

Land use and nitrogen loading in seven estuaries along the southern Gulf of St. Lawrence, Canada



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ABSTRACT

Nitrogen loading from coastal watersheds is a principal factor associated with the decline in eelgrass bed health and cover in estuaries worldwide. We apply the Nitrogen Loading Model (NLM) framework developed in Waquoit Bay, Massachusetts to 7 estuaries in eastern New Brunswick. Using watershed-specific information on human population, wastewater production, atmospheric deposition, and land use in each watershed we estimate annual input of Total Dissolved Nitrogen (TDN) from point and non-point sources. We also estimate flushing time of each estuary using available hydrodynamic and bathymetric data incorporated in a tidal prism model. Finally, we validate the NLM results by testing the link between estimated nitrogen loading, flushing time and nitrogen signals in eelgrass tissue including nitrogen content and stable isotopes. Overall, total nitrogen load (kg TDN yr^{-1}) was strongly dependent on watershed and estuary size, while loading rate per unit watershed area (yield) was linked to watershed population density. Atmospheric deposition was the largest contributor of nitrogen to all estuaries except one, where seafood processing effluent was the greatest source. Stable isotope analysis of eelgrass tissue reflected this distinction, with high $\delta^{15}\text{N}$ values of 8–10‰ related to high wastewater loading, compared to 2–6.5‰ in the other estuaries that receive proportionally more atmospheric deposition. Tissue nitrogen content was positively related to nitrogen yields and loading rate per volume of estuary, highlighting the influence of variable watershed:estuary size ratio. Multiple regression analysis identified a significant interaction between nitrogen yield and flushing time on eelgrass tissue nitrogen content and isotopes, pointing to the mitigating effect an estuary's quick flushing time can have on the expression of nitrogen enrichment in primary producers. The compilation of new information on nitrogen loading to east Canadian estuaries is a novel contribution from a region where human influences are still at a relatively low level, and hence will add to existing information from cold temperate, mainly forested watershed–estuary environments.

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1. Introduction

Anthropogenic nitrogen loading from coastal watersheds is one of the most influential degraders of macrophyte habitats in receiving estuaries worldwide (Hauxwell et al., 2003; Lotze et al., 2006; 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 (Nixon and Pilson, 1983; Bowen and Valiela, 2001a; Bricker et al., 2007, 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 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 (Short et al., 2011; Schmidt et al., 2012). Yet the susceptibility to algal blooms and eutrophication can also be significantly influenced by the residence time of water within the bay or estuary (Valiela et al., 1997b; Ferreira et al., 2005). More extensive distributions, and more frequent episodes of eutrophic concentrations of chlorophyll a and harmful algal blooms have been shown to occur in systems that have longer flushing and residence times (Monbet, 1992;

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Ferreira et al., 2005; Bricker et al., 2007, 2008; Latimer and Charpentier, 2010).

Eelgrass tissue has been shown to be effective at integrating both higher ambient nitrogen concentrations (Nixon and Pilson, 1983; Hemminga and Duarte, 2000), and the stable isotope (SI) signatures (the ratio of $\delta^{15}\text{N}/\delta^{14}\text{N}$) of the dominant sources of nitrogen entering the system (Valiela et al., 1997b; McClelland and Valiela, 1998; Middelburg and Nieuwenhuize, 2001; Xue et al., 2009; Schubert et al., 2013). Generally, wastewater from human or animal wastes has a higher SI signature than nitrogen sourced from synthetic agricultural fertilizer, atmospheric deposition, or un-impacted groundwater (Lepoint et al., 2004; Cole et al., 2006; Schubert et al., 2013).

Eelgrass has been classified as an Ecologically Significant Species (ESS) in Atlantic Canada for its unique role in providing essential habitat for many associated species and sediment stabilization in soft-bottom bays and estuaries (DFO, 2009, 2011). In eelgrass dominated estuaries in eastern New Brunswick, both point and non-point sources of anthropogenic nitrogen have been identified as potential contributors to excessive nitrogen loading (Lotze et al., 2003; Plante and Courtenay, 2008; Therriault et al., 2008) and resulting eutrophication symptoms (Schmidt et al., 2012). Yet there remains a lack of specific and quantifiable information regarding the sources and magnitude of nutrient loading in different estuaries. Biologically verified quantitative estimates of the nitrogen loading, however, are necessary to develop management strategies for coastal habitat conservation and maintenance (Lajtha et al., 1995). Furthermore, estimates of the relative contribution of nitrogen from different human activities allow local and regional managers to make effective decisions regarding how to best manage nitrogen loading (Johnes, 1996).

The purpose of this research was to apply a Nitrogen Loading Model (NLM) framework (Valiela et al., 1997a, 2000) to a selection of watersheds and associated estuaries in eastern New Brunswick of various sizes, characteristics and human activities to estimate nitrogen loading from both point and non-point sources entering each estuary per year. To account for the mitigating effect of tidal flushing, we also calculated flushing time using a tidal prism model (Gregory et al., 1993; Grant et al., 2005). Finally, to validate the model results in the field we measured the nitrogen content and isotopic signatures of eelgrass tissue in each receiving estuary and determined their relationship with NLM and flushing time estimates. Overall, our results provide insight into the magnitude and sources of nitrogen loading in a region of relatively low human impact yet increasing signs of estuarine eutrophication.

2. Methods

2.1. Study area

This study focuses on seven watersheds and estuaries along the eastern coast of New Brunswick (Fig. 1), a temperate region experiencing modified continental climate (Koutitonsky et al., 2004). This region is characterized by semi-regular diurnal tides, although inconsistencies to this pattern are common (Dutil et al., 2012). Along the New Brunswick coast, surface water velocities are slow ($0.06\text{--}0.02\text{ m s}^{-1}$), and the tidal current ranges from $>0.12\text{--}0.04\text{ m s}^{-1}$ (Koutitonsky et al., 2004; Dutil et al., 2012). In each watershed, one or two main rivers drain into the bay except in Baie St. Simon Sud, 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 estuaries (Patriquin and Butler, 1976; Thibault et al.,

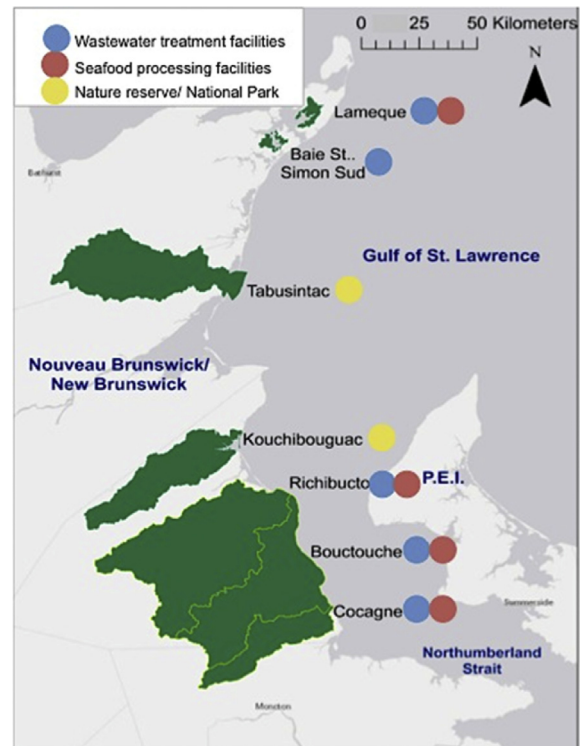


Fig. 1. Map of seven watersheds in eastern New Brunswick for which a nitrogen loading model (NLM) was developed and ground-truthed in the associated estuaries. Image created using ArcGIS (ESRI, 2011).

2000). There are small urban centers (generally <5000 persons) throughout the region, but predominantly the populations are rural and coastal (Table 1). Between 2011 and 2014, bivalve aquaculture (primarily the American oyster *Crassostrea virginica*) was active in all estuaries except for Kouchibouguac (National Park) and Lamèque.

