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Denitrification hot spots: dominant role of invasive macrophyte *Trapa natans* in removing nitrogen from a tidal river

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Abstract. Rivers receive large amounts of nitrogen (N) from their watershed and are the final sites of nutrient processing before delivery to coastal waters. Transformations of dissolved inorganic N (DIN) to gaseous N within rivers can impact both coastal eutrophication and greenhouse gas emissions. Vegetated shallows of rivers are sites of active metabolism and may act as hot spots for N transformation, but little is known about the variability of denitrification within shallows or the role of vegetation structure in controlling this variability. We measured in situ N loss and accumulation of N_2 and N_2O in vegetated shallows of the tidal Hudson River and used regression models to determine the role of plant species in different monospecific beds in ecosystem N loss. N_2 production was highly variable between vegetated shallows and was associated with species-driven differences in dissolved oxygen (DO) dynamics during the ebb tide. N_2 production was extremely high (37–71 mmol $N{\cdot}m^{-2}{\cdot}d^{-1}$) in beds with invasive floating-leaved plants (*Trapa natans*) but was insignificant in submersed native vegetation (*Vallisneria americana*). In *Trapa* sites, N_2 production was strongly related to metabolism. Change in DO concentrations in the surrounding water due to atmospheric venting by the plants during ebb tide, combined with changes in water temperatures, explained 87% of the variation of the observed N_2 production. Despite these high denitrification losses, beds acted as N_2O sinks where N_2O concentrations became undersaturated during ebb tide. An estimate of summertime N_2 production in *Trapa* beds, based on continuously measured oxygen and temperature by moored sondes, suggests that these beds are a major seasonal hot spot for N removal. Large *Trapa* beds represent only 2.7% of the total area of the tidal Hudson, but they remove between 70% and 100% of the total N retained in this river reach during summer months. Although they are active for only three months of the year, *Trapa* shallows contribute to as much as 25% of the annual N removal. *Trapa* activity represents an important ecosystem service, modulated by its impacts on DO as a function of *Trapa*'s growth form trait and modulated by the physical properties of the environment.

Key words: denitrification; ecosystem service; invasive species; nitrogen; nitrous oxide; N retention; river; Trapa natans; *vegetated shallows.*

Introduction

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

Riverine networks can be hot spots (McClain et al. 2003) of N transformations and loss to gaseous N production (Piña-Ochoa and Alvarez-Cobelas 2006). These systems occupy less than 1% of the earth's surface, but denitrification and N_2O production within these ecosystems has been estimated to account for 30% of terrestrial values (Seitzinger et al. 2006). Until recently, small headwater streams have been the focus of studies examining N uptake and loss on the landscape. Smaller streams usually experience higher N cycling rates owing to their higher benthic to surface water ratios (Bernot and Dodds 2005). Larger rivers have been less well studied, but are generally thought to be of lesser importance than headwater streams for N removal (Alexander et al. 2000, Peterson et al. 2001). Some recent studies suggest, however, that large rivers may play an important role in N uptake (Stanley and Maxted 2008,

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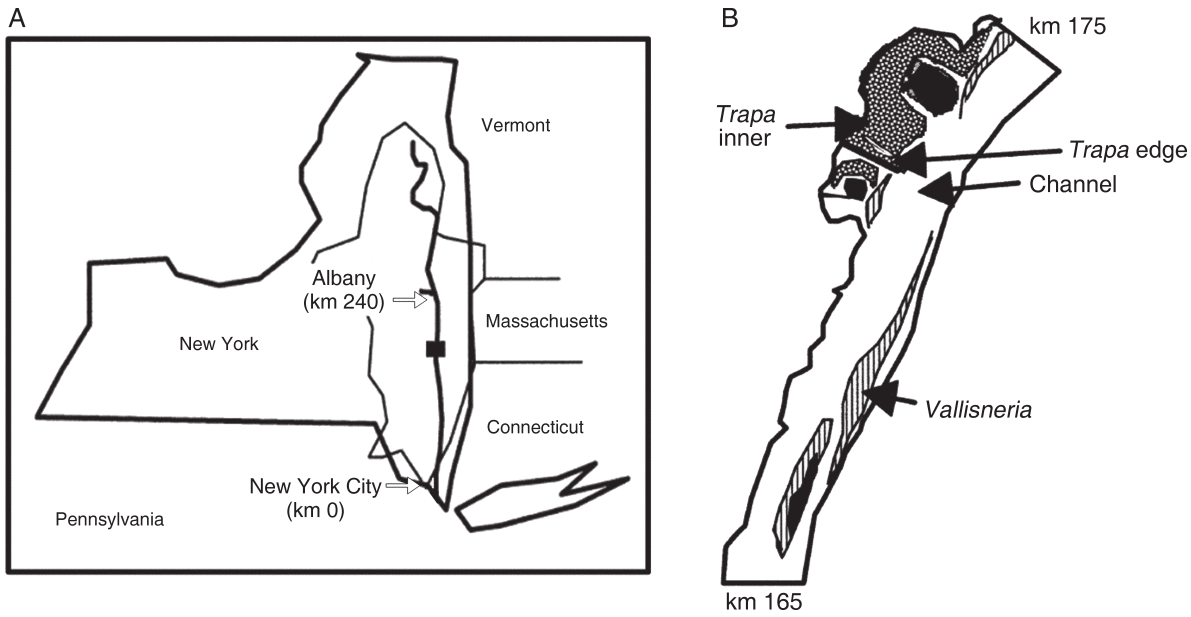

