	-	39 137		
	Dry Direct	3.86	-	-	14 736		
[RB]	Wet Indirect	5.67	2.43	8.10	93 776	61 768	207 995
	Wet Direct	5.67	2.43	8.10	41 455		
	Dry Indirect	5.67	-	-	114 219		
	Dry Direct	3.97	-	-	20 313		
[KB]	Wet Indirect	5.67	2.43	8.10	33 694	17 594	57 279
	Wet Direct	5.67	2.43	8.10	11 808		
	Dry Indirect	5.67	-	-	23 586		
	Dry Direct	3.97	-	-	5 786		
[TB]	Wet Indirect	5.21	2.23	7.44	46 390	40 632	78 863
	Wet Direct	5.21	2.23	7.44	27 270		
	Dry Indirect	5.21	-	-	32 473		
	Dry Direct	3.65	-	-	13 362		
[BSS]	Wet Indirect	5.01	2.15	7.16	1 937	8 875	3 293
	Wet Direct	5.01	2.15	7.16	5 957		
	Dry Indirect	5.01	-	-	1 356		
	Dry Direct	3.51	-	-	2 919		
[LM]	Wet Indirect	5.01	2.15	7.16	2 176	11 479	3 699
	Wet Direct	5.01	2.15	7.16	7 704		
	Dry Indirect	5.01	-	-	1 522		
	Dry Direct	3.51	-	-	3 775		

* Wet = in precipitation, Dry= adsorption/particulate deposition, Direct = to estuary surface, Indirect = to watershed surface

** Average of monthly 1992-2008 data from New Brunswick Precipitation Monitoring Network monitoring sites: NBPNCANB1HRC, and NBPNCANB1PTP (NatChem 2012).

***Estimated from DIN ($NH_4^+ + NO_3^-$)

**** Addition of DIN and DON

*****Using equation from NLM, and land-use data (Appendix 2: Table 1). We do not estimate inorganic nitrogen loading as we do not assess denitrification or transformation of nitrogen types during transport through the watershed.

Hydrological and oceanographic estimates

Table 10. Information used to estimate flushing time for each bay assessed in this study. The calculation used to for the estimate is that presented in Gregory et al. (1993), and it represents the time taken to reduce the concentration of a tracer to a third of it's initial concentration (See main text Table 1, **Eq. 11**). (See footnotes for data sources).

Watershed [abbreviation]	Drainage area (km^2)	Estuary surface area (km^2) (High-tide)	Mean Depth (m)	Mean Tidal Range (m)*	Peak Tidal Range (m)	CHS** data tidal range (m)	Tidal Mean Amplitude (m)= 1/2 tidal range
[CN]	332.462	24.4	1.16	1.115	1.13	2-2.5	0.56
[BT]	760.3198	38.1	1.10	1	1.1	2-2.5	0.50
[RB]	1285.778	51.2	2.20	0.9	1.1	2-2.5	0.45
[KB]	530.4149	14.6	1.50	0.8	0.9	2-2.5	0.40
[TB]	712.761	36.7	1.08	1.1	1.3	2-2.5	0.55
[BSS]	21.5719	8.3	1.58	1.6	1.7	2-2.5	0.80
[LM]	32.41	10.8	3.08	1.6	1.7	2-2.5	0.80

Table 10 Continued: Flushing time

Watershed [abbreviation]	Mean Tidal Current (m/s) ***	Flushing time (days)	Estuary Volume (m^3) (high-tide)****	Tidal Volume (m^3) Tidal Prism	Flushing Time (hrs)
[CN]	0.012	1.3	28206320	27182409	32
[BT]	0.012	1.4	41946042	38132765	33
[RB]	0.012	2.8	112601959	46064438	67
[KB]	0.012	2.2	21868556	11663230	53
[TB]	0.004	1.3	39674423	40330713	30
[BSS]	0.004	1.3	13154772	13321289	30
[LM]	0.004	2.2	33168154	17230210	54

* Difference between high and low tide height (Dutil et al. 2012)
** Canadian Hydrogeographic Service (DFO 2014)
***From Dutil et al. (2012)
**** Calculated using the surface area at high-tide, and average depth of the bay

Table 11. Estimate of annual freshwater recharge for each watershed. This gives the theoretical amount of freshwater that is available to enter the watershed and groundwater each year. This method was compared to measured and area weighted average extrapolation of Environment Canada Stream Gauges, and the results were not significantly different (2-tailed, unequal variance t-test, $p=0.45$). We use this method as more of our 7 watersheds have precipitation monitoring stations than have long-term stream gauges.

Watershed [abbreviation]	Average annual precipitation (mm) *	Loss of precipitation freshwater to evapotranspiration (%) ***	% precipitation freshwater remaining after evapotranspiration	Area of drainage (m^2) ***	Recharge volume (m^3/yr)
[CN]	1130.1	55.0	45	332,462,200	1,690,77,429
[BT]	1130.1	55.0	45	760,319,800	386,669,274
[RB]	1160.7	55.0	45	1,285,777,700	671,557,835
[KB]	1160.7	55.0	45	530,415,000	277,034,163
[TB]	1092.0	55.0	45	712,760,700	350,250,608
[BSS]	1050.4	55.0	45	21,571,900	10,196,429
[LM]	1050.4	55.0	45	32,409,800	15,319,199

* Environment Canada Precipitation monitoring sites: average annual precipitation between 1992-2008.
**From (Shiau 1968)
*** Digital data provided by NB DNR. KB, LM and BSS drainage area refined using ArcHydro tool box in ArcMap (ESRI 2011,13)

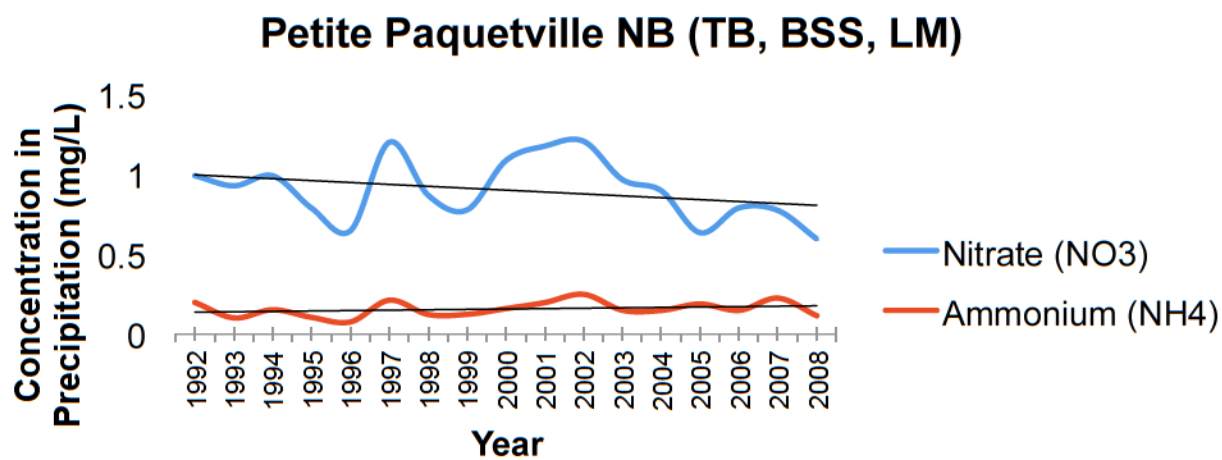
Table 12. Information used to estimate Δ-N for each bay assessed in this study. Δ-N is the difference in ambient nitrogen concentration between bay/ estuarine water and theoretical concentrations in incoming tidal water (No) dependent on the concentration of nitrogen in groundwater entering the bay (Ngw). Q stands for the quantity or volume of water entering the system over a year in either tidal inflow (Qo) or freshwater inflow (Qf). The framework used to for the estimate is presented in (Bugden et al. 2014) and was developed to look at the difference in Nitrate concentration specifically. We use the framework for estimating total dissolved nitrogen (TDN) (See main text Table 1, **Eq. 12,13**).