2.2. NLM selection

We use the framework of the Waquoit Bay Land Margin Ecosystem Research (WBLMER) NLM originally constructed and field validated for Waquoit Bay, Massachusetts (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 this NLM because: 1) it is applicable to coastal watersheds underlain by unconsolidated coarse-grained sediments, where the delivery of nutrients to receiving waters is primarily via groundwater flow, and land cover is mostly forested, residential, and agricultural (Valiela et al., 1997a), as in our study area (Rivard et al., 2008; Pronk and Allard, 2013); 2) it has been applied to numerous watersheds in the northeastern United States and been compared with good agreement to results from other nutrient loading models (Valiela et al., 1997a, 2000; Latimer and Charpentier, 2010); 3) it consists of straightforward additive formulas to calculate the load (kg) of TDN that enters an estuary 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 accessible or can be retrieved from municipal and provincial government sources; 5) in the absence of direct measurements of nitrogen contribution from individual sources, a model, such as the NLM, is vital for developing management strategies.

Table 1
Characteristics of watersheds and estuaries used in this application of the NLM.

Site	Watershed area (ha)	Estuary area (ha)	Watershed: Estuary ratio	Estuary volume ($1 \times 10^7 \text{ m}^3$)	Population (persons)	Pop. density (persons ha^{-1})
Cocagne	33,246	2438	13.6	2.8	12,041	0.36
Bouctouche	76,032	3813	19.9	4.2	25,868	0.34
Richibucto	128,578	5118	25.1	1.1	20,693	0.16
Kouchibouguac	53,042	1458	36.4	2.2	3368	0.06
Tabusintac	71,276	3666	19.4	4.0	5015	0.07
Baie St. Simon Sud	2157	833	2.6	1.3	743	0.34
Lamèque	3241	1077	3.0	3.3	2274	0.70

We ask readers to distinguish our results to those produced from the Estuarine Loading Model, which produces estimates of DIN to estuaries and has been applied in comparable geographies of United States (Valiela et al., 2004).

2.3. NLM application

In our application of the NLM the amount of TDN entering each estuary is predicted by a) calculating the amount of nitrogen entering the estuary directly from both point sources (municipal wastewater treatment facilities (MWWT), seafood processing plants, peat harvesting, atmospheric deposition on estuary 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 nitrogen from non-point sources lost in the terrestrial surface layer, vadose zone, and aquifer while moving through the watershed. The amount of nitrogen 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 infrastructure). The full model design and rationale of loss parameters are described in detail in Valiela et al. (1997a). In the following (and Appendix A1), we only explain any additions or revisions we made to the NLM framework to make it directly applicable to our study region as well as noting where and how we sourced regional data.

2.3.1. Watershed and estuary delineation and land use cover

We used New Brunswick's provincial digital geographic database (GeoNB) to determine the watershed boundaries and area (ha) for 4 study sites (Cocagne, Bouctouche, Richibucto, Tabusintac) as they sufficiently contained all freshwater inputs to each respective estuary (Table 1). Three watersheds (Kouchibouguac, Baie St. Simon Sud, Lamèque) needed further subdivision: we used surface contours from a provincial Digital Terrain Model, GeoNB watercourse data, and the ArcGIS 'ArcHydro' toolbox (ESRI, 2011, 2013; GeoNB, 2012) to create an elevation model predicting the drainage areas around these three estuaries. Although water table contours are more desirable for this, they were not available to us. Next, we used the GeoNB hydrographic data to get the surface area for all 7 estuaries (Table 1) and included only portions of the estuary with the designation of 'Tidal water body' (ESRI, 2011; GeoNB, 2012). The surface area was assumed to present the water body at high-tide (G. Gaudet, NB DNR, pers. comm.). We calculated estuary volume by multiplying surface area by average depth estimates for each estuary (Patriquin and Butler, 1976; Gregory et al., 1993; Plante and Courtenay, 2008; Robichaud and Doiron, 2011). The two smallest watersheds, Baie St. Simon Sud and Lamèque (Fig. 1) have a notably smaller watershed:estuary ratio compared to the 5 larger watersheds (Table 1).

Digital land cover and land use data that were verified between 2002 and 2012 (G. Gaudet, NB DNR, pers. comm.; NB DRN 2012) were used to extract information on the area of forest (provincially harvested and non-harvested), wetlands, peat lands (harvested and

non-harvested), settlement, recreation, industrial, infrastructure, and agricultural areas. Moreover, Irving Canada provided us with commercial forestry freehold land (referred to as commercial forest) 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 (Figure A.2). Watershed population estimates obtained from civic address data (GeoNB, 2012) and Statistics Canada (2014) household data (Table 1, Table A.5).

2.3.2. Point sources

Point sources of nitrogen include those with a direct entry point into the coastal water body, including MWWT outfalls, seafood processing plants, and peat harvest drainage. For these, no loss parameters are included in the calculations, as nitrogen concentrations are measured from effluent exiting treatment lagoons or sedimentation ponds.

MWWT and seafood processing effluent was calculated with data on nitrogen concentration in effluent, the effluent flow rate, and the number of days each facility operated each year (Table 2: Eqs. 1–2, Table A.1, A.2). Effluent discharge rate and nitrogen content for both MWWT and seafood processing facilities were obtained from the New Brunswick Department of Environment and Local Government (F. LeBlanc, pers. comm., DELG, 2012).

We added the contribution of nitrogen from peat harvesting operations to the NLM framework, as this is an important industry in this region. Despite drainage systems and settling ponds that prevent direct flow of runoff into waterways, peat harvesting can contribute significant accumulated nutrient and sediment quantities to receiving water bodies during the preparation of bogs into a harvestable resource (Appendix A.1, Klove, 2001; St. Hilaire et al., 2004, Waddington et al., 2009). We consider peat harvesting a point source as the nitrogen rich drainage water is directed into holding ponds and then into the receiving estuary from a single outfall or drainage ditch (often via a stream or river) (St. Hilaire et al., 2004, Ouellette et al., 2006, Waddington et al., 2009). We used literature data for nitrogen concentration in effluent leaving settling ponds, an average surface water runoff coefficient from peat harvest operations within North America (as there was not one for the region), and federally monitored precipitation data for each watershed (Klove, 2001; Joensuu et al., 2002; Swystun et al., 2013, Table 2: Eq. 2, Appendix A.1, Table A.3, A.4).

2.3.3. Non-point sources

Nitrogen from non-point sources (septic systems, agriculture and turf fertilizer, atmospheric deposition) enters the coastal water body primarily through groundwater transport. Exceptions to this would occur during heavy rainfall events or spring melting, which is not explicitly accounted for in this model. We applied loss parameters supplied by the WBLEMR NLM (see Valiela et al., 1997a, 2000) to represent the loss of nitrogen due to retention, dilution

Table 2List of equations used in this application of the NLM. Input data and more in depth methodology can be found in [Appendix A](#) of the supplementary material.