FIG. 1. (A) Location of the tidal Hudson River from Albany to New York City (river km 0), New York. (B) River reach where the study took place. Hatched areas are *Vallisneria* beds, dark stippled areas are *Trapa* beds, black is intertidal or permanently exposed islands or jetties, and white represents open water sites. The four sites sampled in 2006 are indicated by arrows; the transect sampled in 2007 runs from the *Trapa* edge site to the *Trapa* inner site. The map is modified from Caraco and Cole (2002).

Tank et al. 2008, Alexander et al. 2009) as the amount of N removed per meter of reach is greater in large rivers than in small streams (Seitzinger et al. 2002).

In the tidal Hudson River, almost 2000 metric tons (Mg) of N is taken up per year. This value is estimated to be greater than N loss in freshwater wetlands of the river's watershed and equal to the sewage load to the River from a major metropolis (Lampman et al. 1999). This N uptake occurs despite a relatively short residence time of water in the tidal freshwater Hudson (TFH; Lampman et al. 1999). The high uptake is somewhat surprising as the Hudson river does not have significant groundwater input or associated active riparian areas (Cooper et al. 1988); the sea-level section of the river lacks flood plains and burial in the main stem of the Hudson is relatively low and does not seem to be a dominant fate of this N (Lampman et al. 1999).

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

METHODS

Site description.—The freshwater tidal Hudson River extends 140 km from Albany toward New York, New York, USA (Fig. 1). Dominant water inputs are from two tributaries (Mohawk and Upper Hudson) that enter near the dam at Troy, New York, while additional tributaries contribute 20% of the total water input and groundwater inputs are insignificant (Lampman et al. 1999). About 15% of the 100-km^2 area of the TFH is occupied by two macrophyte species that occur in nearly monospecific beds that can be more than 1 km^2 in size (Nieder et al. 2004). *Vallisneria americana* is a submersed plant that is native to the Hudson River and is generally associated with elevated oxygen concentrations. Low oxygen conditions are extremely rare, even at night in large dense beds of this macrophyte. *Trapa natans* is an introduced exotic species to the Hudson. It is a floating-leaved plant and oxygen is vented to the atmosphere when leaves reach the water surface, resulting in oxygen-depletion events at low tide, particularly in large beds (Caraco and Cole 2002, Goodwin et al. 2008).

This study took place in a large *Trapa* bed (Inbocht Bay) and in a nearby large *Vallisneria* bed located in the

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

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

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

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

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

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

The partial pressure of N_2O ($p\,N_2O$) was measured by headspace equilibration at ambient temperature (Cole and Caraco 2001). A volume of 1.1 L of water taken at the surface was equilibrated in a gas-tight bottle with ambient air (120 mL), by shaking vigorously for two minutes. After equilibration, triplicate 9-mL samples of headspace gas were injected into pre-evacuated vials with a thick butyl stopper and an aluminum ring. Ambient air concentration samples were also collected and injected into pre-evacuated vials. N_2O was measured by gas chromatography using an ECD detector on a Shimadzu 2014 GC (Shimadzu, Kyoto, Japan) with a Tekmar 7050 autosampler (Tekmar, Vernon, British Columbia, Canada). We used a Poropaq Q (80/100) column (Alltech, Deerfield, Illinois, USA) to separate gases with P5 (95% argon and 5% methane) as the carrier gas. Standards consisted of vials treated exactly as above with N_2O concentrations of 0.22, 1.2, and 2.4 ppm. We corrected the measured value of N_2O from the equilibration for the introduction of 120 mL of air. N_2O concentrations were then calculated using the solubility tables of Weiss and Price (1980).