Watershed [abbreviation]	Drainage area (ha)	No* (mg m3^{-1})	Ngw **(mgL^{-1})	Qf (Annual freshwater Recharge) (m3)	Annual Qo (m^3)***	Qe (=Qf + Qo)****
[CN]	33,246	Theoretical	0.386	169,077,430	19,184,800,504	19,353,877,934
[BT]	76,032	Theoretical	0.332	386,669,274	26,913,342,882	27,300,012,156
[RB]	128,578	Theoretical	0.276	671,557,835	32,511,358,932	33,182,916,767
[KB]	53,042	Theoretical	0.201	277,034,163	8,231,674,170	8,508,708,333
[TB]	71,276	Theoretical	0.278	350,250,608	28,464,610,741	28,814,861,349
[BSS]	2,157	Theoretical	1.109	10,196,429	9,401,899,187	9,412,095,616
[LM]	3,241	Theoretical	3.145	15,319,199	12,160,737,670	12,176,056,869

* This concentration is theoretical as this term is not used in the actual equation.
** This value was derived by dividing the total annual load of N calculated in the NLM by the total amount of freshwater discharge estimates to enter the bay in one year.
***Using tidal prism volumes calculated (Appendix 2: Table 2) for 365 days with tidal cycle of 12.42 hours

Table 12. Continued

Watershed [abbreviation]	Bay Area- High tide (m)	Mean tidal range (m)	Volume of incoming tide (m^3)	Tide cycle (T = 12.42 hours)	TDN Loading (incl. direct deposition) (kg)	Δ-N (mgL^{-1})
[CN]	24378842	1.1	27182409	12.420	96330	0.005
[BT]	38132765	1.0	38132765	12.420	189753	0.007
[RB]	51182709	0.9	46064438	12.420	266108	0.008
[KB]	14579037	0.8	11663230	12.420	79958	0.009
[TB]	36664285	1.1	40330713	12.420	130802	0.005
[BSS]	8325805	1.6	13321289	12.420	15773	0.002
[LM]	10768881	1.6	17230210	12.420	67223	0.006



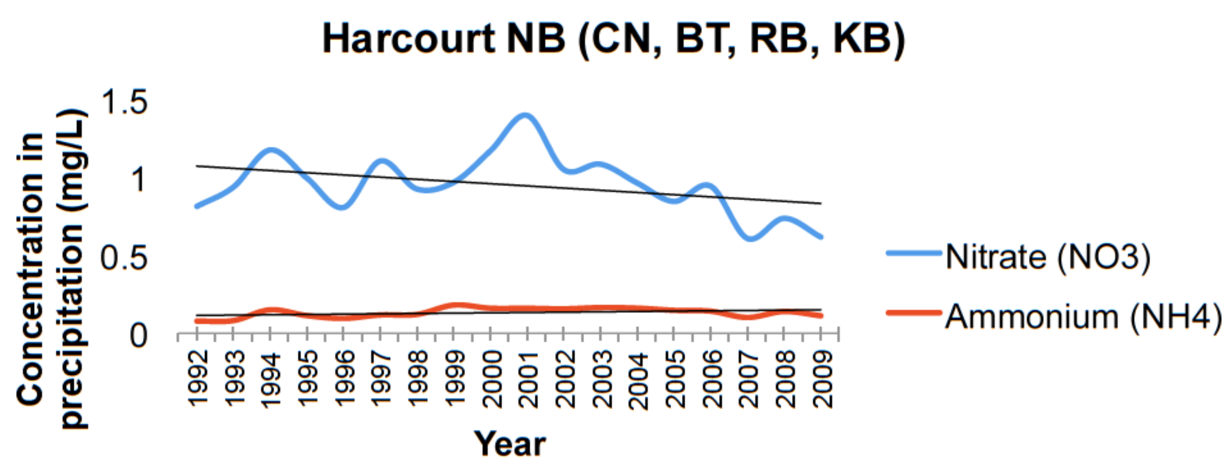


Figure 1. Trends in wet deposition of Nitrate and Ammonium at the two long-term New Brunswick Precipitation Monitoring Network monitoring sites: NBPNCANB1HRC, and NBPNCANB1PTP (NatChem 2012)

Appendix 3. Supplementary Information (Eelgrass Tissue Nitrogen and Isotope samples)

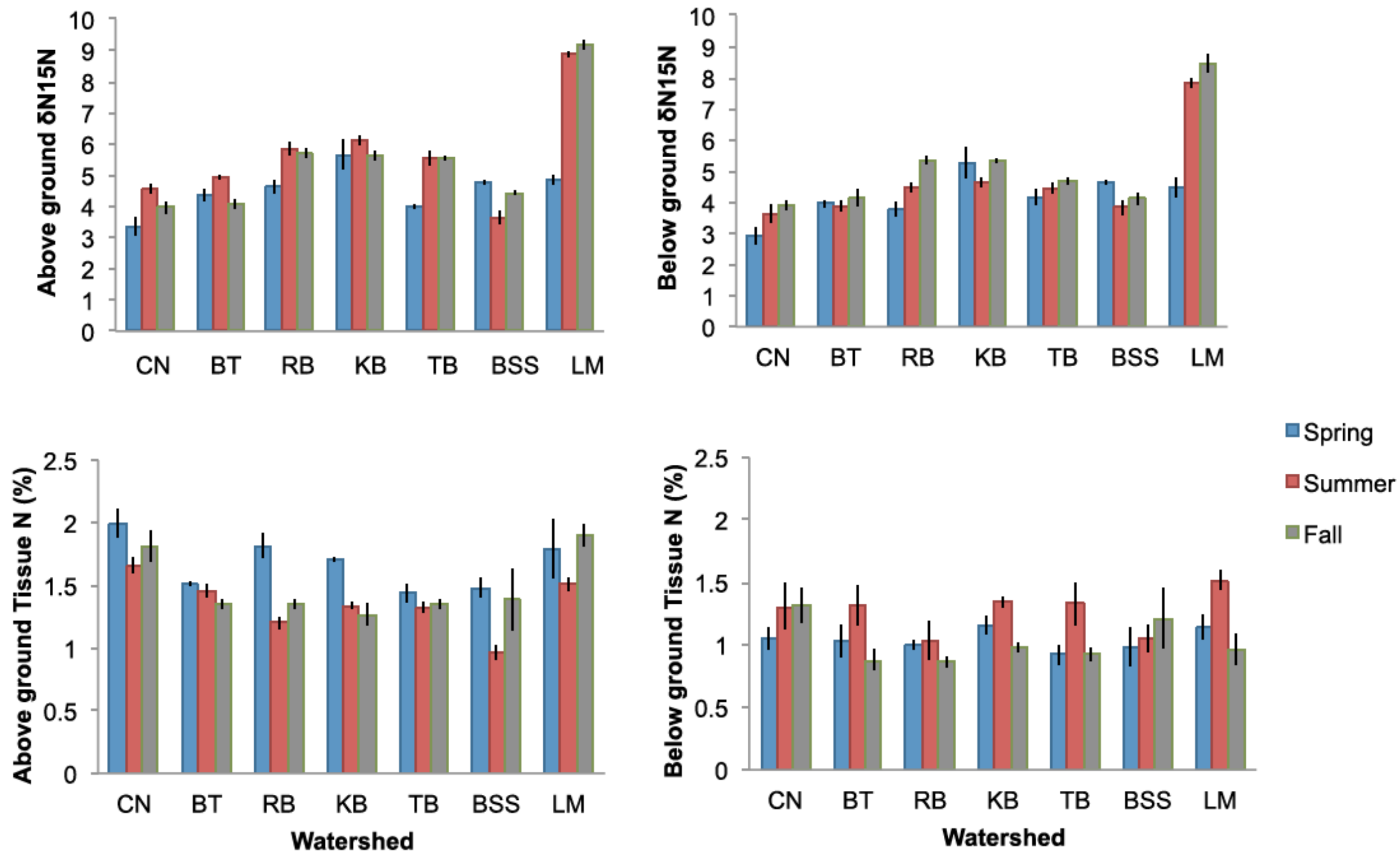


Figure 1. Seasonal measurements of mean (±SE) eelgrass tissue nitrogen isotope values (upper panels) and nitrogen content (lower panels) for above ground (left) and below ground (right) components sampled in May (n=3), August (n=6) and October (n=3) 2013.

Table 1. Results of simple linear regression using estimates from the NLM, Delta-N and flushing time as independent variables and above ground (AG) and below ground (BG) tissue nitrogen stable isotope measured from eelgrass samples collected across all seasons as well as in spring (June), summer (August), and fall (October) 2013. Relationships with a significant p-value (<0.05) are shown in bold.