Point Sources	
MWWT and seafood effluent	Eq.1: $TDN \text{ (kg/yr)} = \text{Effluent flow rate (L/day)} \times \text{TDN concentration in effluent (kg/L)} \times \text{Days/yr in operation.}$
Peat harvesting	Eq.2: $TDN \text{ (kg/yr)} = 0.3642 \text{ (runoff coefficient)} \times \text{Annual precipitation (L/yr)} \times 0.372 \text{ (Concentration of nitrogen in water draining from sedimentation ponds and buffer zone (kg/L))}$
Non-point sources	
Septic system	Eq. 3: $TDN \text{ (kg/yr)} = 4.19 \text{ kg TDN/person/yr} \times \text{Average persons/house} \times \text{Number of houses} > 200 \text{ m} \times 0.60 \text{ (proportion not lost in septic and leaching field)} \times 0.66 \text{ (proportion not lost in plumes, reaching aquifer)} \times 0.65 \text{ (proportion not lost in aquifer, reaching seepage face)}^a$
Agricultural fertilizer	Eq. 4: $TDN \text{ (kg/ha/yr)} = (\text{Applied synthetic fertilizer kg nitrogen} \times 0.61) \times (\text{Area of synthetic fertilizer use (ha)})$
Turf/lawn fertilizer	Eq. 5: $TDN \text{ (kg applied/ha/yr)} = (150 \text{ kg TDN/ha/yr}) \times (\text{Settlement area (ha)} \times 0.3) \times 0.368 \text{ (proportion of settlement area where fertilizer use is reported)} \times 0.61 \text{ (proportion of fertilizer remaining after volatilization)}$
Sum of fertilizer application	Eq. 6: $TDN \text{ (kg/yr)} = (\text{Agricultural fertilizer (kg TDN/yr)} + \text{turf/lawn fertilizer (kgTDN/yr)}) \times 0.61 \text{ (proportion of nitrogen reaching vadose zone)} \times 0.39 \text{ (proportion of nitrogen reaching the aquifer)} \times 0.65 \text{ (proportion not lost in aquifer, reaching seepage face)}$
Indirect atmospheric deposition	Eq. 7: $TDN \text{ (kg/yr)} = \text{Dry or wet deposition (kg TDN/yr)} \times \text{proportion of nitrogen reaching vadose zone (depends on surface type)} \times 0.39 \text{ (proportion of nitrogen reaching the aquifer)} \times 0.65 \text{ (proportion not lost in aquifer, reaching seepage face)}$
Direct atmospheric deposition	Eq. 8: $TDN \text{ (kg/yr)} = \text{Dry deposition (kg TDN/yr)} + \text{wet deposition (kg TDN/yr)}$
Output equations	
NLM- Total nitrogen loading and loading rates	Eq. 9: $\text{Total Loading (kg TDN/yr)} = \text{Sum of point and non-point contributions of TDN}$ Eq. 10: $\text{yield} = (\text{kg TDN/yr})/\text{watershed area (ha)}$ Eq. 11: $\text{loading rate per estuary area} = (\text{kg TDN/yr})/\text{estuary surface area (ha)}$ Eq. 12: $\text{loading rate per estuary volume} = (\text{kg TDN/yr})/\text{estuary volume (m}^3\text{)}$ Eq. 13: $\text{flushing time normalized loading rate per estuary area} = \text{kg TDN/estuary surface area (ha)/yr} \times (1/\text{flushing time (h}^b\text{)})$ Eq. 14: $\text{flushing time normalized loading rate per estuary volume} = \text{kg TDN/estuary volume (m}^3\text{)/yr} \times (1/\text{flushing time (h}^b\text{)})$

^a For houses <200 m from shoreline, don't apply last loss parameter (assume no retention of nitrogen in aquifer).

^b Hours were converted to years (1/hr \times 24hr/1 day \times 365 d/1 yr) so units were common within equation.

and transformation as it traverses the vegetative layer, vadose zone (saturated soil layer), and the aquifer (Table 2: Eqs. (3)–(12)). See Valiela et al. (1997a, 2000) for full description and underlying assumptions of loss parameters.

Septic system nitrogen contribution was calculated using the same inputs as the original model, including the United States Environmental Protection Agency estimate of 4.9 kg nitrogen person⁻¹ yr⁻¹ (Table 2: Eq. (3), Valiela et al., 1997a; USEPA, 2002). We estimated the number of people in a watershed by multiplying the number of civic addresses (residential, commercial, institutional and industrial buildings identification code) in a watershed (ESRI, 2011; NB DNR, 2012) by the average number of persons per household in that region (Statistics Canada, 2011, 2014). Civic addresses within 200 m of a coastal or fresh-water way, which do not have the parameter for nitrogen loss in the aquifer applied to their inputs (Valiela et al., 1997a), were distinguished and enumerated using the buffer tool in ArcMap (Appendix A.1, ESRI, 2011). Approximately 10% of all civic addresses in each watershed are only used for half the year due to tourism or seasonal dwelling (Statistics Canada, 2014; NB Tourism 2012, 2014). We multiply nitrogen contribution from 10% of civic addresses in each watershed by 0.5 to account for this (Table A.5).

To determine nitrogen loading from fertilizer use, we estimated the sum of fertilizer applied to agriculture (Table 2: Eq. (4)) and to turf/lawns (Table 2: Eq. (5)) in each watershed. We assumed agricultural and turf/lawn fertilizers are subject to volatilization rates up to 39% and we apply this loss in addition to the loss parameters noted above for all non-point sources (Valiela et al., 1997a; Latimer and Charpentier, 2010). To estimate agricultural fertilizer nitrogen input we took the average recommended fertilizer amount for all crop types in New Brunswick (Statistics Canada, 2011) and multiplied this by the area in each watershed that reports synthetic fertilizer application for agriculture purposes (Appendix A.1, Table A.6–7, Statistics Canada, 2011). For turf and lawn fertilizer addition we multiplied the proportion of properties in New Brunswick that have a lawn/garden and report fertilizer application (0.368), by the total settlement area in each watershed (Appendix A.1, GeoNB, 2012; Google maps 2014, Statistics Canada, 2012). We then multiplied this area by the New Brunswick recommended

fertilizer application guidelines of 150 kg TDN ha⁻¹ yr⁻¹ (Table 2: Eq. (5), Table A.8, NBDA, 1989, 2001).

We used weekly reports from the Canadian National Atmospheric Chemistry Database sites in eastern New Brunswick to get average wet deposition rates dissolved inorganic nitrogen (DIN), sum of nitrate (NO₃⁻) and ammonium (NH₄) between 1992 and 2008 (Figure A.1, Table A.9, NATChem, 2012). We adjusted precipitation and nitrogen deposition estimates according to regional rates of evapotranspiration, which reduces the amount of nitrogen available for both overland and groundwater transport (Shiau, 1968; Lajtha et al., 1995). No monitoring sites in New Brunswick measure dissolved organic nitrogen (DON) deposition: 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, and DIN comprises the remaining 70% (Valigura et al., 2001; Valiela et al., 2004; Latimer and Charpentier, 2010). Similarly, there are no regional velocity deposition estimates for particulate aerosol nitrogen for Atlantic Canada: we used a 1:1 ratio for wet to dry DIN deposition on terrestrial surfaces based on published literature for dry deposition rates in New England and Eastern Canada (Valigura et al., 2001; Castro and Driscoll, 2002; Valiela et al., 2004; Castro et al., 2013). 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 NO₃⁻ and NH₄ measured at the NatChem stations (Table A.9).

We applied loss parameters to nitrogen deposited on the watershed surface from wet and dry deposition (Table 2: Eq. (7)). We used the compiled information on land use to apply appropriate loss parameters to atmospheric nitrogen at the surface layer, accounting for different sequestration rates of different surface types (e.g. natural cover attenuates more nitrogen than surfaces like parking lots or agricultural land, see 2.3.1 above, Appendix A.1, Figure A.2, Valiela et al., 1997a). Nitrogen that has leached through the surface/vegetative layer is then subject to loss/transformation in the unsaturated zone and the aquifer like other diffuse nitrogen sources (Table 2: Eq. (7), Table A.9).

Direct atmospheric deposition required no loss parameters in the model, as all nitrogen is assumed as subject to removal through

flushing, transformation, or biological uptake (Table 2: Eq. (8)). We assumed that rates of wet deposition of NO_3 , NH_4 , and inferred DON (see above) on the estuary surface were equal to rates on land (Valiela et al., 2004). In line with previous research we reduced the rate of DIN direct dry deposition to 70% of indirect dry deposition to account for the decreased surface area of the water surface relative to land cover (Table A.9, Valigura et al., 2001; Castro and Driscoll, 2002). Similar to indirect dry deposition, we did not include DON in the quantification of direct dry deposition.