Water samples for dissolved nutrients ($NO_3^- + NO_2^-$ [referred to as NO_3^- only for the rest of the text], NH_4^+, dissolved organic carbon [DOC], and PO_4) were collected hourly at each site and filtered immediately

in the field using 25-mm Gelman A/E filters in filter holders (Swinnex; Millipore, Billerica, Massachusetts, USA) and water samples for total analyses (total N and P) were taken directly. All samples were kept in a cold, dark cooler in the field. In the laboratory, samples were acidified to a pH < 2 using 1 mL of 0.5 mol/L H_2SO_4 per 100 mL of sample. Nutrients and DOC were analyzed following procedures described in Lampman et al. (1999). Water samples for chlorophyll *a* (chl *a*) were filtered through Whatman GF/F filters and then filters were frozen prior to analysis. Chl *a* was measured after methanol extraction (Holm-Hansen and Riemann 1978).

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

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

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

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

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

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

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

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

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

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

Changes in nutrient concentrations in the bed were measured over the ebb tide at three sampling dates and were found to be moderately well predicted from O_2 concentrations, with the exception of NH_4^+, which was always found at low concentrations (Table 1). We

TABLE 1. Simple linear regression relationships between the concentration of the different N forms (in μmol/L) and dissolved oxygen (DO) concentrations in *Trapa* beds (in μmol/L).

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

Notes: Degrees of freedom for all models are 1, 23. TN stands for total nitrogen.

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

RESULTS

Changes in gas and nutrient concentrations during ebb tide among sites

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

Relationships to predict changes in N_2

In *Trapa* beds, changes in N_2 were strongly and linearly related to changes in NO_3^- and DO concentrations. The relationships between ΔN_2 and ΔNO_3^- were typically very strong and highly significant, where ΔNO_3^- could explain up to 96% of the variance in ΔN_2 on a given date. The slopes of the relationships varied significantly among sampling dates (ANCOVA, $R^2 = 0.95$; $F = 76.36$; df = 2, 27; $P < 0.001$; Fig. 3A). The shallowest slope of −0.79 was observed in July 2006 and was the closest to a 1:1 relationship where N from nitrate reduction alone could account for all the N_2 produced. In July 2007 and September 2006, slopes were substantially steeper at −2.53 and −4.60, respectively, suggesting that considerably more N_2 was produced per unit NO_3^- consumed. The relationships between ΔN_2 and ΔDO were also very strong and highly significant, where ΔDO explained between 56% and 97% of the observed change in N_2. Again, relationships varied among sampling dates, but less so than with ΔNO_3^-. The relationships determined for September 2006 and July 2007 were not significantly different from one another, with slopes of −0.42 and −0.49, respectively. However the relationship was different in July 2006 where substantially less N_2 was produced with the same change in DO (ANCOVA, $R^2 = 0.95$; $F = 42.22$; df = 2, 35; $P < 0.001$). We found that the different trends observed among sampling dates for both relationships could in part be explained by a significant interaction with temperature (ANCOVA interaction terms, temperature × ΔNO_3^-, $P < 0.001$, and temperature × ΔDO, $P < 0.001$). Thus in July 2006 when water temperatures

TABLE 2. Average and extreme values observed (minimum–maximum) of various physical chemical properties in monospecific *Trapa* and *Vallisneria* beds for N samples in the Hudson River during ebbing tide on different sampling dates.

