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 N2 and N2O 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. N2 production was highly variable between vegetated shallows and was associated with species-driven differences in dissolved oxygen (DO) dynamics during the ebb tide. N2 production was extremely high (37–71 mmol N·m⁻²·d⁻¹) in beds with invasive floating-leaved plants (*Trapa natans*) but was insignificant in submersed native vegetation (*Vallisneria americana*). In *Trapa* sites, N2 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 N2 production. Despite these high denitrification losses, beds acted as N2O sinks where N2O concentrations became undersaturated during ebb tide. An estimate of summertime N2 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 N2O production within these ecosystems has been estimated to account for 30% of terrestrial values (Seitzinger et al. 2006). Until recently, small headwater streams have been the focus of studies examining N uptake and loss on the landscape. Smaller streams usually experience higher N cycling rates owing to their higher benthic to surface water ratios (Bernot and Dodds 2005). Larger rivers have been less well studied, but are generally thought to be of lesser importance than headwater streams for N removal (Alexander et al. 2000, Peterson et al. 2001). Some recent studies suggest, however, that large rivers may play an important role in N uptake (Stanley and Maxted 2008,
Tank et al. 2008, Alexander et al. 2009) as the amount of N removed per meter of reach is greater in large rivers than in small streams (Seitzinger et al. 2002).

In the tidal Hudson River, almost 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 N2 and N2O 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-km² area of the TFH is occupied by two macrophyte species that occur in nearly monospecific beds that can be more than 1 km² in size (Nieder et al. 2004). *Vallisneria americana* is a submerged 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...
sondes are provided in Goodwin et al. (2008). Drift corrections, deployment, and recovery of the surface on a permanently moored buoy in 7 m of water. Water site in the main stem of the river, 2 m below the sediment. Sondes were also set in a nearby open-channel at 700 m from the edge of the bed, 0.2–0.3 m above the bed. On 17 July and 13 September 2006, the Trapa biomass was at its annual maximum and 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 km² 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 O₂ m⁻² h⁻¹) is slightly greater than respiration, resulting in a slightly positive oxygen balance in these beds and a general increase in oxygen concentrations during daytime ebb tides (Caraco and Cole 2002).

Field sampling.—Sampling was conducted when Trapa biomass was at its annual maximum and plant leaves were floating at the surface. To access our sites in the Trapa bed, we used a channel at the eastern edge of the bay which connected the main stem of the river to the back of the bed. On 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 (N₂) 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 HgCl₂, 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 N₂ concentrations in water were measured using a quadrupole inlet mass spectrometer (MIMS; Bay Instruments, Easton, Maryland, USA) and N₂ production was determined by looking at changes in N₂: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%). N₂ concentration was determined from N₂:Ar ratio as

$$[N₂] = \frac{(N₂:Ar)_{spl}}{(N₂:Ar)_{std}} \times (N₂)_{sat}$$

where (N₂:Ar)_{spl} is the measured ratio of the water sample, (N₂:Ar)_{std} is the measured ratio of the standard (both corrected for instrument drift), and (N₂)_{sat} is the N₂ 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 N₂O (p N₂O) 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. N₂O was measured by gas chromatography using an ECD detector on 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 NO₃⁻ only for the rest of the text, NH₄⁺, dissolved organic carbon [DOC], and PO₄ 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 H2SO4 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 N2 production.**—“Denitrification” and “N2 production” are used interchangeably in the text, although we recognize that some of the N2 could have been produced via the anammox pathway. N2 production was estimated as the deviation in the concentration of N-N2 (ΔN2) in the *Trapa* bed relative to concentrations 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 (ΔADO, ΔO2, and ΔNO3

**NO3

**[N2]** production was determined two ways. First, using the model described above that combines ΔADO and temperature to determine an average summertime loss estimate during the ebb period. Second, we used the ratio of N2 produced per unit O2 consumed (0.303, equivalent to the slope for our linear regression model between ΔN2 and ΔDO only) combined with a previously measured rate of areal respiration (233 mmol O2 m⁻² d⁻¹ for sediment, submerged *Trapa* leaves, stems, and roots) in *Trapa* beds (Caraco and Cole 2002). A range of N2 production was determined to ways: by using long-term monitoring of O2 concentrations in the beds as compared to the channel, measured with sondes during ebbing 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 O2 changes at the scale of the whole bed.