2.3.4. NLM output calculations

Once applicable loss parameters were applied to all non-point sources (Table 2: Eqs. (3), (4), (6) and (7)) the volume of TDN from all point and non-point sources within a watershed was summed to produce an estimate for the cumulative amount of nitrogen entering each estuary annually (Table 2: Eq. (9)). Loading rates were calculated by dividing the total load by the respective area or volume measure (Eqs. (10)–(12), Table 2, Patriquin and Butler, 1976; Gregory et al., 1993; Plante and Courtenay, 2008; Robichaud and Doiron, 2011). In addition, loading rates per area and per volume of estuary were normalized with estimated flushing times (Eqs. (13)–(14), Table 2).

The error estimates shown in Fig. 2a are the maximum and minimum loading given the variation in all sources for which we were able to obtain effluent concentration or precipitation data from multiple years (atmospheric deposition, MWWT, seafood processing plants, peat harvesting). Other estimates of nitrogen 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 and we were not able to calculate a range in loading for these sources.

2.4. Tidal flushing time

We used methods specified by Gregory et al. (1993) and refined in Grant et al. (2005) to calculate flushing time (t_f), defined as the time required to reduce the concentration of a tracer throughout an estuary to $1/e$ of its initial concentration (Eq. (15), Dame 1996). All data incorporated in the tidal flushing estimates were gathered from the Department of Fisheries and Oceans tidal database or from literature specific to this region.

$$t_f = -1\omega \left[\ln \left(\frac{(V_T^*)}{(V_T + V_P + V_{FW})} \right) \right]^{-1} \quad (15)$$

* 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 ω is the time of the tidal cycle (h), V_T is the total volume of the estuary (m^3), V_P is the volume of the mean tidal prism (m^3), and V_{FW} is the volume of freshwater inflow to the estuary during the period of ω (m^3). Mean tidal volume (V_T) was calculated using a simple tidal prism, incorporating the mean tidal range and surface area of each estuary (Table A.10) (Gregory et al., 1993; Dutil et al., 2012; NB DNR, 2012). Generally, semidiurnal tides have a consistent ω (~12.42 h), but in this region of the southern Gulf of St. Lawrence tides are mostly mixed semi-diurnal with one of the 2 daily flood tides being smaller than the other, thereby increasing the time needed to replace the tidal prism (V_P) to >12.42 h. To calculate a more accurate estimate for ω we used curves for tidal levels over 21 consecutive days and summed the hourly volumetric increase of each flood tide (~2 each day) then divided by the number of mean tidal volumes (V_T) that could be exchanged in that volume. We included flushing owing to a river input for all estuaries

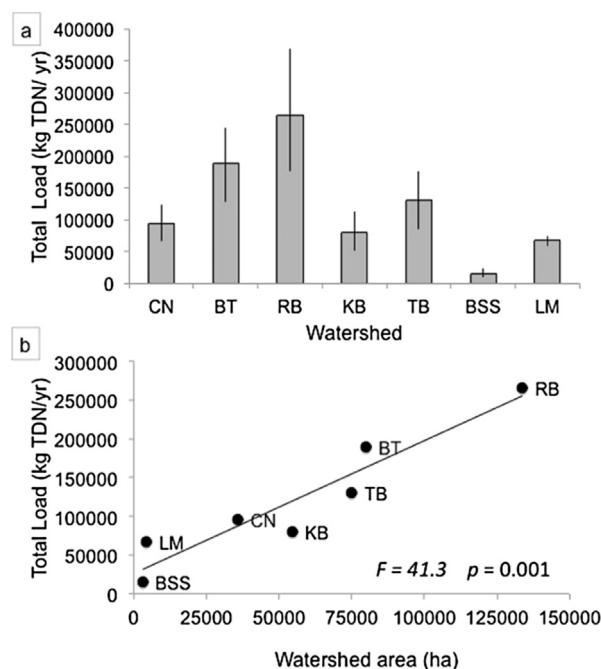


Fig. 2. a) Mean total dissolved nitrogen (TDN) load to each estuary over one year. Error bars represent the minimum and maximum loading given the variability in precipitation, evapotranspiration, freshwater recharge, and concentration of nitrogen in MWWT and seafood effluent; b) Relationship between watershed size and total nitrogen load (CN = Cocagne, BT = Bouctouche, RB = Richibucto, KB = Kouchibouguac, TB = Tabusintac, BSS = Baie St. Simon Sud, LM = Lamèque).

except Baie St. Simon Sud, which lacks a true riverine source of freshwater. Average annual river discharge was calculated from in situ Environment Canada loggers (Table A.11, Strain and Yeats, 1999). Assumptions of this model include that the incoming tidal water and estuarine water mix completely on each flood tide, and that the same volume is exported permanently on the following ebb tide.

2.5. Sampling and processing of field data

Six replicate eelgrass tissue samples were collected in 0.0365 m^2 sediment cores in 6 predefined quadrats laid in a 400 m^2 (50 m \times 4 m) area of continuous eelgrass parallel to shore in all seven estuaries in Summer (July 29th–August 12th) 2013 at long-term sampling locations (Lotze et al., 2003; Schmidt et al., 2012; see McIver, 2015 for full sampling methods). All tissue samples were rinsed, placed in labeled bags, and stored on ice until returned to the lab, where they were stored in a fridge (4 °C) for a maximum of 7 days until processed.

In the lab, roots/rhizomes were separated from the shoots and these above and below-ground components were treated individually for the rest of the analysis. We removed all epiphytic algae and invertebrates with freshwater and a razor blade. Cleaned tissue was dried in the oven at 80 °C for 48 h. 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 sent to the University of California Davis Stable Isotope facility for analysis of % tissue nitrogen and nitrogen isotopes ($\delta^{15}\text{N}$).

2.6. Linking NLM to field data

We both qualitatively and quantitatively compared proportions of loading from human/animal derived wastewater (MWWT,

seafood processing, septic systems), fertilizer applications (agricultural, and turf/lawn use), and atmospheric deposition (sum of indirect and direct) to $\delta^{15}\text{N}$ values in above- and below-ground eelgrass tissue ($n = 6/\text{site}$). For the quantitative comparison we used simple linear regression to assess the relationship between the mass of wastewater loading per area estuary, and $\delta^{15}\text{N}$: if wastewater loading is high and a dominant source of loading we would expect to see a higher $\delta^{15}\text{N}$ signature in eelgrass tissues from the receiving estuary (Valiela et al., 1997b; McClelland and Valiela, 1998). Additionally, we used linear regression to assess the relationship between NLM estimates (total nitrogen load, nitrogen yield, nitrogen loading rates) with the nitrogen content (%) in above and below-ground eelgrass tissue ($n = 6/\text{site}$). Furthermore, we integrated loading rates and flushing times by normalizing nitrogen loading rates (kg N m^{-3} estuary yr^{-1} , and kg N ha^{-1} estuary yr^{-1}) with estimated flushing time (hours, adjusted to years to be consistent in equation), and related these normalized rates to % nitrogen in above- and below-ground eelgrass tissue (Table 2: Eqs. (13)–(14)). Lastly, we used multiple linear regression to test whether nitrogen yield or loading rates interact with flushing times in explaining the variance in nitrogen content of eelgrass tissues. 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.

3. Results

3.1. Land use patterns

The five larger watersheds in the southern portion of the study region (Fig. 1) are mainly covered by forest, wetland, and natural scrubland (83–96% of watershed surface). 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 $\leq 16\%$ of the overall watershed size. Throughout all watersheds, agricultural land does not comprise more than 11% of land-use, and is less than 1% of watershed surface area in 2 watersheds. In contrast to the larger watersheds, the smaller, northern watersheds have higher population densities, less natural land cover (49–50%), and more land dedicated to peat harvesting, agriculture and settlement (Figure A.2). Pastureland accounts for 0–60% of agricultural land, and synthetic fertilizer is applied to 10–100% of agricultural lands depending on the watershed (Statistics Canada, 2011). We estimate that lawn and turf fertilizer (non-agricultural) is applied to less than 4% of total watershed area (Table A.7, Figure A.2).