Model Prediction	df	AG δ^{15}N (all seasons)				BG δ^{15}N (all seasons)			
		F-stat	Residual st. error	Adj. R^2	p- value	F-stat	Residual st. error	Adj. R^2	p- value
Load (kg TDN yr^{-1})	1, 79	0.11	1.55	-0.01	0.74	2.68	1.36	0.02	0.11
Loading rate (kg TDN ha $watershed^{-1}$yr-1)	**1, 79**	**46.72**	**0.37**	**0.36**	**1.47×10^{-9}**	**73.81**	**0.99**	**0.48**	**6.12×10^{-13}**
Loading rate (kg TDN ha $bay^{-1} yr^{-1}$)	**1, 79**	**50.06**	**1.22**	**0.40**	**5.08×10^{-10}**	**28.86**	**1.18**	**0.26**	**7.63×10^{-7}**
Loading rate (kg TDN m^3 $bay^{-1} yr^{-1}$)	1, 79	2.435	1.56	0.02	0.12	6.78	1.32	0.068	0.01
Δ-N (mg/L)	1, 79	**6.54**	**1.49**	**0.06**	**0.01**	1.37	1.37	0.01	0.25
Flushing time (hrs)	1, 79	**30.62**	**1.32**	**0.27**	**3.85×10^{-7}**	**19.95**	**1.23**	**0.19**	**2.61×10^{-5}**
Wastewater load (kg TDN yr^{-1})	1, 79	**17.03**	**1.38**	**0.17**	**9.0×10^{-5}**	**19.6**	**1.27**	**0.19**	**3.0×10^{-5}**
Wastewater loading rate (kg TDN ha $watershed^{-1}$yr-1)	1, 79	**130.9**	**1.11**	**0.62**	**$<2.2 \times 10^{-16}$**	**74.76**	**0.92**	**0.45**	**4.3×10^{-13}**
Wastewater loading rate (kg TDN ha bay^{-1}yr-1)	1, 79	**97.13**	**1.15**	**0.55**	**2.1×10^{-15}**	**65.28**	**0.99**	**0.44**	**5.6×10^{-12}**
Wastewater loading rate (kg TDN m^3 $bay^{-1} yr^{-1}$)	1, 79	**25.78**	**1.34**	**0.24**	**2.5×10^{-6}**	**21.37**	**1.22**	**0.20**	**1.4×10^{-5}**
		Spring				Spring			
Load (kg TDN yr^{-1})	1, 19	0.40	0.79	-0.03	0.53	2.67	0.77	0.08	0.12
Loading rate (kg TDN ha $watershed^{-1}$yr-1)	1, 19	0.56	0.79	-0.02	0.46	0.60	0.81	-0.02	0.45
Loading rate (kg TDN ha bay^{-1}yr-1)	1, 19	1.85	0.76	0.04	0.19	0.20	0.82	-0.04	0.66

Loading rate (kg TDN m^3 $bay^{-1}yr^{-1}$)	1, 19	0.62	0.79	-0.02	0.44	0.57	0.81	-0.02	0.46
Δ-N (mg/L)	1, 19	3.01	0.74	0.09	0.10	**0.44**	**1.22**	**0.19**	**$4.0x10^{-5}$**
Flushing time (hrs)	1, 19	**5.76**	**0.70**	**0.19**	**0.03**	0.44	0.82	-0.03	0.51
Wastewater load (kg TDN yr^{-1})	1, 19	**23.75**	**1.38**	**0.23**	**$1.0x10^{-4}$**	**15.26**	**1.25**	**0.06**	**$2.0x10^{-4}$**
Wastewater loading rate (kg TDN ha $watershed^{-1}$yr-1)	1, 19	4.78	0.79	-0.02	0.48	0.37	0.82	-0.03	0.55
Loading rate (kg TDN ha bay^{-1}yr-1)	1, 19	0.14	0.80	-0.05	0.72	0.01	0.82	-0.05	0.91
Wastewater loading rate (kg TDN m^3 $bay^{-1}yr^{-1}$)	1, 19	0.35	0.79	-0.03	0.56	0.85	0.81	-0.01	0.37
				Summer				Summer	
Load (kg TDN yr^{-1})	1, 38	0.04	1.65	-0.03	0.84	1.367	1.45	0.01	0.25
Loading rate (kg TDN ha $watershed^{-1}$yr-1)	1, 38	**35.48**	**1.19**	**0.47**	**$6.5x 10^{-7}$**	**96.58**	**0.78**	**0.72**	**$7.36x 10^{-12}$**
Loading rate (kg TDN ha bay^{-1}yr-1)	1, 38	**69.26**	**0.98**	**0.64**	**$4.3x 10^{-10}$**	**21.90**	**1.17**	**0.36**	**$3.78x 10^{-5}$**
Loading rate (kg TDN m^3 $bay^{-1}yr^{-1}$)	1, 38	0.29	1.64	-0.02	0.60	4.11	1.40	0.08	0.05
Δ-N (mg/L)	1, 38	**6.23**	**1.53**	**0.12**	**0.02**	0.40	1.47	-0.02	0.53
Flushing time (hrs)	1, 38	**21.51**	**1.32**	**0.35**	**$4.1x10^{-5}$**	**11.90**	**1.29**	**0.22**	**$2.0x10^{-3}$**
Wastewater load (kg TDN yr^{-1})	1, 38	**23.33**	**1.30**	**0.36**	**$2.0x10^{-5}$**	**16.53**	**1.23**	**0.29**	**$2.0x10^{-4}$**
Wastewater loading rate (kg TDN ha $watershed^{-1}$yr-1)	1, 38	**83.98**	**0.92**	**0.68**	**$3.65x10^{-11}$**	**185.5**	**0.61**	**0.83**	**$5.6 x 10^{-16}$**
Loading rate (kg TDN ha bay^{-1}yr-1)	1, 38	**83.56**	**0.93**	**0.68**	**$3.9x10^{-11}$**	**122.80**	**0.79**	**0.76**	**$2.6 x 10^{-13}$**

Wastewater loading rate (kg TDN m^3 bay^{-1} yr^{-1})	1, 38	**25.35**	**1.28**	**0.38**	**$1.2x10^{-5}$**	**25.74**	**1.34**	**0.39**	**$1.13x10^{-5}$**
				Fall				Fall	
Load (kg TDN yr^{-1})	1, 19	0.37	1.73	-0.03	0.55	0.25	1.551	-0.04	0.62
Loading rate (kg TDN ha $watershed^{-1}$yr-1)	1, 19	**36.59**	**1.02**	**0.64**	**$8.09x 10^{-6}$**	**40.37**	**0.88**	**0.66**	**$4.27x10^{-6}$**
Loading rate (kg TDN ha bay^{-1}yr-1)	1, 19	**11.24**	**1.38**	**0.34**	**$3.0x10^{-3}$**	**15.73**	**1.16**	**0.42**	**$8.0x10^{-4}$**
Loading rate (kg TDN m^3 $bay^{-1}$$yr^{-1}$)	1, 19	3.02	1.62	0.09	0.10	2.35	1.47	0.06	0.14
Δ-N (mg/L)	1, 19	0.37	1.73	-0.03	0.55	0.77	1.53	-0.01	0.39
Flushing time (hrs)	1, 19	**8.69**	**1.44**	**0.28**	**$8.0x10^{-3}$**	**10.50**	**1.25**	**0.32**	**$4.0x10^{-3}$**
Wastewater load (kg TDN yr^{-1})	1, 19	**5.80**	**1.53**	**0.19**	**0.03**	**8.81**	**1.29**	**0.29**	**0.01**
Wastewater loading rate (kg TDN ha $watershed^{-1}$yr-1)	1, 19	**68.21**	**0.82**	**0.77**	**$1.0x10^{-7}$**	**82.67**	**0.68**	**0.81**	**$2.4x10^{-8}$**
Wastewater loading rate (kg TDN ha bay^{-1}yr-1)	1, 19	**41.31**	**0.91**	**0.67**	**$3.7x10^{-6}$**	57.05	0.78	0.73	**$4.0x10^{-7}$**
Wastewater loading rate (kg TDN m^3 $bay^{-1}$$yr^{-1}$)	1, 19	**7.39**	**1.48**	**0.24**	**0.02**	10.89	1.25	0.33	**$4.0x10^{-3}$**

Table 2. Results of simple linear regression using estimates from the NLM, Delta-N and flushing time as independent variables and above ground (AG) and below ground (BG) percent tissue nitrogen measured from eelgrass samples collected across all seasons as well as in spring (June), summer (August), and fall (October) 2013. Relationships with a significant p-value (<0.05) are shown in bold.