**Tidal inputs to beds (Nin) as NO3

**[N2]** and organic N were calculated as follows:

\[
N_{\text{in}} = \left| N \right| \times \text{TD} \times 1.92
\]

where \(|N|\) represents the average N concentrations of the various N species (in μmol N/L with TN = 55, NO3⁻ = 30 and NH4⁺ = 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-km² 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 (\(N_{\text{out}}\)) to the channel needed to be accounted for at more refined intervals (15 minutes). This was calculated using the following approach:

\[
N_{\text{out}} = \sum_{t=1}^{n} \frac{|N_t| \times (Z_t - Z_{t+1})}{D_{\text{tot}}}
\]

where \(N_t\) is the modeled N concentration (TN, NO3⁻, or NH4⁺ 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 \(D_{\text{tot}}\) is the total number of days used to estimate \(N_{\text{out}}\).

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 O2 concentrations, with the exception of NH4⁺, which was always found at low concentrations (Table 1).
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/m² scaled up to 4 km² for Trapa beds. N bound in plant biomass was calculated based on Trapa density (50 plants per m²), 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% (Table 2) while N_2 on a given date. The slopes of the relationships varied significantly among sampling dates (ANCOVA, R² = 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 N_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^−.

### 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 N_2 on a given date. The slopes of the relationships varied significantly among sampling dates (ANCOVA, R² = 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.

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<tbody>
<tr>
<td>DO (mg/L)</td>
<td>4.02 (0.53–7.64)</td>
<td>7.03 (6.73–7.58)</td>
<td>5.66 (0.48–9.39)</td>
<td>8.46 (8.16–8.78)</td>
<td>4.33 (2.10–6.61)</td>
<td></td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>26.42 (25.66–27.44)</td>
<td>26.29 (25.77–27.65)</td>
<td>19.72 (18.05–20.82)</td>
<td>20.35 (20.18–20.70)</td>
<td>23.38 (22.16–24.26)</td>
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<tr>
<td>NO_3^− (µmol N/L)</td>
<td>16.83 (8.84–36.41)</td>
<td>30.32 (29.45–34.00)</td>
<td>18.05 (12.1–26.33)</td>
<td>26.24 (24.84–27.67)</td>
<td>17.02 (1.43–28.57)</td>
<td></td>
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<tr>
<td>NH_4^+ (µmol N/L)</td>
<td>1.92 (0.98–3.58)</td>
<td>2.47 (2.09–2.82)</td>
<td>1.14 (0.44–1.58)</td>
<td>0.79 (0.15–1.20)</td>
<td>5.13 (0.00–15.71)</td>
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<tr>
<td>TN (µmol N/L)</td>
<td>35.32 (17.07–54.34)</td>
<td>49.64 (47.15–57.43)</td>
<td>45.01 (24.84–36.75)</td>
<td>45.79 (40.54–49.59)</td>
<td>45.79 (40.54–49.59)</td>
<td></td>
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<tr>
<td>DOC (mg/L)</td>
<td>4.64 (3.58–8.22)</td>
<td>4.34 (3.73–4.89)</td>
<td>3.82 (3.67–4.13)</td>
<td>3.69 (3.41–3.91)</td>
<td>3.82 (3.67–4.13)</td>
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<tr>
<td>PO_4 (µmol P/L)</td>
<td>0.49 (0.16–0.90)</td>
<td>0.80 (0.77–0.84)</td>
<td>0.33 (0.09–0.54)</td>
<td>0.44 (0.27–0.32)</td>
<td>0.33 (0.09–0.54)</td>
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<tr>
<td>TP (µmol P/L)</td>
<td>1.54 (0.90–2.56)</td>
<td>2.21 (1.88–3.24)</td>
<td>1.74 (0.76–4.82)</td>
<td>1.74 (0.76–4.82)</td>
<td>1.74 (0.76–4.82)</td>
<td></td>
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<tr>
<td>Chl a (µg/L)</td>
<td>2.07 (0.69–3.51)</td>
<td>2.98 (2.48–3.41)</td>
<td>3.97 (1.49–6.80)</td>
<td>4.12 (3.27–5.08)</td>
<td>4.12 (3.27–5.08)</td>
<td></td>
</tr>
</tbody>
</table>