3.2. Predicted nitrogen loading and flushing time

Estimated annual TDN loads (kg yr^{-1}) are highest in the largest watersheds (Richibucto, Bouctouche), reflecting the positive relationship between watershed size and total nitrogen loading

(Table 3, Fig. 2). This relationship highlights the high contribution of nitrogen from atmospheric deposition that is ubiquitous across the region (Fig. 3a, Table A.9).

In contrast, the higher nitrogen yields ($\text{kg TDN ha watershed}^{-1} \text{ yr}^{-1}$) and loading rates per ha estuary reflect anthropogenic nitrogen contributions additional to atmospheric deposition, namely point sources and wastewater contributions (Fig. 3a, Table 3). This is evidenced by the strong positive correlation between nitrogen yields and population density throughout the study region (Fig. 3b). The highest estimated nitrogen yield is to the Lamèque estuary, which receives the majority of its annual nitrogen input from seafood processing effluent (Fig. 3a). Here, the nitrogen yield is more than double that of the other watersheds assessed, and is more than $10\times$ higher than nitrogen yields from the watersheds with protected natural areas (Kouchibouguac and Tabusintac, Table 3).

Human derived wastewater (MWWT and septic systems) contributes 10–17% of total nitrogen loading in watersheds with both these disposal treatments in place. In the watersheds with only septic systems (and no real urban settlements), nitrogen from human wastewater contributes less than 6% of total nitrogen loading (Fig. 3a). Loading from septic systems is higher where there are higher population densities, particularly where many civic addresses are within 200 m of the shoreline (Cocagne, Bouctouche, Richibucto, Table A.5). The high proportion of indirect atmospheric deposition and small contributions from point sources, septic systems, and fertilizer additions reflect the large amount of forest and wetland cover and low population densities in the Kouchibouguac and Tabusintac watersheds. The contribution from peat harvesting is minimal in most watersheds throughout the region ($<2\%$), with the exception of Baie St. Simon Sud, where more of the natural peat land has been exploited: here peat harvesting contributes an estimated 13% to the total annual nitrogen load (Fig. 3a).

Estuary size is also important to consider in terms of nitrogen loading rates in the estuaries which have small watershed:estuary area ratios: Direct atmospheric deposition contributes a larger proportion of nitrogen to estuaries where the watershed:estuary area ratio is small (Table 1, Fig. 3a). Specifically, Baie St. Simon Sud has the second highest estimated nitrogen yield of the 7 estuaries. However, this is because we include direct atmospheric deposition in our nitrogen yield estimates, and it is the largest source of nitrogen to this estuary. When nitrogen loading for Baie St. Simon Sud is put in the context of estuary surface area, the loading rate in this estuary is the smallest of all the estuaries assessed (Table 3).

Adjusting ω based on the measured flood tide amplitude at each (or adjacent) tidal inlet increased it from the regular semi-diurnal (12.42 h) to between 26 and 37 h, and was therefore an important adjustment. The three estuaries in the northern part of the Strait (Tabusintac, Baie St. Simon Sud, and Lamèque) had faster cycles than the four estuaries in the southern portion of the strait, likely due to the faster slightly higher average tidal amplitude in this northern part of the Strait (Dutil et al., 2012). However,

Table 3
Estimated total nitrogen (TDN) load per year and nitrogen loading rates per area of watershed (yield), area of estuary surface, and volume of estuary (\pm range of loading given range in atmospheric deposition, freshwater recharge, MWWT and seafood processing). Flushing time and freshwater recharge for each estuary are also shown. See input data in Appendix A.

	Cocagne	Bouctouche	Richibucto	Kouchi-bouguac	Tabu-sintac	Baie St. Simon Sud	Lamèque
Total load (kg TDN yr^{-1})	94,562 \pm 29,028	188,324 \pm 58,724	265,027 \pm 96,749	79,795 \pm 30,841	130,624 \pm 45,466	15,772 \pm 6803	67,223 \pm 7632
Yield ($\text{kg TDN h watershed}^{-1} \text{ yr}^{-1}$)	2.84 \pm 0.87	2.47 \pm 0.77	2.06 \pm 0.75	1.50 \pm 0.58	1.83 \pm 0.64	7.31 \pm 3.15	20.74 \pm 2.36
Loading rate ($\text{kg TDN ha estuary}^{-1} \text{ yr}^{-1}$)	38.79 \pm 11.91	49.39 \pm 15.40	51.78 \pm 51.99	54.73 \pm 21.15	35.63 \pm 12.40	18.94 \pm 8.17	62.42 \pm 7.09
Loading rate ($\text{kg TDN m}^3 \text{ estuary}^{-1} \text{ yr}^{-1}$)	0.0034 \pm 0.001	0.0045 \pm 0.001	0.0024 \pm 0.001	0.0037 \pm 0.001	0.0033 \pm 0.001	0.0012 \pm 0.001	0.002 \pm 0.002
Flushing time (h)	54	56	91	64	34	39	64
Freshwater recharge volume ($\text{m}^3 \text{ yr}^{-1}$)	1.7×10^8	3.9×10^8	6.7×10^8	2.7×10^8	3.5×10^8	1.0×10^7	1.5×10^7

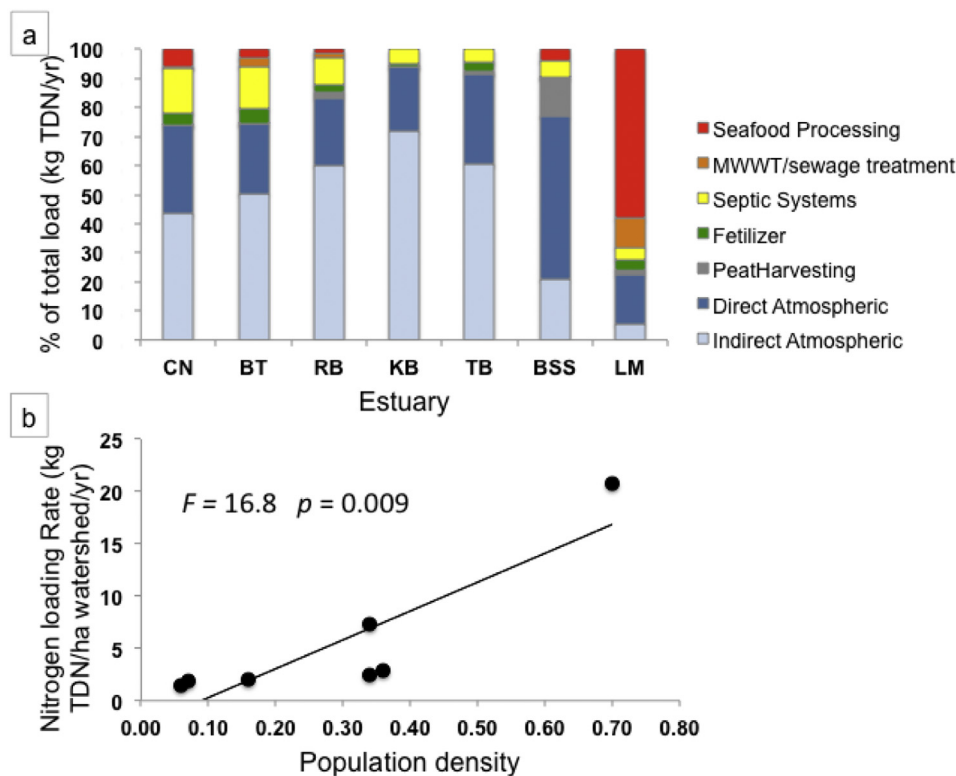


Fig. 3. a) Proportion of nitrogen loading from each source considered in the NLM, and b) Relationship between population density and nitrogen loading rate per ha of watershed (nitrogen yield) in 7 watersheds in eastern New Brunswick (CN = Cocagne, BT = Bouctouche, RB = Richibucto, KB = Kouchibouguac, TB = Tabusintac, BSS = Baie St. Simon Sud, LM = Lamèque). Indirect atmospheric deposition is the largest source of nitrogen in the large watersheds.

estuarine volume was the biggest factor determining estuarine flushing time, with the longest estimated flushing times (91 and 64 h) found in the largest estuaries (Richibucto, Kouchibouguac and Lamèque, respectively, Table 3, Table A.10). Freshwater recharge to the estuaries was small in comparison to the influence of tidal volume in all watersheds assessed (Table 3, Table A.10, A.11).