Parameter	July 2006		September 2006		July 2007
	Trapa (N = 10)	*Vallisneria* (N = 6)	*Trapa* (N = 11)	*Vallisneria* (N = 6)	*Trapa* (N = 23)
DO (mg/L)	4.02 (0.53–7.64)	7.03 (6.73–7.58)	5.66 (0.48–9.39)	8.46 (8.16–8.78)	4.33 (2.10–6.61)
Temperature (°C)	26.42 (25.66–27.44)	26.29 (25.77–27.65)	19.72 (18.05–20.82)	20.35 (20.18–20.70)	23.38 (22.16–24.26)
NO_3^- (μmol N/L)	16.83 (0.84–36.41)	30.32 (29.45–34.00)	18.05 (1.21–26.33)	26.24 (24.84–27.67)	17.02 (1.43–28.57)
NH_4^+ (μmol N/L)	1.92 (0.98–3.58)	2.47 (2.09–2.82)	1.14 (0.44–1.58)	0.79 (0.15–1.20)	5.13 (0.00–15.71)
TN (μmol N/L)	35.32 (17.07–54.34)	49.64 (47.15–57.43)	45.01 (24.88–67.65)	45.79 (40.54–49.59)	
DOC (mg/L)	4.64 (3.58–3.82)	4.34 (3.73–4.89)	3.82 (3.67–4.13)	3.69 (3.41–3.91)	
PO_4 (μmol P/L)	0.49 (0.16–0.90)	0.80 (0.77–0.84)	0.33 (0.09–0.54)	0.44 (0.27–0.52)	1.80 (0.32– 2.61)
TP (μmol P/L)	1.54 (0.90–2.56)	2.21 (1.88–3.24)	1.74 (0.76–4.82)	1.21 (1.13–1.29)	
Chl *a* (μg/L)	2.07 (0.69–3.51)	2.98 (2.48–3.41)	3.97 (1.49–6.80)	4.12 (3.27–5.08)	

Notes: Key to abbreviations: DOC, dissolved organic carbon; TP, total phosphorus; chl *a*, chlorophyll *a*. Data are not available when cells are empty.

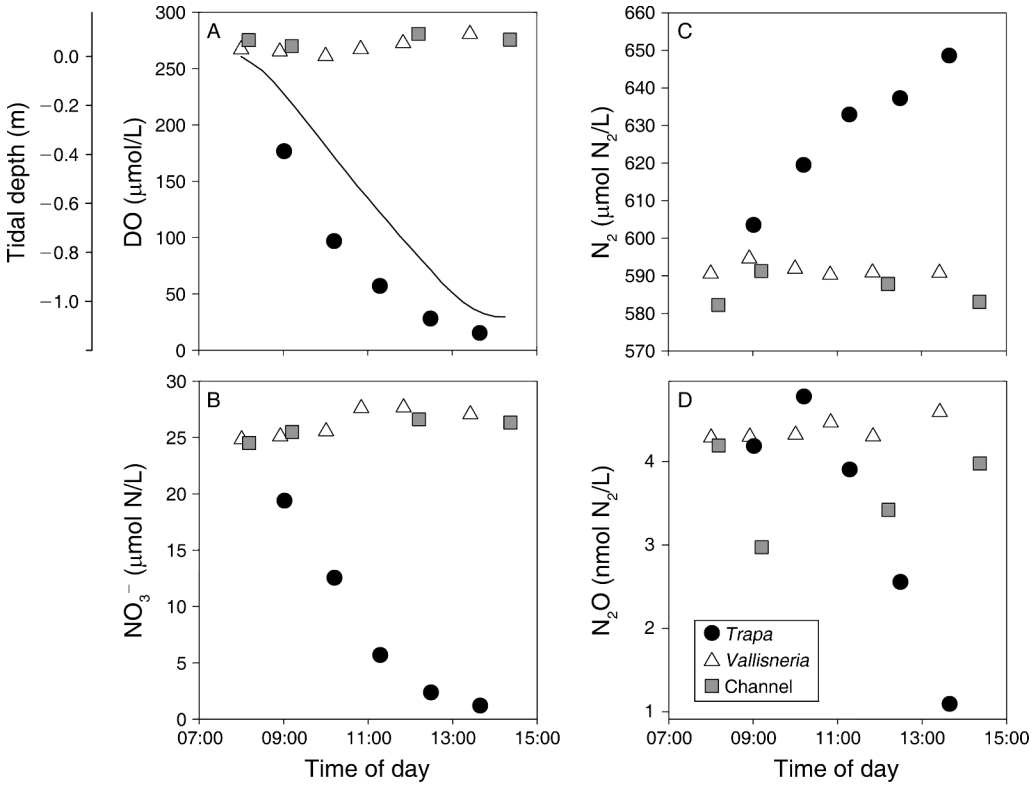

FIG. 2. Dynamics of (A) dissolved oxygen (DO) concentrations (mg/L) and delta tidal depth (m) represented by the symbols and the line respectively, (B) NO_3^-, (C) N_2, and (D) N_2O concentrations measured in September 2006 during ebbing tide at different sites.

were warmer, less N_2 was produced for the same change in DO and NO_3^- as compared to the two other sampling dates.