Notes: Key to abbreviations: DOC, dissolved organic carbon; TP, total phosphorus; chl a, chlorophyll a. Data are not available when cells are empty.
were warmer, less N\textsubscript{2} was produced for the same change in DO and NO\textsubscript{3}/C\textsubscript{0} as compared to the two other sampling dates.

To estimate N\textsubscript{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 \(\Delta N\textsubscript{2}\) and \(\Delta NO\textsubscript{3}/C\textsubscript{0}\) 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 \(\Delta N\textsubscript{2}\) and \(\Delta DO\) was much stronger with an adjusted \(R^2\) of 0.56, suggesting that overall change in N\textsubscript{2} was more tightly coupled with changes in DO regardless of timing. \(\Delta N\textsubscript{2}\) was also negatively related with temperature suggesting that some of the observed change in N\textsubscript{2} was a function of a change in physical solubility and not necessarily biological production. However the best and most parsimonious model to predict \(\Delta N\textsubscript{2}\) used both temperature and \(\Delta DO\) as predictor variables (AIC = 274.17; Table 3).

To estimate the variability in N\textsubscript{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\textdegree{} to 4\textdegree{}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\textsubscript{2} production. N\textsubscript{2} production was highly variable within a single day and during summer (Fig. 4C), with excess N\textsubscript{2} varying throughout the summer from 1 to >150 \(\mu\)mol N/L. Based on this model, N\textsubscript{2} production was on average 47 \(\mu\)mol N/L per ebbing tide or around 7 \(\mu\)mol N L\textsuperscript{-1}h\textsuperscript{-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

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**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\textsubscript{3}/C\textsubscript{0}, (C) N\textsubscript{2}, and (D) N\textsubscript{2}O concentrations measured in September 2006 during ebbing tide at different sites.
the beds (55–82%) was transformed to N₂ gas and permanently eliminated from the ecosystem. Transformation from the DIN pool to N₂ gas was the main loss term, where N₂ 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 N₂ 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 stock 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 N₂ 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 N₂ concentrations in native *Vallisneria* beds. Our average daily rate estimates of N₂ production of 88 ± 51 μmol N L⁻¹ 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 N₂ production was occurring during ebbing tide only when rates were actually measured. The average hourly rate of N₂ production during ebb was extremely high (7 μmol N L⁻¹ 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 N₂ production in *Trapa* beds was intimately linked with localized O₂ consumption and system metabolism. The contrasting O₂ 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,

![Graphs showing regression relationships between DIN and N₂ production](image)

**Fig. 3.** (A) Simple linear regression relationships between ΔNO₃⁻ and ΔN₂ for each sampling date: July 2006, ΔN₂ = (−0.79 × ΔNO₃⁻) + 0.83 with R² = 0.55, n = 9, F test, P ≤ 0.01; September 2006, ΔN₂ = (−4.60 × ΔNO₃⁻) + 12.47 with R² = 0.96, n = 11, F test, P ≤ 0.0001; and July 2007, ΔN₂ = (−2.53 × ΔNO₃⁻) − 3.14 with R² = 0.89, n = 13, F test, P ≤ 0.0001. (B) Simple linear regression relationships between ΔDO and ΔN₂ for each sampling date: July 2006, ΔN₂ = (−0.13 × ΔDO) + 0.39 with R² = 0.56, n = 9, F test, P ≤ 0.01; September 2006, ΔN₂ = (−0.42 × ΔDO) + 3.48 with R² = 0.97, n = 11, F test, P ≤ 0.0001; and July 2007, ΔN₂ = (−0.49 × ΔNO₃⁻) − 9.25 with R² = 0.93, n = 21, F test, P ≤ 0.0001.