3.3. Field verification

Nitrogen 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 (Fig. 4). Still, overall isotopic patterns between sites were similar for both components, and 1-way ANOVAs with protected post-hoc tests revealed significantly higher values in Lamèque compared to all other sites (above ground $\delta^{15}\text{N}$ vs. estuary: $F[6,74] = 24.77$ $p < 0.001$, below ground $\delta^{15}\text{N}$ vs. estuary: $F[6,74] = 25.13$ $p < 0.001$) (Mclver, 2015). This is correlated with the much higher proportion of wastewater loading (Figs. 3a and 4). We found significant positive relationships between wastewater nitrogen loading (proportion of total), and wastewater loading rate ($\text{kg wastewater N ha}^{-1}$ estuary yr^{-1}) with $\text{NO}_3^- \delta^{15}\text{N}$ in both AG and BG tissue (Fig. 4a, A.4, Table A.12); however, these relationships were strongly driven by the much higher wastewater loading in Lamèque. Lowest $\delta^{15}\text{N}$ values were recorded in Cocagne and Bouctouche, indicative of higher proportions of synthetic fertilizer additions (Figs. 3a and 4). The remaining estuaries had intermediate isotopic values (Fig. 4) that could be indicative of atmospheric loading as well as background levels.

Tissue nitrogen content was significantly different for AG and BG eelgrass components (Studentized 2-tailed t -test of equal variance,

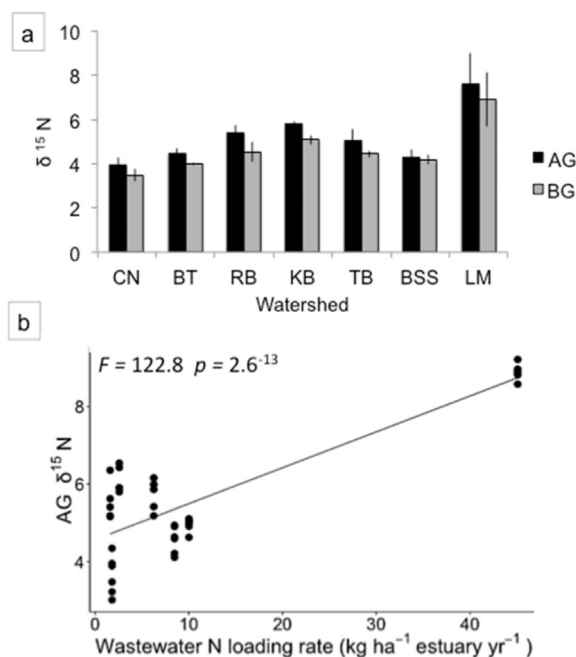


Fig. 4. a) Average $\delta^{15}\text{N}$ for aboveground (AG) and belowground (BG) eelgrass tissue sampled between July 29–August 8th 2013 from Cocagne (CN), Bouctouche (BT), Richibucto (RB), Kouchibouguac (KB), Tabusintac (TB), Baie St. Simon Sud (BSS), and Lamèque (LM) estuaries. Error bars show standard error ($n = 6/\text{site}$), and b) relationships between above ground eelgrass tissue $\delta^{15}\text{N}$ signatures and the estuary area normalized wastewater loading rate (sum of municipal, septic, and seafood processing). The trendlines show the relationship between average tissue isotope values from summer and the model result.

$p < 0.001$), and overall average BG tissue nitrogen was lower and less variable between sites than AG tissue (Figure A.3). Average tissue content was highest in Lamèque and Cocagne, while Kouchibouguac, Tabusintac, and Baie St. Simon Sud had the lowest values.

We found significant positive relationships between both regular and normalized nitrogen loading rates per estuary volume and area and AG tissue % nitrogen content (Fig. 5a–d, Table A.12), although the normalization did not improve the relationships. Total nitrogen load and nitrogen yield were not significantly correlated with AG tissue nitrogen content, and BG tissue nitrogen showed no strong relationships with any results of the NLM or flushing time (Table A.12).

Multiple regression analysis illustrated significant positive relationships between AG tissue nitrogen and both the individual and interaction terms of flushing time and NLM model results (Nitrogen yield, nitrogen loading rates per area and volume of estuary) (Table 4). Furthermore, there were no significant relationships between BG tissue nitrogen and nitrogen yield, or nitrogen loading rate per volume of estuary (Table A.13).

4. Discussion

Overall, the NLM framework provides estimates of total annual TDN loading to a estuary, while also revealing which anthropogenic sources are the largest contributors. It is an important first step in understanding how to manage nitrogen sources within these coastal watersheds.

4.1. Nitrogen loading and sources

The dominance of atmospheric deposition to the total nitrogen loading in most watersheds we assess highlights the low human population density and associated wastewater and agricultural nitrogen production. Given that surface area of the watershed and estuary is used to determine atmospheric deposition loading, the strong relationship between watershed size and total nitrogen load is not an unexpected result (Figs. 2b and 3a). Still, the strength of

the relationship illustrates how dominant atmospheric deposition is compared to other sources.

In eastern New Brunswick, nitrate is the primary type of DIN in atmospheric deposition with approximately 6 times more nitrate than ammonium in precipitation consistently throughout the last 2 decades (NATChem, 2012). It is likely that reactive nitrogen deposited through wet and dry deposition is largely transported from outside the region, from areas with higher population densities and industrial practices (Bowen and Valiela, 2001b; Valigura et al., 2001). Conversely, volatilization of ammonium from local agricultural practices contributes relatively little to overall nitrogen loading, which reflects the small concentration of livestock in this region relative to dense agricultural regions like south western Ontario (Yang, 2006; Huffman et al., 2008; Yang et al., 2011). We propose our estimates of atmospherically derived nitrogen ($< 11 \text{ kg TDN ha}^{-1} \text{ yr}^{-1}$) are lower than those from the north eastern United States ($12 \text{ kg TDN ha}^{-1} \text{ yr}^{-1}$, Bowen and Valiela, 2001b) due to lower ammonium deposition in this region, but not significantly smaller than other estimates 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 nitrogen yield and loading rates show a clear footprint of human settlement and activities within watersheds, namely in the smaller watersheds with higher population densities (Fig. 3b, Table 1). The relationship we found between population density and nitrogen yield is similar to the relationship between increased housing development and nitrogen loading rates in Waquoit Bay (Short and Burdick, 1996). In Lamèque, the high nitrogen loading rate (per ha estuary) reflects the land use patterns and the higher proportion of nitrogen from point sources, including wastewater from MWWT and seafood processing. Conversely, the five larger watersheds are predominantly forested and naturally vegetated with scrubland, wetland, or unexploited peatland (Figure A.2). Of these, the lowest nitrogen loading rates per ha estuary are from watersheds with the smallest population density and small or no point source contributions of wastewater: a RAMSAR wetland protection area borders a large portion of the Tabusintac estuary,