Model Prediction	df	AG % Tissue Nitrogen (All Seasons)				BG % Tissue Nitrogen (All Seasons)			
		F-stat	Residual st. error	Adj. R^2	p	F-stat	Residual st. error	Adj. R^2	P
Load (kg TDN yr^{-1})	1, 79	0.02	0.29	-0.01	0.90	0.93	0.27	-0.01	0.34
Loading rate (kg TDN ha $watershed^{-1}$yr-1)	1, 79	3.85	0.29	0.03	0.05	0.02	0.27	-0.01	0.89
Loading rate (kg TDN ha bay^{-1}yr-1)	1, 79	**9.89**	**0.28**	**0.10**	**$3.0x10^{-3}$**	0.11	0.27	-0.01	0.74
Wastewater loading rate (kg TDN m^3 bay^{-1} yr^{-1})	1, 79	2.65	0.29	0.02	0.11	0.24	0.27	-0.01	0.56
Δ-N (mg/L)	1, 79	1.19	0.29	0.01	0.28	0.25	0.27	-0.01	0.62
Flushing time (hrs)	1, 79	0.19	0.29	-0.01	0.67	1.42	0.27	0.01	0.24
		Spring				Spring			
Nitrogen load (kg TDN yr^{-1})	1, 19	0.17	0.26	-0.04	0.68	0.47	0.16	-0.03	0.50
Loading rate (kg TDN ha $watershed^{-1}$yr-1)	1, 19	0.24	0.26	-0.04	0.63	1.00	0.16	-0.01	0.33
Loading rate (kg TDN ha $watershed^{-1}$yr-1)	1, 19	2.57	0.25	0.07	0.13	2.44	0.16	0.07	0.14
Loading rate (kg TDN m^3 $bay^{-1}$$yr^{-1}$)	1, 19	$8.0x10^{-4}$	0.26	-0.05	0.98	0.04	0.17	-0.05	0.84
Δ-N (mgL^{-1})	1, 19	1.30	0.25	0.02	0.27	1.24	0.16	0.01	0.28
Flushing time (hrs)	1, 19	2.56	0.24	0.07	0.13	0.98	0.16	-0.01	0.36

			Summer				Summer		
Nitrogen load (kg TDN yr^{-1})	1, 38	0.27	0.25	-0.02	0.61	0.05	0.32	-0.03	0.82
Loading (kg TDN ha $watershed^{-1}$yr-1)	1, 38	0.56	0.25	-0.01	0.46	0.08	0.32	-0.03	0.79
Loading rate (kg TDN ha $bay^{-1} yr^{-1}$)	1, 38	**12.26**	**0.22**	**0.22**	**1.0×10^{-3}**	2.0×10^{-3}	0.32	-0.03	0.96
Loading rate (kg TDN m^3 $bay^{-1} yr^{-1}$)	1, 38	**15.43**	**0.21**	**0.27**	**4.0×10^{-4}**	1.24	0.32	0.01	0.27
Δ-N (mgL^{-1})	1, 38	3.52	0.24	0.06	0.07	2.0×10^{-4}	0.32	-0.03	0.99
Flushing time (hrs)	1, 38	0.11	0.25	-0.02	0.74	0.82	0.32	-0.01	0.37
			Fall				Fall		
Nitrogen load (kg TDN yr^{-1})	1, 19	1.42	0.29	0.02	0.25	4.52	0.23	0.15	0.05
Loading rate (kg TDN ha $watershed^{-1}$yr-1)	1, 19	**9.36**	**0.25**	**0.30**	**0.01**	0.01	0.25	-0.05	0.94
Loading rate (kg TDN ha $bay^{-1} yr^{-1}$)	1, 19	0.79	0.30	-0.01	0.39	3.59	0.23	0.12	0.07
Loading rate (kg TDN m^3 $bay^{-1} yr^{-1}$)	1, 19	0.67	0.30	-0.02	0.42	0.77	0.25	-0.01	0.39
Δ-N (mgL^{-1})	1, 19	1.02	0.30	0.01	0.33	3.61	0.23	0.12	0.07
Flushing time (hrs)	1, 19	4.0×10^{-3}	0.30	-0.05	0.95	2.60	0.24	0.07	0.12

Table 3. Multiple regression results of total nitrogen loading rate per unit bay area, flushing time, and the interaction of these factors related to above (AG) and below ground (BG) isotope content of eelgrass tissue sampled in across all seasons as well as in spring (June), summer (August), and fall (October). The tissue characteristics are analyzed individually to represent any seasonal patterns, as well as grouped to understand any overarching patterns throughout the growing season. Relationships with a significant p-value (<0.05) are shown in bold.

	AG $\delta^{15}N$ (‰) All seasons							BG $\delta^{15}N$ (‰) All Seasons						
	df	Coeff	SE	p-value	F-stat	Overall Adj. R2	Overall p-value	df	Coeff	SE	p-value	F-stat	Overall Adj. R2	Overall p-value
Flushing Time	3,78	**0.16**	**0.02**	**1.57×10^{-12}**	**29.02**	**0.51**	**1.05×10^{-12}**	3,78	**0.13**	**1.72×10^{-2}**	**1.87×10^{-10}**	**24.65**	**0.47**	**2.78×10^{-11}**
Nitrogen Load (kg TDN ha bay^{-1} yr^{-1})		**2.0×10^{-5}**	**5.33×10^{-6}**	**6.64×10^{-4}**					**1.17×10^{-5}**	**4.94×10^{-6}**	**0.021**			
Flushing time + Nitrogen Load		**-5.25×10^{-7}**	**1.04×10^{-7}**	**2.56×10^{-6}**					**-4.08×10^{-7}**	**9.66×10^{-8}**	**6.64×10^{-5}**			

	Summer							Summer						
	df	Coeff	SE	p-value	F-stat	Overall Adj. R2	Overall p-value	df	Coeff	SE	p-value	F-stat	Overall Adj. R2	Overall p-value
Flushing Time	3,36	**0.21**	**0.02**	**1.27×10^{-11}**	**35.15**	**0.72**	**5.54×10^{-11}**	3,35	**0.15**	**2.38×10^{-2}**	**2.53×10^{-7}**	**17.45**	**0.57**	**4.26×10^{-7}**
Nitrogen Load (kg TDN ha bay^{-1} yr^{-1})		**3.0×10^{-4}**	**6.00×10^{-6}**	**7.95×10^{-6}**					**1.59×10^{-5}**	**6.80×10^{-6}**	**0.03**			
Flushing time + Nitrogen Load		**-7.7×10^{-7}**	**1.17×10^{-7}**	**1.08×10^{-7}**					**-5.16×10^{-7}**	**1.34×10^{-7}**	**5.0×10^{-5}**			

	Spring							Spring						
	df	Coeff	SE	p-value	F-stat	Overall Adj. R2	Overall p-value	df	Coeff	SE	p-value	F-stat	Overall Adj. R2	Overall p-value
Flushing Time	3,17	**0.01**	**0.02**	**0.04**	3.89	0.30	0.03	3,17	0.04	2.46×10^{-2}	0.090	2.57	0.19	0.09
Nitrogen Load (kg TDN ha bay^{-1} yr^{-1})		-9.2×10^{-8}	6.4×10^{-6}	0.99					6.40×10^{-7}	7.12×10^{-6}	0.93			

	df	Coeff	SE	p-value	F-stat	Overall Adj. R2	Overall p-value	df	Coeff	SE	p-value	F-stat	Overall Adj. R2	Overall p-value
Flushing time + Nitrogen Load		-8.7x 10^{-8}	1.24x 10^{-7}	0.50					-1.25x 10^{-7}	1.38x 10^{-7}	0.38			
	Fall							Fall						
	df	Coeff	SE	p-value	F-stat	Overall Adj. R2	Overall p-value	df	Coeff	SE	p-value	F-stat	Overall Adj. R2	Overall p-value
Flushing Time	3,17	**0.17**	**0.04**	**5.0x 10^{-4}**	**9.03**	**0.552**	**8.0x 10^{-4}**	3,17	**0.16**	**3.23x 10^{-2}**	**1.0x 10^{-4}**	**11.60**	**0.61**	**2.0x 10^{-4}**
Nitrogen Load (kg TDN ha bay^{-1} yr^{-1})		**1.4x 10^{-5}**	**1.1x 10^{-5}**	**0.23**					**1.48x 10^{-5}**	**9.32x 10^{-6}**	**0.13**			
Flushing time + Nitrogen Load		**-5.0x 10^{-7}**	**2.19x 10^{-7}**	**0.04**					**-4.85x 10^{-7}**	**1.81x 10^{-7}**	**0.02**			

Table 4. Multiple regression results of total nitrogen load, flushing time, and the interaction of these factors related to above (AG) and below ground (BG) isotope content of eelgrass tissue sampled in across all seasons as well as in spring (June), summer (August), and fall (October). The tissue characteristics are analyzed individually to represent any seasonal patterns, as well as grouped to understand any overarching patterns throughout the growing season.. Relationships with a significant p-value (<0.05) are shown in bold.