To estimate N_2 production from the *Trapa* sites at a larger spatial scale, we used a simple and a multiple regression approach using ordinary least squares (OLS) regression to develop different predictive models (Table 3). The overall relationship between ΔN_2 and ΔNO_3^- was significant but weak with an adjusted R^2 of 0.20. This is not surprising given the variability observed among dates. The global relationship between ΔN_2 and ΔDO was much stronger with an adjusted R^2 of 0.56, suggesting that overall change in N_2 was more tightly coupled with changes in DO regardless of timing. ΔN_2 was also negatively related with temperature suggesting that some of the observed change in N_2 was a function of a change in physical solubility and not necessarily biological production. However the best and most parsimonious model to predict ΔN_2 used both temperature and ΔDO as predictor variables (AIC = 274.17; Table 3).

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

Nitrogen mass balance in Trapa *beds*

Mass balance revealed more than 7000 kg N/d on average enters the *Trapa* beds of the TFH (Fig. 5) during ebb tide. Tidal inputs were mainly in the form of DIN representing 57% of the total N tidal input. Groundwater N inputs were considered negligible at 98 kg N/d (Cooper et al. 1988, Nystrom 2010). Half of N input (48%) was exported by tidal outputs from the beds to the main channel. Tidal outputs were mainly in the form of organic N with DIN representing less then a quarter of total N outputs. The majority of N entering

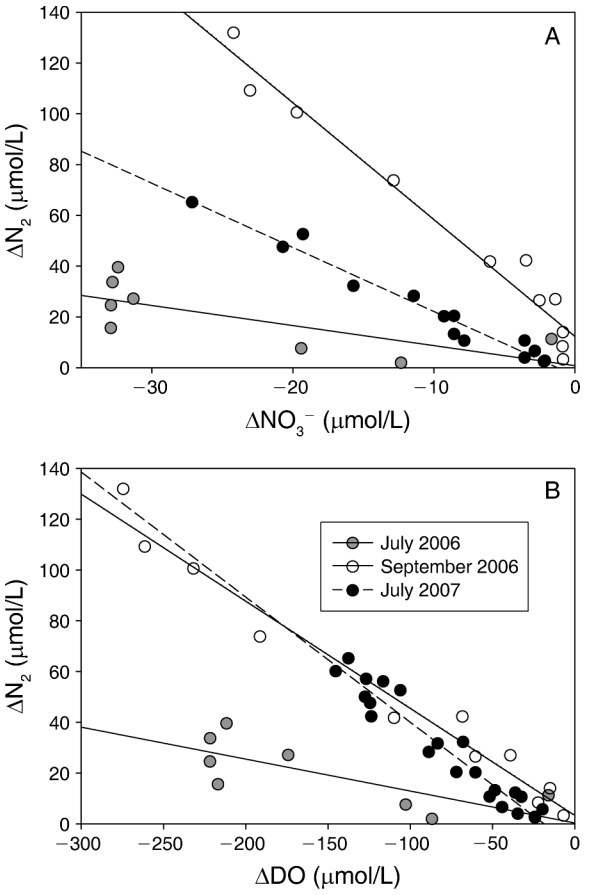

FIG. 3. (A) Simple linear regression relationships between ΔNO_3^- and ΔN_2 for each sampling date: July 2006, $\Delta N_2 = (-0.79 \times \Delta NO_3^-) + 0.83$ with $R^2 = 0.55$, $n = 9$, F test, $P \leq 0.01$; September 2006, $\Delta N_2 = (-4.60 \times \Delta NO_3^-) + 12.47$ with $R^2 = 0.96$, $n = 11$, F test, $P \leq 0.0001$; and July 2007, $\Delta N_2 = (-2.53 \times \Delta NO_3^-) - 3.14$ with $R^2 = 0.89$, $n = 13$, F test, $P \leq 0.0001$. (B) Simple linear regression relationships between ΔDO and ΔN_2 for each sampling date: July 2006, $\Delta N_2 = (-0.13 \times \Delta DO) + 0.39$ with $R^2 = 0.56$, $n = 9$, F test, $P \leq 0.01$; September 2006, $\Delta N_2 = (-0.42 \times \Delta DO) + 3.48$ with $R^2 = 0.97$, $n = 11$, F test, $P \leq 0.0001$; and July 2007, $\Delta N_2 = (-0.49 \times \Delta NO_3^-) - 9.25$ with $R^2 = 0.93$, $n = 21$, F test, $P \leq 0.0001$.

the beds (55–82%) was transformed to N_2 gas and permanently eliminated from the ecosystem. Transformation from the DIN pool to N_2 gas was the main loss term, where N_2 production accounted for 96–143% of DIN inputs. Despite the close correspondence between the N input and output terms on average, an estimated 205–2150 kg of extra N per day would be required to fuel our estimates of N_2 production. We calculate a sediment standing stock of 96 000 kg N in the *Trapa* beds, that would in theory be able to supply up to 1000 kg of N per day for 90 days. Moreover the standing sock of N in sediment could be partially replenished each year by senescing *Trapa* representing an estimated standing stock of approximately 37 000 kg N.