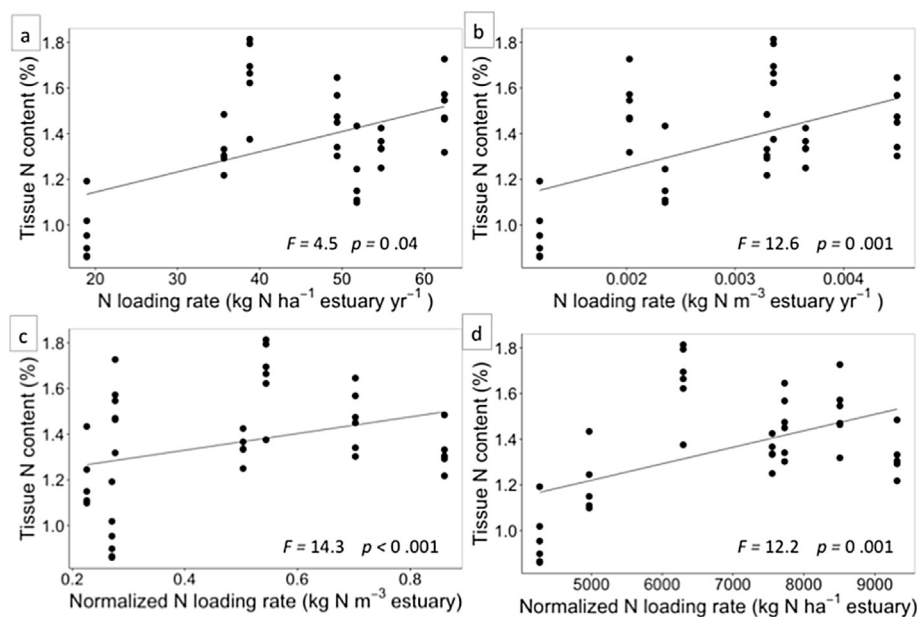


Fig. 5. Relationships between nitrogen content (%) of all AG eelgrass tissue sampled in summer 2013 ($n = 6/\text{site}$) and a) Nitrogen loading rate per m^3 estuary, b) Nitrogen loading rate per unit estuary area, c) Nitrogen loading rate (per estuary volume) normalized with flushing time, d) Nitrogen loading rate (per estuary surface area) normalized with flushing time (See Table 2 for equations).

Table 4

Results of multiple regression using yield and loading rate estimates from the NLM and flushing time as independent variables, and AG nitrogen content from eelgrass samples collected in July/August ($n = 6/\text{site}$) as dependent variables. A significance level of $p = 0.05$ was used. Results for multiple regressions with BG nitrogen content in supplementary material (Table A.13).

Multiple regression	AG % tissue nitrogen				
	DF	Standard deviation of coefficient	<i>p</i> -values	<i>F</i> Value	Overall- <i>p</i> value
Flushing Time	3, 35	0.003	4.9×10^{-4}	11.18	2.7×10^{-5}
Yield (kg TDN ha watershed ⁻¹ yr ⁻¹)		0.04	3.4×10^{-6}		
Flushing time \times Yield		6.9×10^{-4}	2.4×10^{-6}		
Flushing Time	3, 35	0.01	3.1×10^{-5}	20.43	7.9×10^{-8}
Loading rate (kg TDN ha estuary ⁻¹ yr ⁻¹)		0.01	1.5×10^{-7}		
Flushing time \times loading rate		2.3×10^{-4}	4.8×10^{-6}		
Flushing Time	3.35	0.008	0.04	6.83	9.6×10^{-4}
Loading rate (kg TDN m ⁻³ estuary yr ⁻¹)		16.6	0.01		
Flushing time \times loading rate		3.18	0.05		

and Baie St. Simon Sud has minimal septic input and only a small amount of nitrogen contribution from the seafood processing facility. These estuaries have also exhibited reduced eutrophic symptoms in eelgrass habitats relative to Bouctouche, Cocagne and Lamèque over the past decade (Lotze et al., 2003; Schmidt et al., 2012). However, watershed:estuary area is important to consider as the higher estimated loading rate in Kouchibouguac reflects the higher watershed:estuary area and not the watershed activities: Kouchibouguac is predominantly covered in National Park forest, and has the lowest predicted nitrogen yield. Conversely, the high proportion of nitrogen from direct atmospheric deposition contributed to Baie St. Simon Sud estuary drives the high nitrogen yield there. The nitrogen yield does not directly represent nitrogen contributions from human activities in that watershed, but instead reflects the small watershed:estuary area.

4.2. Eelgrass tissue characteristics and nitrogen loading

The significantly elevated $\text{NO}_3^- \delta^{15}\text{N}$ signature in tissues from Lamèque (8–10‰), where wastewater is the dominant nitrogen source to the estuary, suggests that eelgrass tissue is incorporating the nitrogen signal from wastewater sourced nitrogen (Figs. 3–4, Figure A.4, McClelland and Valiela, 1998; Cole et al., 2006). Wastewater has characteristically higher $\text{NO}_3^- \delta^{15}\text{N}$ signatures resulting from volatilization of ^{14}N rich ammonia during initial treatment (Macko and Ostrom, 1994). Lamèque is the only site we study in the region with a dominant loading source that is non-atmospheric, and the positive relationship between wastewater nitrogen loading and $\delta^{15}\text{N}$ signatures is primarily driven by the values in Lamèque (Figure A.4, Table A.12). Results of tissue $\text{NO}_3^- \delta^{15}\text{N}$ analysis are less distinct in the other six estuaries, with values falling in a range indicative of atmospheric deposition (+2 to +6‰) or synthetic agricultural and lawn/turf fertilizers (–4 to +4‰) (Figs. 3a and 4, Kendall, 1998; Lepoint et al., 2004; Cole et al., 2006). The slightly lower signature in Bouctouche and Cocagne estuaries may reflect the higher input of synthetic fertilizers to these estuaries (Fig. 3a), however even in these watersheds fertilizer input contributes less than 6% of the total estimated nitrogen load. Therefore, the minimal input of fertilizers in the sparsely populated and non-agriculturally intensive watersheds other than Lamèque suggest that the isotope signatures there reflect the large proportion of atmospherically derived nitrogen loading (Figs. 3a and 4). We note that isotope values representative of atmospheric deposition and fertilizer nitrogen are not clearly distinct from values typical of background levels of $\text{NO}_3^- \delta^{15}\text{N}$ in bedrock and pristine groundwater (ranging from +2 to +8‰) (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 an estuary (not 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 $\text{NO}_3^- \delta^{15}\text{N}$ and sources of nitrogen in each estuary and note the potential ability of these primary producers to integrate nitrogen signals from nitrogen (namely wastewater) sources in the Lamèque watershed, as has been shown in bays with eelgrass in New England, the Baltic Sea, and elsewhere (Voss and Struck, 1997; McClelland and Valiela, 1998; Voss et al., 2000; Cole et al., 2006).

4.3. Tidal influence and estuarine flushing time

We compared our simple flushing time estimates to more spatially explicit models of flushing for the whole Richibucto estuary (Guyondet et al., 2013). Of the water renewal estimates proposed for the three distinct areas of the Richibucto bay and estuary (Main Harbor, North Arm, and Baie du Village, Guyondet et al., 2013), our estimate for this site is within the range for the Main Harbor (5–20 days), but underestimates flushing time proposed for the other two arms of the estuary where renewal estimates are longer by 10–20 days. Therefore, we note that our simple hydrodynamic 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 nitrogen of eelgrass shoots/blades with flushing time normalized nitrogen loading per estuary area or estuary volume indicates that both nitrogen loading rates and flushing time may affect the legacy of nitrogen loading and thereby the ambient concentration of nitrogen in the estuarine water column that is available to eelgrass (Fig. 4). This simple regression result, however, was not different or better than the model where regular (non-normalized) loading rate was used, thereby it did not provide sufficient information as to the importance of flushing time separate or in concert with loading rate on nitrogen availability in these systems. Here, our multiple regression analysis provides valuable information on the importance of both individual factors and their interaction. The results are a further indication that estuary size and hydrodynamics influence the amount of nitrogen available to eelgrass and other primary producers. However, nitrogen loading rate is more significantly correlated with tissue nitrogen characteristic than flushing time (Table 4). This suggests that in the southern Gulf of St. Lawrence, despite the capacity of tidal flushing to reduce the production of phytoplankton and macroalgae in estuaries (Valiela et al., 2000), high levels of nitrogen loading may not be able to be mitigated by tidal flushing alone.