	AG Tissue N (%) All Seasons							BG Tissue N (%) All Seasons						
	df	Coeff	SE	p-value	F-stat	Overall Adj. R2	Overall p-value	df	Coeff	SE	p-value	F-stat	Overall Adj. R2	Overall p-value
Flushing Time	3,78	0.01	4.88x 10^{-3}	**0.01**	2.42	0.05	0.07	3,77	-1.86x 10^{-3}	4.63x 10^{-3}	0.69	0.548	-0.02	0.65
Nitrogen Load (kg TDN yr^{-1})		3.32x 10^{-6}	1.40x 10^{-3}	**0.02**					-1.50x 10^{-7}	1.33x 10^{-6}	0.91			
Flushing time + Nitrogen Load		-7.149 x10^{-8}	2.72x 10^{-8}	**0.01**					-1.32x 10^{-9}	2.6x 10^{-8}	0.96			
	Summer							Summer						
	df	Coeff	SE	p-value	F-stat	Overall Adj. R2	Overall p-value	df	Coeff	SE	p-value	F-stat	Overall Adj. R2	Overall p-value
Flushing Time	3,36	**1.74x1 0^{-7}**	**4.96x 10^{-3}**	**1.0x 10^{-2}**	**7.40**	**0.33**	**6.0x 10^{-4}**	3,35	-2.65x 10^{-3}	8.05x 10^{-3}	0.74	0.43	-0.05	0.73

Nitrogen Load (kg TDN yr^{-1})		**6.56×10^{-6}**	**1.41×10^{-6}**	**04.28 $\times 10^{-5}$**					1.11×10^{-9}	2.30×10^{-6}	0.63			
Flushing time + Nitrogen Load		**-1.26×10^{-7}**	**2.74×10^{-8}**	**5.09×10^{-5}**					-1.29×10^{-8}	4.53×10^{-8}	0.78			
	Spring							Spring						
	df	Coeff	SE	p-value	F-stat	Overall Adj. R2	Overall p-value	df	Coeff	SE	p-value	F-stat	Overall Adj. R2	Overall p-value
Flushing Time	3,17	6.02×10^{-3}	8.78×10^{-3}	0.50	0.80	-0.03	0.51	3,17	9.76×10^{-3}	5.41×10^{-3}	0.09	1.39	0.06	0.28
Nitrogen Load (kg TDN yr^{-1})		-4.67×10^{-7}	2.54×10^{-6}	0.86					1.15×10^{-5}	1.56×10^{-6}	0.47			
Flushing time + Nitrogen Load		4.92×10^{-9}	4.92×10^{-8}	0.92					-3.67×10^{-8}	3.03×10^{-8}	0.24			
	Fall							Fall						
	df	Coeff	SE	p-value	F-stat	Overall Adj. R2	Overall p-value	df	Coeff	SE	p-value	F-stat	Overall Adj. R2	Overall p-value
Flushing Time	3,17	7.84×10^{-3}	1.04×10^{-2}	0.88	0.63	-0.06	0.61	3,17	-1.24×10^{-2}	7.54×10^{-3}	0.12	2.46	0.18	0.10
Nitrogen Load (kg TDN yr^{-1})		4.75×10^{-7}	3.01×10^{-6}	0.46					-4.0×10^{-6}	2.18×10^{-6}	0.08			
Flushing time + Nitrogen Load		-3.40×10^{-8}	5.83×10^{-8}	0.57					5.59×10^{-8}	4.22×10^{-8}	0.18			

Appendix 4: Chapter 3 Supplementary Information

Table 1. Results of field survey from August, 2013 (quadrat variables) and June, August and September for water column variables. Average values are shown, ± Standard error.

Site	CN	BT	RB	KB	TB	BSS	LM
Sediment organic content (n=6)	1.5± 0.08	1.7±0.09	1.6± 0.12	1.9+ 0.09	6.5± 1.00	4.1± 0.43	2.1± 0.14
Microphytobenthos (Chl*a* ugL^{-1}) (n=6)	399.3± 52.0	411.8± 10.44	454.5± 55.88	270.2± 51.49	229.1± 6.78	352.8± 22.29	432.1± 17.66
Eelgrass shoot density (n=11)	33± 1.61	18±1.31	20±1.67	23± 1.22	18±3.30	37± 3.00	48± 7.60
Eelgrass canopy height (n=11)	34± 1.90	46±1.83	34± 1.79	62± 1.19	35± 2.45	30± 1.59	25± 2.41
Eelgrass % Cover (n=11)	92± 6.26	96± 1.48	91± 4.82	98± 1.24	72± 9.71	100± 0.45	85± 9.50
AG eelgrass biomass (g m$^{1-1}$) (n=6)	496± 56	818± 78	910± 198	1369± 55	711± 163	751± 80	722± 226
BG eelgrass biomass (g m$^{1-1}$) (n=6)	2448± 449	1712± 281	1416± 293	2203± 149	1751± 417	2002± 134	2425±304
Epiphytic % cover (n=11)	56.4± 2.14	45±5.04	72.7± 2.97	26.4± 2.70	25.9± 6.47	53.2± 5.69	50.9± 3.49
Benthic % cover (n=11)	0± 0	0± 0	0.2± 0.12	0.9± 0.91	4.5± 1.06	0± 0	1.8± 1.02
Chl*a* (n=9)	1.40± 0.52	1.44± 0.45	1.64± 0.52	3.46± 1.16	0.75± 0.33	0.14± 0.04	0.69± 0.21
TPM (n=9)	51.2± 11.4	50.6± 14.4	52.2± 18.1	38.1± 8.8	55.7± 13.7	23.1± 2.8	44.3± 11.2
POM (n=9)	13.24± 3.6	12.81± 4.2	9.89± 3.6	2.41± 4.6	20.36± 8.3	5.65± 1.3	10.54± 3.7
PIM (n=9)	37.97± 8.9	37.77± 10.7	42.35± 14.6	35.70± 11.1	35.32± 6.2	17.48± 2.2	33.76± 8.6

Table 2. Results of univariate PERMANOVA and ANOVA with eelgrass/ eutrophic measurements as dependent variables and Site (bays) as the independent variable. Both the ANOVA and PERMANOVA results are shown to illustrate there is no difference in parametric and non-parametric test results. Protected post-hoc tests were done using the parametric Tukey's Honestly Significant Difference test (Tables 3-15 below). Significance levels: 0.05=*, 0.01=**, ≤0.001=***. Mean Sq. error is the sum of squared divided by the degrees of freedom in the ANOVA, representing the proportion of variance explained by site in the squared units of the measured variable. √V are estimates of the components of variance for each of the factors in the PERMAOVA model, and is the square root of the Mean Sq. error. Negative V indicates there is no evidence against the null hypothesis. Variables that violated the assumptions of homogeneity of variance are shown in italics: the significance of these results is not expressed with confidence as a result of breaking the assumptions.[1,2]

Variable	PERMANOVA			ANOVA	
	df (Watershed | Residuals)	√V	Pseudo- F	Mean Sq. error	F
CH	6	26.35	11.59***	694.10	11.59***
Residuals	67	7.74		59.90	
Shoot density	6	119.63	10.11***	14312	10.11***
Residuals	67	37.61		1416	
log(AG Biomass)	6	0.71	2.93*	0.50	2.93*
Residuals	32	0.41		0.17	
log(BG Biomass)	6	0.57	2.05	0.32	2.05
Residuals	32	0.4		0.16	
epiphytic algae	6	0.45	8.84***	0.21	8.42***
Residuals	67	0.14		0.02	
benthic algae	6	0.03	2.36	0.001	2.36
Residuals	67	0.02		0.001	
microphytobenthos	6	183.70	3.98**	33744	3.98**
Residuals	32	92.04		8471	
Sediment organic	6	0.04	12.42***	0.002	12.42
Residuals	32	0.01		0.0001	
Eelgrass AG tissue N (%)	6	0.68	8.51***	0.46	8.51***
Residuals	74	0.23		0.05	
Eelgrass BG tissue N (%)	6	0.40	2.49*	0.16	2.49*
Residuals	74	0.25		0.06	