Discussion

Trapa *beds as hot spots*

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

We found that N_2 production in *Trapa* beds was intimately linked with localized O_2 consumption and system metabolism. The contrasting O_2 dynamics in exotic *Trapa* beds during ebb tide as compared to native *Vallisneria* has been previously described in great detail (Caraco and Cole 2002, Goodwin et al. 2008). Briefly,

TABLE 3. Results of simple regressions and multiple regressions of change in N_2 (ΔN_2, in μmol N/L) with change in oxygen (ΔDO, in μmol/L), change in nitrate (ΔNO_3^-, in μmol N/L), and temperature (Temp, in °C).

Model	*N*	*P*	*F*	df	R^2	AIC
Simple regression models						
$\Delta N_2 = -0.30(\Delta DO) + 2.22$	41	<0.0001	52.03	1, 39	0.56	317.17
$\Delta N_2 = -7.30(\text{Temp}) + 201.33$	41	<0.0001	24.40	1, 39	0.37	321.73
$\Delta N_2 = -1.31(\Delta NO_3^-) + 14.95$	35	0.0044	9.33	1, 33	0.20	332.32
Multiple regression models						
$\Delta N_2 = -0.28(\Delta DO) - 6.51(\text{Temp}) + 154.06$	41	<0.0001	132.70	2, 38	0.87	**274.17**
$\Delta N_2 = -9.45(\text{Temp}) - 1.98(\Delta NO_3^-) + 222.45$	35	<0.0001	88.39	2, 32	0.84	279.20
$\Delta N_2 = -0.51(\Delta DO) + 1.75(\Delta NO_3^-) + 3.01$	35	<0.0001	32.34	2, 32	0.65	309.34
$\Delta N_2 = -0.21(\Delta DO) - 7.35(\text{Temp}) - 0.58\ (\Delta NO_3^-) + 171.34$	35	<0.0001	84.24	2, 32	0.88	274.20

Note: The best model determined using the Akaike information criterion (AIC) is shown in boldface type.

when the rosette leaves of *Trapa* reach the surface, the plant vents O_2 to the atmosphere, depleting O_2 in the surrounding water during ebb tide; during rising tide, O_2 and nutrients from the main channel replenish the beds due to the physical exchange of water. The cycle of O_2 loss begins anew upon ebbing tide. Suboxic conditions created directly by *Trapa* under ebb tide favor microbial transformations that remove inorganic N species and produce N_2 gas (canonical denitrification and anaerobic ammonium oxidation). Although the loss of nitrate and a decrease in the N:P ratio had been previously reported in these beds (Caraco and Cole 2002), our study provides conclusive evidence that the N loss observed during ebb tide was a function of N_2 production, thus representing permanent N loss from the ecosystem.

Denitrification losses were clearly the dominant fate of N in the beds during ebb tide whereby gaseous production of N_2 represented between 55% and 82% of total N inputs to the beds (Fig. 5). Numerous studies have found a positive relationship between N availability and denitrification in a range of aquatic systems (Saunders and Kalff 2001, Seitzinger et al. 2006). A recent evaluation of denitrification losses in streams found that high rates of N loss were closely associated with elevated concentrations of N (Mulholland et al. 2008). Our stronger link of N_2 production with O_2 may better reflect the dynamics that would influence the N available for denitrification beyond NO_3^- concentration in the system. Indeed in a sediment denitrification review by Fennel et al. (2009), the authors suggest that sediment oxygen demand is a more useful metric to predict denitrification in bottom waters than NO_3^- concentration because of the multiple microbial N transformations influenced by O_2 concentration that supply the substrates and create the optimal conditions required for N_2 production.