In contrast to above ground tissue, we found no strong relationships between nitrogen loading rates and below-ground

nitrogen content. Eelgrass roots and rhizomes, which may only account for 50% of the plants nitrogen intake, 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, below-ground tissue may not directly reflect the ambient nitrogen content of the water column, which in addition to ammonium may have higher nitrate concentrations from atmospheric deposition and land runoff. Eelgrass shoots may better reflect the ambient DIN available in the water column than roots and rhizomes (Hemminga and Duarte, 2000).

4.4. Broader regional context

In this study we consider both the magnitude of an impact (nitrogen loading) and the sensitivity of the system (flushing time) as principal determinants of the eutrophication risk. One of the benefits of using the NLM framework to estimate the magnitude of nutrient loading to an estuary is that it has had wide-scale application to numerous coastal embayments in the continental United States, and has shown good agreement with other nitrogen loading models (Bowen and Valiela, 2001a; Latimer and Charpentier, 2010; Giordano et al., 2011). The results from our application of the NLM fit at the lower end of the gradient of nitrogen loading rates predicted by the Latimer and Charpentier (2010) NLM application to 74 watersheds of various size and nitrogen loading rates in the north eastern United States. They are also mostly lower than nitrogen loading rates ($\text{kg N ha}^{-1} \text{ estuary yr}^{-1}$) produced from a land-use based nitrogen loading model applied to estuaries in Prince Edward Island, Canada (Jiang et al., 2011; Bugden et al., 2014). Flushing times are not available yet for all these estuaries that have published nitrogen loading rates. However when we compare estuaries from these cold, temperate regions (New Brunswick, Prince Edward Island, north eastern United States) that have both loading rates and flushing time estimates we gain a clearer picture of the relative susceptibility to nutrient impacts in eastern New Brunswick estuaries (Fig. 6a).

Compared to nitrogen loading rates and water residence times in estuaries in Prince Edward Island and the north eastern United States, the estuaries in New Brunswick have low loading rates, most falling into the lower, or 25th percentile. Only Lamèque, with the significant nitrogen input from a point source, has a loading rate similar to those in the more heavily populated north eastern United States, or more agriculturally intensive Prince Edward Island.

Flushing times in the seven New Brunswick estuaries (at least the central bays of each estuary) are relatively short compared to

other estuaries in the wider mid-Atlantic region (Fig. 6b). Although flushing times for the estuaries from PEI and the United States included in this comparison were calculated using different flushing models (see Latimer and Charpentier, 2010 and Bugden et al., 2014 for full methodology), they are useful for general comparisons as they all use tidal prism calculations. More than 50% of the estuaries with both flushing times and nitrogen loading rates available in PEI and the eastern United States have flushing times that are substantially longer than 192 h (8 days). Our estimates of flushing times for the seven New Brunswick estuaries are all <192 h; the estimated range from Guyondet et al. (2013) for Richibucto estuary (120–480 h) is longer than ours (91 h), but is still shorter than a large proportion of the estuaries we compare with in the other regions (Fig. 6b).

Therefore, we suggest the estuaries we assess in New Brunswick have a cumulatively lower risk for potential negative effects of nitrogen loading and resulting eutrophication. However, we stress that exceptions are present in New Brunswick, notably in Lamèque, where both nitrogen yields and nitrogen loading rates per ha estuary are comparable to those in highly impacted estuaries throughout this wider Atlantic region (Bowen and Valiela, 2001; Bricker et al., 2008; Latimer and Charpentier, 2010). Additionally, the impacts of current nitrogen loading in this region are not inconsequential, and we highlight the increased presence of eutrophication symptoms previously identified in estuaries for which we have estimated higher nitrogen yields and loading rates (Cocagne, Bouctouche, Lamèque) relative to the estuaries with lower nutrient input (Tabusintac, Kouchibouguac; Lotze et al., 2003; Plante and Courtenay, 2008; Schmidt et al., 2012; Turcotte-Lanteigne and Ferguson, 2013). These primary symptoms of eutrophication may be a precursor to increased canopy loss if nitrogen loadings are increased or not mitigated. Further comparison of NLM estimates (this study) with measured characteristics of eelgrass bed structure and eutrophication signs in these estuaries can be found in Mclver 2015.

4.5. Conclusions and management implications

We suggest that estuaries with significant sources of nitrogen additional to atmospheric deposition (e.g. seafood processing plants, MWWT, septic systems), and estuaries with a reduced capacity to remove excess nitrogen through tidal circulation and flushing are at a higher risk of exacerbating eutrophic condition in eelgrass habitats. At the landscape scale ecosystems such as wetlands and riparian buffer zones have an inherently elevated capacity to conserve nitrogen as a result of their position near the

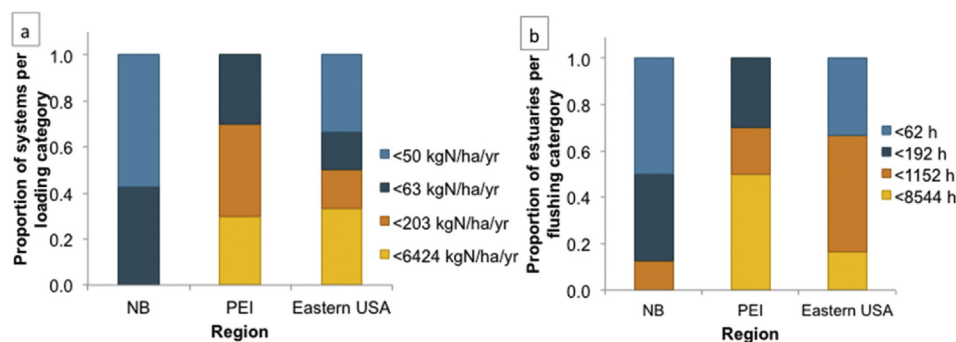


Fig. 6. Summary of (a) nitrogen loading rate ($\text{kg N ha}^{-1} \text{ estuary yr}^{-1}$) and (b) flushing times for 7 New Brunswick estuaries (NB, this study, and flushing time for Richibucto estuary from Guyondet et al., 2013), 10 Prince Edward Island estuaries* (PEI, Meeuwig et al., 1998, Bugden et al., 2014), and 8 north-eastern United States estuaries (Abdelrhman, 2005; Bricker et al., 2008; Latimer and Charpentier, 2010). Although there are estimates of nitrogen loading rates for many more estuaries, particularly in the United States, those with both nitrogen loading rates and flushing or residence time calculations are fewer. * Estimates for PEI estuaries were made for May–October only, and estimated NO_3^- -N. This is the largest type of nitrogen entering estuaries on PEI due to the high rate of fertilizer application (Bugden et al., 2014).

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, given that most nitrogen loading in eastern New Brunswick is from non-point sources, protecting remaining wetland areas and re-establishing and maintaining riparian buffer zones would be a beneficial for preventing further increases in nitrogen loading.

This study provides a quantification of the magnitude and different sources of annual nitrogen loading representative of the time period 2002–2012 to seven estuaries in eastern New Brunswick. Additionally we provide simple and user-friendly estimates of hydrodynamics and influence of tidal flushing on nutrient loading in these estuaries through flushing time calculations (Gregory et al., 1993; Grant et al., 2005; Bugden et al., 2014). 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. This research is important for filling in the knowledge gap of nitrogen loading to temperate, forested watersheds where human impact is relatively little and nitrogen yields are low, compared to more heavily impacted estuaries in this broader coastal region.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.ecss.2015.08.011>.

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