Eelgrass AG tissue C (%)	6	4.24	2.41*	17.97	2.46*
Residuals	74	2.73		7.47	
Eelgrass BG tissue C (%)	6	3.63	0.77	13.15	0.77
Residuals	74	4.13		17.01	
Eelgrass AG $\delta^{15}N$	6	4.62	24.77***	21.37	24.77***
Residuals	74	0.93		0.86	
Eelgrass BG $\delta^{15}N$	6	4.10	25.13***	16.80	25.13***
Residuals	74	0.82		0.67	
Eelgrass AG $\delta^{13}C$	6	3.30	46.32***	10.89	46.31***
Residuals	74	0.49		0.24	
Eelgrass BG $\delta^{13}C$	6	2.91	35.87***	8.46	35.87***
Residuals	74	0.49		0.24	

1. All water column variables broke assumptions of both PERMANOVA and ANOVA, resulting in significantly different results between parametric and non-parametric tests. Therefore we do not include the resuls for watercolumn Chl*a*, TPM, PIM, POM.
2. For the variables identified as not meeting assumptions (and water column variables), transformations ($\log_{10}$, square and quarter root) were performed. Log_{10} transformation improved result for AG and BG biomass, but not other variables.

Tables 3-15. Tukey multiple comparisons of means, with 95% family-wise confidence level, was conducted as a post-hoc test for univariate ANOVASs with the effect of Site (bay) on eelgrass/ eutrophic measurements in 7 bays in eastern New Brunswick. Post-hoc tests were only conducted when the variation in the response variable met the assumption of homogeneity of dispersion (Anderson, 2003). Abbreviations of sites are used: CN= Cocagne, BT= Bouctouche, RB= Richibucto, KB= Kouchibouguac, TB= Tabusintac, BSS= Baie St. Simon Sud, LM= Lamèque; as well as abbreviations for tissue components: AG= above ground tissue, BG= below ground tissue.

Table 3. Output of protected pair-wise difference tests comparing eelgrass canopy height between all 7 sites sampled in eastern NB in August 2013 (n=11).

Site	difference	lower	upper	p adjusted
BT-BSS	16.80	2.09	31.51	**0.02**
CN-BSS	7.40	-7.31	22.11	0.69
KB-BSS	31.00	16.29	45.71	**0.00**
LM-BSS	-0.40	-15.11	14.31	1.00
RB-BSS	5.40	-9.31	20.11	0.90
TB-BSS	6.40	-8.31	21.11	0.81
CN-BT	-9.40	-24.11	5.31	0.42
KB-BT	14.20	-0.51	28.91	0.06
LM-BT	-17.20	-31.91	-2.49	**0.01**
RB-BT	-11.40	-26.11	3.31	0.21
TB-BT	-10.40	-25.11	4.31	0.31
KB-CN	23.60	8.89	38.31	**0.00**
LM-CN	-7.80	-22.51	6.91	0.63
RB-CN	-2.00	-16.71	12.71	1.00
TB-CN	-1.00	-15.71	13.71	1.00
LM-KB	-31.40	-46.11	-16.69	**0.00**
RB-KB	-25.60	-40.31	-10.89	**0.00**
TB-KB	-24.60	-39.31	-9.89	**0.00**
RB-LM	5.80	-8.91	20.51	0.87
TB-LM	6.80	-7.91	21.51	0.76
TB-RB	1.00	-13.71	15.71	1.00

Table 4. Output of protected pair-wise difference tests comparing eelgrass shoot density between all 7 sites sampled in eastern NB in August 2013 (n=11).

Site	difference	lower	upper	p adjusted
BT-BSS	-1.3E+02	-206.92	-60.28	**0.00**
CN-BSS	-6.0E+01	-133.32	13.32	0.17
KB-BSS	-1.2E+02	-194.12	-47.48	**0.00**
LM-BSS	-6.3E+01	-136.52	10.12	0.13
RB-BSS	-1.2E+02	-194.12	-47.48	**0.00**
TB-BSS	-1.1E+02	-186.12	-39.48	**0.00**
CN-BT	7.4E+01	0.28	146.92	0.05
KB-BT	1.3E+01	-60.52	86.12	1.00
LM-BT	7.0E+01	-2.92	143.72	0.07
RB-BT	1.3E+01	-60.52	86.12	1.00
TB-BT	2.1E+01	-52.52	94.12	0.97
KB-CN	-6.1E+01	-134.12	12.52	0.16
LM-CN	-3.2E+00	-76.52	70.12	1.00
RB-CN	-6.1E+01	-134.12	12.52	0.16
TB-CN	-5.3E+01	-126.12	20.52	0.29
LM-KB	5.8E+01	-15.72	130.92	0.20
RB-KB	2.8E-14	-73.32	73.32	1.00
TB-KB	8.0E+00	-65.32	81.32	1.00
RB-LM	-5.8E+01	-130.92	15.72	0.20
TB-LM	-5.0E+01	-122.92	23.72	0.36
TB-RB	8.0E+00	-65.32	81.32	1.00

Table 5. Output of protected pair-wise difference tests comparing eelgrass AG wet weight (g/m^2) between all 7 sites sampled in eastern NB in August 2013 (n=6).

Site	difference	lower	upper	p adjusted
BT-BSS	97.69	-572.24	767.62	1.00
CN-BSS	-229.31	-899.24	440.62	0.93
KB-BSS	511.61	-158.32	1181.54	0.23
LM-BSS	1.35	-668.58	671.28	1.00
RB-BSS	54.36	-615.57	724.29	1.00
TB-BSS	56.92	-613.01	726.85	1.00
CN-BT	-327.00	-996.93	342.93	0.71
KB-BT	413.92	-256.01	1083.85	0.46
LM-BT	-96.34	-766.27	573.59	1.00
RB-BT	-43.33	-713.26	626.60	1.00
TB-BT	-40.78	-710.71	629.15	1.00
KB-CN	740.92	70.99	1410.85	**0.02**
LM-CN	230.66	-439.27	900.59	0.93
RB-CN	283.67	-386.26	953.60	0.83
TB-CN	286.23	-383.70	956.16	0.82
LM-KB	-510.26	-1180.19	159.67	0.23
RB-KB	-457.25	-1127.18	212.68	0.35
TB-KB	-454.69	-1124.62	215.24	0.35
RB-LM	53.01	-616.92	722.94	1.00
TB-LM	55.57	-614.36	725.50	1.00
TB-RB	2.55	-667.38	672.48	1.00

Table 6. Output of protected pair-wise difference tests comparing epiphyte algae % cover in eelgrass habitat between all 7 sites sampled in eastern NB in August 2013 (n=11).

Site	difference	lower	upper	p adjusted
BT-BSS	-0.05	-0.36	0.26	1.00
CN-BSS	0.13	-0.18	0.44	0.83
KB-BSS	-0.23	-0.54	0.09	0.28
LM-BSS	0.09	-0.22	0.41	0.96
RB-BSS	0.29	-0.02	0.60	0.08
TB-BSS	-0.29	-0.60	0.02	0.08
CN-BT	0.18	-0.13	0.49	0.55
KB-BT	-0.18	-0.49	0.13	0.55
LM-BT	0.14	-0.17	0.45	0.78
RB-BT	0.34	0.03	0.65	**0.03**
TB-BT	-0.24	-0.55	0.07	0.22
KB-CN	-0.36	-0.67	-0.05	**0.02**
LM-CN	-0.04	-0.35	0.28	1.00
RB-CN	0.16	-0.15	0.47	0.66
TB-CN	-0.42	-0.73	-0.11	**0.00**
LM-KB	0.32	0.01	0.63	**0.04**
RB-KB	0.52	0.21	0.83	**0.00**
TB-KB	-0.06	-0.38	0.25	1.00
RB-LM	0.20	-0.11	0.51	0.43
TB-LM	-0.38	-0.70	-0.07	**0.01**
TB-RB	-0.58	-0.89	-0.27	**0.00**

Table 7. Output of protected pair-wise difference tests comparing microphytobenthos concentration in eelgrass habitats between all 7 sites sampled in eastern NB in August 2013. (n=6)

Site	difference	lower	upper	p adjusted
BT-BSS	62.11	-123.06	247.28	0.93
CN-BSS	22.96	-162.21	208.13	1.00
KB-BSS	-66.34	-251.51	118.84	0.91
LM-BSS	96.81	-88.36	281.98	0.65
RB-BSS	90.76	-94.41	275.94	0.71
TB-BSS	-117.12	-302.29	68.05	0.43
CN-BT	-39.15	-224.32	146.02	0.99
KB-BT	-128.44	-313.61	56.73	0.33
LM-BT	34.71	-150.47	219.88	1.00
RB-BT	28.66	-156.51	213.83	1.00
TB-BT	-179.23	-364.40	5.95	**0.06**
KB-CN	-89.29	-274.46	95.88	0.73
LM-CN	73.85	-111.32	259.03	0.86
RB-CN	67.81	-117.36	252.98	0.90
TB-CN	-140.08	-325.25	45.09	0.24
LM-KB	163.15	-22.02	348.32	0.11
RB-KB	157.10	-28.07	342.27	0.14
TB-KB	-50.78	-235.96	134.39	0.97
RB-LM	-6.05	-191.22	179.12	1.00
TB-LM	-213.93	-399.10	-28.76	**0.02**
TB-RB	-207.88	-393.06	-22.71	**0.02**

Table 8. Output of protected pair-wise difference tests comparing sediment organic content (%) in eelgrass habitat between all 7 sites sampled in eastern NB in August 2013 (n=6).

