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Denitrification Capacity in a Subterranean Estuary below a Rhode Island Fringing Salt Marsh

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ABSTRACT: Coastal waters are severely threatened by nitrogen (N) loading from direct groundwater discharge. The subterranean estuary, the mixing zone of fresh groundwater and sea water in a coastal aquifer, has a high potential to remove substantial N. A network of piezometers was used to characterize the denitrification capacity and groundwater flow paths in the subterranean estuary below a Rhode Island fringing salt marsh. ^{15}N-enriched nitrate was injected into the subterranean estuary (in situ push-pull method) to evaluate the denitrification capacity of the saturated zone at multiple depths (125–300 cm) below different zones (upland-marsh transition zone, high marsh, and low marsh). From the upland to low marsh, the water table became shallower, groundwater dissolved oxygen decreased, and groundwater pH, soil organic carbon, and total root biomass increased. As groundwater approached the high and low marsh, the hydraulic gradient increased and deep groundwater upwelled. In the warm season (groundwater temperature >12°C), elevated groundwater denitrification capacity within each zone was observed. The warm season low marsh groundwater denitrification capacity was significantly higher than all other zones and depths. In the cool season (groundwater temperature <10.5°C), elevated groundwater denitrification capacity was only found in the low marsh. Additions of dissolved organic carbon did not alter groundwater denitrification capacity suggesting that an alternative electron donor, possibly transported by tidal inundation from the root zone, may be limiting. Combining flow paths with denitrification capacity and saturated porewater residence time, we estimated that as much as 29–60 mg N could be removed from 1 l of water flowing through the subterranean estuary below the low marsh, arguing for the significance of subterranean estuaries in annual watershed scale N budgets.

Introduction

Approximately 60% of coastal rivers and bays in the United States have been moderately to severely degraded by nutrient pollution (Howarth et al. 2002). Nitrogen (N) loading accelerates eutrophication in estuarine waters and can spur harmful algal blooms, hypoxia, the decline of eelgrass, and the destruction of critical spawning habitats (Valiela et al. 1990; Nixon 1995; Oviatt et al. 1995; Short and Burdick 1996; Nixon et al. 2001). Direct groundwater discharge in some coastal settings can be a substantial contributor of nitrate to estuaries (Giblin and Gaines 1990; Valiela et al. 1990, 1992; Barlow 2003). As this nitrate-laden groundwater flows toward the coast, it mixes with sea water that has infiltrated the aquifer in a transition zone, sometimes referred to as the zone of dispersion (Barlow 2003) or the subterranean estuary (Moore 1999), producing groundwater of intermediate salinity. Coastal aquifers with groundwater flow and subterranean estuaries are common along the eastern coast of the U.S. (Barlow 2003) and in other parts of the world's coastline where sand deposits exist (Burnett et al. 2001). In these areas the subterranean estuary is a saturated zone of permeable mineral deposits with flow responding to changes in pressure rather than capillary tension. These subterranean estuaries need to be explored to determine if they are substantial sinks for watershed N to help account for the large amounts of missing or unaccounted N in numerous watershed studies (Howarth et al. 1996, 2003; Valiela et al. 1997; Brawley et al. 2000; Castro et al. 2003).

Riparian zones, the land-water margin between uplands and streams, can markedly decrease the flux of groundwater N from watersheds (Hill 1996; Correll 1997; Lowrance et al. 1997), even when groundwater flows primarily through sandy subsoils (Gold et al. 2001; Kellogg et al. 2005). Like riparian zones, subterranean estuaries are similarly poised at the land-water margin and may contain similar attributes—most notably sources of electron donors and an anoxic environment (Seitzinger 1988;

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Korom 1992; Hedin et al. 1998; Nowicki et al. 1999; Joye 2002) both of which have been shown to support groundwater denitrification, the microbially mediated transformation of nitrate to gaseous N (N_2 and N_2O).

Most studies of N cycling at the land-water margin of estuaries have focused on the organically-enriched peat at the marsh surface (Kaplan et al. 1979; Childers and Day 1988; Childers 1994; Anderson et al. 1997; Wigand et al. 2004). These studies have generally found that N is transformed in the surface soil and can serve as a potential sink of N, especially from tidal input (LaMontagne and Valiela 1995; LaMontagne et al. 2002). Groundwater movement through the subterranean estuary, which can extend in depth down to several meters depending on the total thickness of the aquifer (Barlow 2003), is often the major mechanism transporting N to estuaries in areas underlain by unconsolidated coarse sediments (Capone and Bautista 1985; Valiela et al. 1990, 1992; Portnoy et al. 1998). Groundwater flux may bypass peat at the marsh surface due to its low hydraulic conductivity (K). Groundwater is more likely to flow in the more conductive sandy mineral subsoil below the salt marsh peat at the soil surface.