**Table 3.** Results of simple regressions and multiple regressions of change in N₂ (ΔN₂, in μmol N/L) with change in oxygen (ΔDO, in μmol/L), change in nitrate (ΔNO₃⁻, in μmol N/L), and temperature (Temp, in °C).

<table>
<thead>
<tr>
<th>Model</th>
<th>N</th>
<th>P</th>
<th>F</th>
<th>df</th>
<th>R²</th>
<th>AIC</th>
</tr>
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<tbody>
<tr>
<td>ΔN₂ = -0.30(ΔDO) + 2.22</td>
<td>41</td>
<td>&lt;0.0001</td>
<td>52.03</td>
<td>1.39</td>
<td>0.56</td>
<td>317.17</td>
</tr>
<tr>
<td>ΔN₂ = -7.30(Temp) + 201.33</td>
<td>41</td>
<td>&lt;0.0001</td>
<td>24.40</td>
<td>1.39</td>
<td>0.37</td>
<td>321.73</td>
</tr>
<tr>
<td>ΔN₂ = -1.31(ΔNO₃⁻) + 14.95</td>
<td>35</td>
<td>0.0044</td>
<td>9.53</td>
<td>1.33</td>
<td>0.20</td>
<td>332.32</td>
</tr>
</tbody>
</table>

Multiple regression models

<table>
<thead>
<tr>
<th>Model</th>
<th>N</th>
<th>P</th>
<th>F</th>
<th>df</th>
<th>R²</th>
<th>AIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔN₂ = -0.28(ΔDO) − 6.53(Temp) + 154.06</td>
<td>41</td>
<td>&lt;0.0001</td>
<td>132.70</td>
<td>2.38</td>
<td>0.87</td>
<td>274.17</td>
</tr>
<tr>
<td>ΔN₂ = -9.45(Temp) − 1.98(ΔNO₃⁻) + 222.45</td>
<td>35</td>
<td>&lt;0.0001</td>
<td>88.39</td>
<td>2.32</td>
<td>0.84</td>
<td>279.20</td>
</tr>
<tr>
<td>ΔN₂ = -0.51(ΔDO) + 1.75(ΔNO₃⁻) + 3.01</td>
<td>35</td>
<td>&lt;0.0001</td>
<td>32.34</td>
<td>2.32</td>
<td>0.65</td>
<td>309.34</td>
</tr>
<tr>
<td>ΔN₂ = -0.21(ΔDO) − 7.35(Temp) − 0.58(ΔNO₃⁻) + 171.34</td>
<td>35</td>
<td>&lt;0.0001</td>
<td>84.24</td>
<td>2.32</td>
<td>0.88</td>
<td>274.20</td>
</tr>
</tbody>
</table>

*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 $\Delta$N$_2$ and $\Delta$NO$_3^-$ 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$_3^-$ fuelled 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.

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.
Contrary to expectation, high rates of N\textsubscript{2}O production were not observed in \textit{Trapa} beds. In fact, we measured a decrease in N\textsubscript{2}O concentration with increasing N\textsubscript{2}O production, suggesting that N\textsubscript{2}O produced in the beds was ultimately reduced to N\textsubscript{2}. Observations of net N\textsubscript{2}O 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\textsubscript{2}O sink in conditions of low mineral N and large moisture content. One possible mechanistic explanation for this N\textsubscript{2}O consumption is that the enzyme NOR, responsible for N\textsubscript{2}O reduction to N\textsubscript{2}, is more sensitive to oxygen than other denitrification enzymes (Knowles 1982) and hypoxic-anoxic conditions in \textit{Trapa} beds could have enhanced its efficiency at reducing N\textsubscript{2}O.

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, \textit{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, \textit{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). \textit{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 \textit{M. faya} have been reported to double or triple N\textsubscript{2}O 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 \textit{Trapa} beds were found to be hot spots of N removal, whereas beds of native \textit{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 \textit{Trapa} area represents only 2.7% of this 110-km\textsuperscript{2} reach of the TFH, our study suggests that around 70% to greater than 100% of this daily removal occurred in the \textit{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 O₂ and N cycling, whereby *Trapa* vents O₂ 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 O₂ 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 O₂ 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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**Literature Cited**