Site	difference	lower	upper	p adjusted
BT-BSS	-0.02	-0.04	0.00	**0.01**
CN-BSS	-0.03	-0.04	-0.01	**0.00**
KB-BSS	-0.02	-0.04	0.00	**0.01**
LM-BSS	-0.02	-0.04	0.00	**0.04**
RB-BSS	-0.02	-0.04	-0.01	**0.00**
TB-BSS	0.02	0.00	0.04	**0.01**
CN-BT	0.00	-0.02	0.02	1.00
KB-BT	0.00	-0.02	0.02	1.00
LM-BT	0.00	-0.02	0.02	0.99
RB-BT	0.00	-0.02	0.02	1.00
TB-BT	0.05	0.03	0.07	**0.00**
KB-CN	0.00	-0.02	0.02	1.00
LM-CN	0.01	-0.01	0.02	0.95
RB-CN	0.00	-0.02	0.02	1.00
TB-CN	0.05	0.03	0.07	**0.00**
LM-KB	0.00	-0.02	0.02	1.00
RB-KB	0.00	-0.02	0.02	1.00
TB-KB	0.05	0.03	0.07	**0.00**
RB-LM	-0.01	-0.02	0.01	0.98
TB-LM	0.04	0.02	0.06	**0.00**
TB-RB	0.05	0.03	0.07	**0.00**

Table 9. Output of protected pair-wise difference tests comparing AG tissue N content (%) in eelgrass habitat between all 7 sites sampled in eastern NB in June, August, and October 2013 (n=12).

Site	difference	lower	upper	p adjusted
BT-BSS	0.46	0.24	0.69	**0.00**
CN-BSS	0.74	0.51	0.96	**0.00**
KB-BSS	0.36	0.14	0.59	**0.00**
LM-BSS	0.58	0.35	0.80	**0.00**
RB-BSS	0.23	0.00	0.45	**0.04**
TB-BSS	0.35	0.12	0.57	**0.00**
CN-BT	0.28	0.05	0.50	**0.01**
KB-BT	-0.10	-0.33	0.12	0.79
LM-BT	0.11	-0.11	0.34	0.68
RB-BT	-0.24	-0.46	-0.01	**0.04**
TB-BT	-0.12	-0.34	0.11	0.66
KB-CN	-0.38	-0.60	-0.15	**0.00**
LM-CN	-0.16	-0.39	0.06	0.29
RB-CN	-0.51	-0.74	-0.29	**0.00**
TB-CN	-0.39	-0.62	-0.17	**0.00**
LM-KB	0.21	-0.01	0.44	0.07
RB-KB	-0.13	-0.36	0.09	0.50
TB-KB	-0.02	-0.24	0.21	1.00
RB-LM	-0.35	-0.57	-0.12	**0.00**
TB-LM	-0.23	-0.45	0.00	**0.04**
TB-RB	0.12	-0.11	0.34	0.64

Table 10. Output of protected pair-wise difference tests comparing BG tissue N content (%) in eelgrass habitat between all 7 sites sampled in eastern NB in June, August, and October 2013 (n=12).

Site	difference	lower	upper	p adjusted
BT-BSS	0.11	-0.38	0.60	0.99
CN-BSS	-0.02	-0.50	0.47	1.00
KB-BSS	-0.14	-0.63	0.35	0.97
LM-BSS	-0.13	-0.62	0.36	0.97
RB-BSS	-0.11	-0.60	0.38	0.99
TB-BSS	-0.33	-0.82	0.16	0.36
CN-BT	-0.12	-0.61	0.36	0.98
KB-BT	-0.25	-0.73	0.24	0.69
LM-BT	-0.24	-0.73	0.25	0.71
RB-BT	-0.22	-0.71	0.27	0.78
TB-BT	-0.44	-0.92	0.05	0.11
KB-CN	-0.12	-0.61	0.37	0.98
LM-CN	-0.12	-0.61	0.37	0.99
RB-CN	-0.10	-0.59	0.39	1.00
TB-CN	-0.31	-0.80	0.18	0.42
LM-KB	0.00	-0.48	0.49	1.00
RB-KB	0.03	-0.46	0.51	1.00
TB-KB	-0.19	-0.68	0.30	0.88
RB-LM	0.02	-0.47	0.51	1.00
TB-LM	-0.19	-0.68	0.29	0.86
TB-RB	-0.21	-0.70	0.27	0.80

Table 11. Output of protected pair-wise difference tests comparing AG tissue C content (%) in eelgrass habitat between all 7 sites sampled in eastern NB in June, August, and October 2013 (n=12).

Site	difference	lower	upper	p adjusted
BT-BSS	-3.00	-11.32	5.32	0.91
CN-BSS	-1.24	-9.56	7.09	1.00
KB-BSS	1.15	-7.17	9.47	1.00
LM-BSS	-5.14	-13.46	3.18	0.46
RB-BSS	-4.00	-12.32	4.33	0.73
TB-BSS	-3.31	-11.63	5.01	0.86
CN-BT	1.77	-6.56	10.09	0.99
KB-BT	4.15	-4.17	12.48	0.69
LM-BT	-2.14	-10.46	6.19	0.98
RB-BT	-0.99	-9.31	7.33	1.00
TB-BT	-0.31	-8.63	8.02	1.00
KB-CN	2.39	-5.93	10.71	0.97
LM-CN	-3.90	-12.22	4.42	0.75
RB-CN	-2.76	-11.08	5.56	0.94
TB-CN	-2.07	-10.39	6.25	0.98
LM-KB	-6.29	-14.61	2.03	0.24
RB-KB	-5.15	-13.47	3.17	0.46
TB-KB	-4.46	-12.78	3.86	0.62
RB-LM	1.14	-7.18	9.46	1.00
TB-LM	1.83	-6.49	10.15	0.99
TB-RB	0.69	-7.63	9.01	1.00

Table 12. Output of protected pair-wise difference tests comparing AG N isotopes in eelgrass tissue between all 7 sites sampled in eastern NB in June, August, and October 2013 (n=12).

Site	difference	lower	upper	p adjusted
BT-BSS	1.19	0.47	1.90	**0.00**
CN-BSS	0.88	0.16	1.59	**0.01**
KB-BSS	2.34	1.62	3.05	**0.00**
LM-BSS	5.14	4.43	5.85	**0.00**
RB-BSS	1.95	1.23	2.66	**0.00**
TB-BSS	1.77	1.06	2.48	**0.00**
CN-BT	-0.31	-1.02	0.41	0.82
KB-BT	1.15	0.44	1.86	**0.00**
LM-BT	3.95	3.24	4.67	**0.00**
RB-BT	0.76	0.05	1.47	**0.03**
TB-BT	0.58	-0.13	1.30	0.17
KB-CN	1.46	0.74	2.17	**0.00**
LM-CN	4.26	3.55	4.97	**0.00**
RB-CN	1.07	0.35	1.78	**0.00**
TB-CN	0.89	0.18	1.60	**0.01**
LM-KB	2.80	2.09	3.52	**0.00**
RB-KB	-0.39	-1.10	0.32	0.60
TB-KB	-0.57	-1.28	0.15	0.19
RB-LM	-3.19	-3.91	-2.48	**0.00**
TB-LM	-3.37	-4.08	-2.66	**0.00**
TB-RB	-0.18	-0.89	0.54	0.98

Table 13. Output of protected pair-wise difference tests comparing BG N isotopes in eelgrass tissue between all 7 sites sampled in eastern NB in June, August, and October 2013 (n=12).