Average daily N input and output estimates for the 4-km^2 patches occupied by large *Trapa* beds were well balanced, with an estimated 7194 kg N/d entering these shallow beds and between 7399 and 9344 kg N/d exiting, 3954–5899 kg N/d of it as N_2 gas. Surprisingly, almost all of the N_2 production could have been fuelled by DIN loading to the beds, when comparing the lower estimate of N_2 production to our mass balance terms. Although we saw strong relationships between ΔN_2 and ΔNO_3^- in the *Trapa* beds by date, the slopes of these relationships suggested that depending on the date, change in NO_3^- concentration alone was unable to account for all N_2 produced (Fig. 3). Given the variability in the month-to-month relationships of NO_3^- vs. N_2, NO_3^- must have been internally produced, likely via nitrification. Nitrification in oxic sediments can be an important source of NO_3^- fuelling coupled nitrification-denitrification (Seitzinger 1988) and the rapid cycling of N stored in the sediment was the most likely source to fuel these reactions. The huge variability in N_2 production can also be linked to the variable rates of O_2 loss in *Trapa* beds (Goodwin et al. 2008, Fig. 3B). Gradual O_2 loss would promote nitrification and enhance N_2 production beyond NO_3^- concentration. However, a rapid loss of O_2 caused by enhanced respiratory losses at very high temperatures or an incomplete replenishment of O_2 to the bed would hinder the nitrification–denitrification coupling and N_2 losses would likely reflect the available NO_3^- concentrations only.

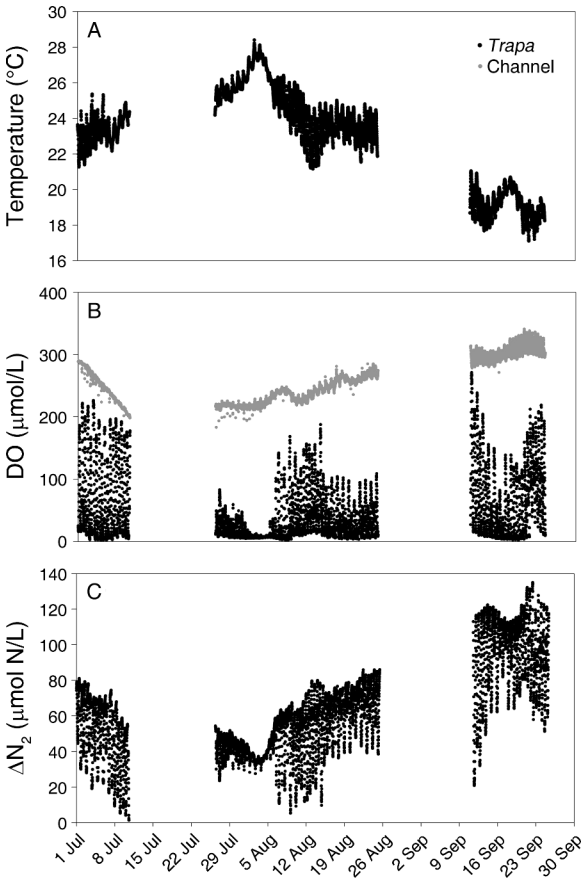

FIG. 4. (A) Temperature change in the inner *Trapa* bed site and (B) DO values for the inner *Trapa* bed site (black dots) and channel site (gray dots), both measured at 15-minute intervals in summer 2006 during periods of ebb tide. (C) Depiction of modeled ΔN_2 using measured DO and temperature as predictor variables (Table 3) during ebb tide. These measurements from midsummer to fall cover the period of maximum floating leaved *Trapa* biomass in the river.

Although other studies have shown direct N uptake by *Trapa* plants to be a significant N sink, removing between 15% and 85% of available dissolved inorganic nitrogen (Tsuchiya and Iwakuma 1993), direct uptake by plants in the TFH was by comparison a small and temporary N loss term. *Trapa* N uptake represents approximately 7% of the total N removed from the TFH. Furthermore this would be only a temporary N storage term as plants would likely release this N during their decay and serve to partially replenish the *Trapa* bed sediment with N.