Site	difference	lower	upper	p adjusted
BT-BSS	-0.16	-1.13	0.80	1.00
CN-BSS	-0.34	-1.31	0.62	0.92
KB-BSS	0.66	-0.31	1.62	0.35
LM-BSS	3.82	2.86	4.79	**0.00**
RB-BSS	0.49	-0.48	1.45	0.69
TB-BSS	0.46	-0.50	1.43	0.73
CN-BT	-0.18	-1.15	0.78	1.00
KB-BT	0.82	-0.15	1.78	0.14
LM-BT	3.98	3.02	4.95	**0.00**
RB-BT	0.65	-0.32	1.61	0.37
TB-BT	0.62	-0.34	1.59	0.41
KB-CN	1.00	0.03	1.96	**0.04**
LM-CN	4.16	3.20	5.13	**0.00**
RB-CN	0.83	-0.14	1.79	0.13
TB-CN	0.80	-0.16	1.77	0.15
LM-KB	3.17	2.20	4.13	**0.00**
RB-KB	-0.17	-1.14	0.79	1.00
TB-KB	-0.19	-1.16	0.77	0.99
RB-LM	-3.34	-4.30	-2.37	**0.00**
TB-LM	-3.36	-4.33	-2.40	**0.00**
TB-RB	-0.02	-0.99	0.94	1.00

Table 14. Output of protected pair-wise difference tests comparing AG C isotopes in eelgrass tissue between all 7 sites sampled in eastern NB in June, August, and October 2013 (n=12).

Site	difference	lower	upper	p adjusted
BT-BSS	1.46	0.55	2.37	**0.00**
CN-BSS	1.29	0.39	2.20	**0.00**
KB-BSS	0.99	0.08	1.90	**0.03**
LM-BSS	1.87	0.96	2.78	**0.00**
RB-BSS	2.07	1.16	2.98	**0.00**
TB-BSS	-1.08	-1.99	-0.17	**0.01**
CN-BT	-0.16	-1.07	0.74	1.00
KB-BT	-0.47	-1.38	0.44	0.66
LM-BT	0.41	-0.50	1.32	0.78
RB-BT	0.61	-0.30	1.52	0.36
TB-BT	-2.54	-3.44	-1.63	**0.00**
KB-CN	-0.31	-1.21	0.60	0.93
LM-CN	0.57	-0.33	1.48	0.43
RB-CN	0.77	-0.13	1.68	0.13
TB-CN	-2.37	-3.28	-1.46	**0.00**
LM-KB	0.88	-0.03	1.79	0.06
RB-KB	1.08	0.17	1.99	**0.01**
TB-KB	-2.07	-2.97	-1.16	**0.00**
RB-LM	0.20	-0.71	1.11	0.99
TB-LM	-2.95	-3.85	-2.04	**0.00**
TB-RB	-3.15	-4.05	-2.24	**0.00**

Table 15. Output of protected pair-wise difference tests comparing BG C isotopes in eelgrass tissue between all 7 sites sampled in eastern NB in June, August, and October 2013 (n=12).

Site	difference	lower	upper	p adjusted
BT-BSS	1.03	-0.02	2.08	0.06
CN-BSS	0.80	-0.25	1.85	0.23
KB-BSS	-0.10	-1.15	0.95	1.00
LM-BSS	1.10	0.04	2.15	**0.04**
RB-BSS	0.83	-0.22	1.88	0.19
TB-BSS	-1.06	-2.11	-0.01	0.05
CN-BT	-0.23	-1.28	0.82	0.99
KB-BT	-1.13	-2.18	-0.08	**0.03**
LM-BT	0.06	-0.99	1.12	1.00
RB-BT	-0.20	-1.25	0.85	1.00
TB-BT	-2.09	-3.14	-1.04	**0.00**
KB-CN	-0.90	-1.95	0.15	0.13
LM-CN	0.29	-0.76	1.35	0.97
RB-CN	0.03	-1.02	1.08	1.00
TB-CN	-1.86	-2.91	-0.81	**0.00**
LM-KB	1.20	0.15	2.25	**0.02**
RB-KB	0.93	-0.12	1.99	0.11
TB-KB	-0.96	-2.01	0.09	0.09
RB-LM	-0.26	-1.31	0.79	0.98
TB-LM	-2.16	-3.21	-1.11	**0.00**
TB-RB	-1.89	-2.95	-0.84	**0.00**

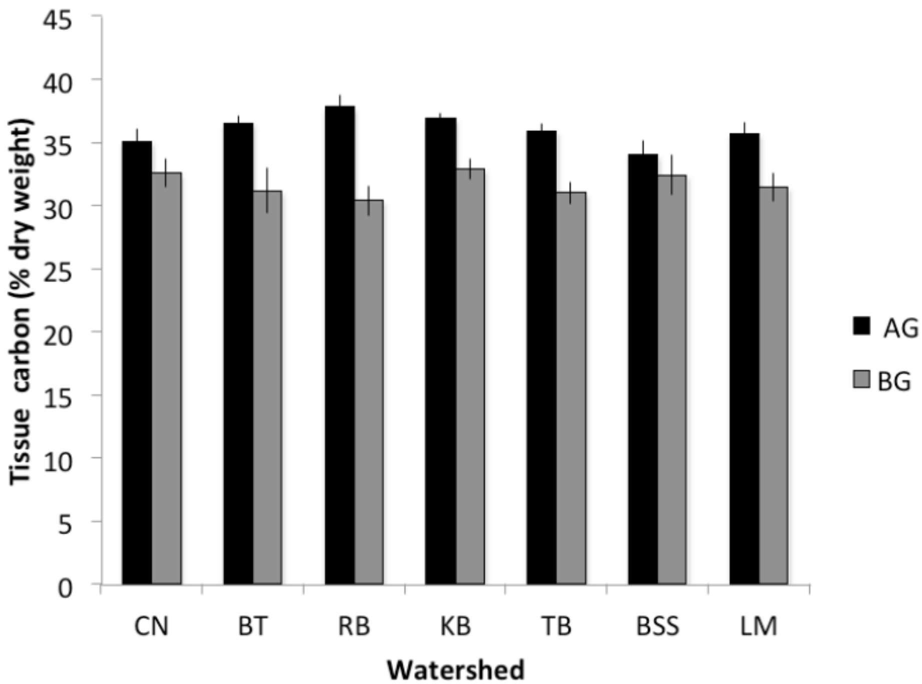


Figure 1. Above and Below ground carbon content of eelgrass tissue sampled in 7 bays in eastern New Brunswick in spring summer and fall of 2013. Average values are shown, ± Standard error . No significant differences between eelgrass tissue C from different bays was identified

Table 17. Results of *envfit* Analysis. The *envfit()* function is available through the package "*vegan*" in R. It illustrates the linear correlation between the site variables (left column) and the 2D nMDS ordination of eelgrass and eutrophic variables (eelgrass shoot density and CH, epiphytic and benthic algae % cover, microphytobenthos, and summer AG abd BG Tissue N, $\delta^{15}N$, $\delta^{13}C$). Significance levels: 0.05=*, 0.01=**, ≤0.001=***

	nMDS1	nMDS2	r2	Pr(>r)
N load (kgTDN yr^{-1})	-1.00	-0.07	0.05	0.398
N loading rate (kgTDN ha watershed^{-1}yr^{-1})	-0.89	0.45	0.31	0.002**
N loading rate (kgTDN ha bay^{-1}yr^{-1})	-0.65	0.76	**0.82**	**0.001*** **
N loading rate (kgTDN m^{3}bay^{-1}yr^{-1})	-0.01	1.00	0.13	0.081
Δ-N (mgL^{-1})	-0.44	0.90	0.33	0.001***
Flushing time (h)	-0.83	0.56	**0.31**	**0.004** **
Sampling depth (m)	-1.00	-0.04	0.43	0.001***
Aquaculture active lease (ha)	0.11	-0.99	**0.50**	**0.001*** **
Aquaculture bag count (bags bay^{-1})	-0.16	-0.99	0.15	0.052
Aquaculture stocking density (bags ha bay^{-1})	0.21	-0.98	**0.69**	**0.001*** **
Aquaculture stocking density (bags ha lease^{-1})	0.37	-0.93	0.11	0.133