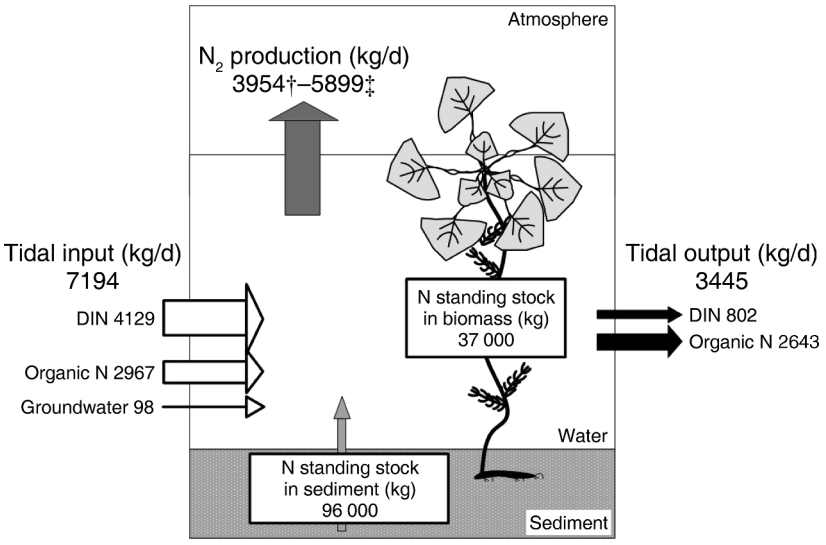

FIG. 5. Fate of N in the 4-km^2 *Trapa* vegetated shallows of the tidal freshwater Hudson (TFH) during the summer months represented using a mass balance approach. Arrows represent the amount of N entering or exiting the beds via different processes in kg N per day. Tidal inputs represent N entering bed with the water during rising tide. Tidal outputs represent N flushed out of the beds with ebbing tide. N_2 production represents N permanently lost to the atmosphere in gas form. N stored in *Trapa* and sediments (kg N) are reported. DIN stands for dissolved inorganic nitrogen.
† Value estimated using areal system respiration.
‡ Value estimated using measured O_2 during ebb tide.

Contrary to expectation, high rates of N_2O production were not observed in *Trapa* beds. In fact, we measured a decrease in N_2O concentration with increasing N_2 production, suggesting that N_2O produced in the beds was ultimately reduced to N_2. Observations of net N_2O consumption in aquatic systems remain rare (Beaulieu et al. 2008, Baulch et al. 2011). However, a review by Chapuis-Lardy et al. (2007) reported that soils could be an important N_2O sink in conditions of low mineral N and large moisture content. One possible mechanistic explanation for this N_2O consumption is that the enzyme NOR, responsible for N_2O reduction to N_2, is more sensitive to oxygen than other denitrification enzymes (Knowles 1982) and hypoxic-anoxic conditions in *Trapa* beds could have enhanced its efficiency at reducing N_2O.

Species can matter in ecosystem function

When compared to native vegetation, invasive plant species are known to strongly influence N dynamics by either altering rates of key microbial processes or modifying standing stocks (Ehrenfeld 2003), but impacts vary widely among species. For example, *Phragmites australis*, an invasive perennial wetland grass is reported to have 60% more N bound in its biomass, and its dominance accelerates the rate of N mineralization as compared to native vegetation (Windham and Ehrenfeld 2003). This species apparently can access the dissolved organic N more effectively and has higher affinity for DIN than does native vegetation (Mozdzer et al. 2010). Alternatively, *Microstegium vimineum*, another invasive wetland grass, has lower N requirements and reduced N remineralization rates when compared to a diverse native community (DeMeester and Richter 2010). This invasive plant lowered the redox potential of the soils, thereby reducing the rates of soil decomposition. N-fixing invasive species are also well known for their impacts on altering N cycling dynamics through their capacity to increase inorganic N pools, influencing overall mineralization and nitrification rates (D'Antonio and Corbin 2003). *Myrica faya*, an exotic N-fixing shrub has completely modified ecosystem properties in Hawaii and is now the largest N source to this once N-limited system (Vitousek and Walker 1989). Although this input of new N to an N-limited system may be perceived as positive, negative impacts may be observed at larger scales. Invasive N-fixing Kudzu and *M. faya* have been reported to double or triple N_2O emissions per unit area as compared to native vegetation (Hall and Asner 2007, Hickman et al. 2010) resulting in a decrease of air quality.

Our study clearly shows that the presence of an exotic and invasive macrophyte significantly enhances the permanent loss of N from the TFH, thereby playing a positive role in whole ecosystem function. Sites invaded by large *Trapa* beds were found to be hot spots of N removal, whereas beds of native *Vallisneria* did not demonstrate significant rates of N loss. The TFH reach is estimated to remove 2000 Mg N/yr or 5480 kg N/d (Lampman et al. 1999). Although the *Trapa* area represents only 2.7% of this 110-km^2 reach of the TFH, our study suggests that around 70% to greater than 100% of this daily removal occurred in the *Trapa* vegetated shallows during the summer months. Further-

more, if we consider that *Trapa* rosette leaves are emergent for only 90 days in a year, *Trapa* beds could remove between 331 and 556 Mg, an impressive 18–27% of the annual N retention, making the summer months a serious "hot moment" of N removal.

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

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

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