Several mechanisms have the potential to provide labile carbon (C) and promote denitrification in the mineral subsoil of nitrate-enriched subterranean estuaries. The water table of the subterranean estuary rises and falls as the piezometric head at the land-water margin changes in response to tidal action (Gardner et al. 2002). These water table fluctuations can bring dissolved organic carbon (DOC) originating from the surficial sources into the groundwater of the subterranean estuary. Potential sources of DOC to the subsurface include peat deposits, roots, rhizomes, and organic debris accumulated through surface wrack. Buried organic matter also may be found within the mineral deposits of the subterranean estuary as a result of historic flooding events, sea level rise, or roots and rhizomes that occasionally occur at deeper depths (Stolt and Rabenhorst 1991).

Denitrification in the subterranean estuary may be substantial. Talbot et al. (2003) tracked high concentrations of groundwater nitrate through the upland into the subterranean estuary down to 8.5 m below the land surface. They suggested denitrification was responsible for removing nitrate in the saline groundwater of the subterranean estuary. Tobias et al. (2001c) tracked the fate of an introduced ^{15}N-enriched nitrate plume in the shallow groundwater upgradient from a fringing salt marsh. Following this plume in the subterranean estuary, they found 90% groundwater nitrate removal in the surface 10 cm with denitrification as the primary removal mechanism. At their site, a lower permeability, finer textured layer confined groundwater flow to the marsh surface. Ueda et al. (2003) found qualitative evidence for denitrification 0–15 cm below the water table in the subterranean estuary at a sandy beach location.

Numerous riparian investigators have argued that groundwater flow paths are critically important in determining the significance of the groundwater N sink function (Cirmo and McDonnell 1997; Cey et al. 1999; Devito et al. 2000; Hill et al. 2000; Maitre et al. 2003). While groundwater may enter a salt marsh via surface seeps at the upland-marsh boundary (Howes et al. 1996), groundwater may also travel in long, deep groundwater flow paths via submarsh flow through coarse unconsolidated mineral subsoil (Schultz and Ruppel 2002) that compose the subterranean estuary. Following these flow paths and studying N transformations in mineral deposits underlying salt marshes is critical to understanding the extent of nitrate removal taking place in the subterranean estuary prior to groundwater discharge into the estuary.

Researchers studying N cycling at the salt marsh surface (Kaplan et al. 1979; Valiela and Teal 1979b; Wigand et al. 2004) and in deeper groundwater flow in the subterranean estuary (Tobias et al. 2001a) and freshwater riparian zones (Nelson et al. 1995; Kellogg et al. 2005) have found conflicting results in seasonal denitrification trends. Potential seasonal trends in nitrate transformations within the subterranean estuary warrant further attention to understand annual N cycling.

In addition to interest in water quality, there are concerns that N sink areas may be important sources of N_2O, a potent greenhouse gas (Prather et al. 1995). Small areas of the landscape with high rates of subsurface denitrification due to anthropogenic loading, such as riparian wetlands, may be significant sources of this gas at the regional scale (Groffman et al. 1998, 2000; Hefting et al. 2003), and we suggest that the subterranean estuary warrants consideration in regional assessments of the effects of humans on atmospheric chemistry and physics.

In this study, a network of piezometers was used to characterize ambient groundwater, determine groundwater flow paths, and evaluate denitrification capacity (with ^{15}N-enriched nitrate) during different seasons in the subterranean estuary below a Rhode Island (U.S.) fringing salt marsh. We examined multiple depths (125–300 cm) in three different zones of the subterranean estuary below the salt marsh: the upland-marsh transition zone, the high marsh, and the low marsh. We also explored how the addition of DOC to the aquifer affected denitrification in the subterranean estuary.

Methods

STUDY AREA

We examined the subterranean estuary below a fringing salt marsh along Brushneck Cove, a tidally influenced cove of Greenwich Bay, Rhode Island (41°41′N, 71°24′W). The upland of the study area was dominated by a mixed oak (*Quercus* species) forest in a community park. The study area extended 25 m from the open water to the upland edge. While our specific study site was approximately 30 m wide, the entire salt marsh stretches at least 2 km on the western side of the cove. Three zones were classified at this salt marsh: the upland-marsh border, hereafter referred to as the transition zone, the high marsh, and the low marsh.

In all of these zones, we assessed the in situ groundwater denitrification capacity in the sandy subsoil underlying the peat and mineral soil layers at the marsh surface. The transition zone had a thin organically-enriched horizon (4 cm thick) above at least 400 cm of sand. Transition zone soils were somewhat poorly drained Typic Psammaquents (Soil Survey Staff 1999) and were tidally inundated intermittently. The high and low marshes had surface peat deposits 50–120 cm thick underlain by sand to at least 300 cm. High and low marsh soils were very poorly drained Terric Sulfihemists (Soil Survey Staff 1999) and were tidally inundated twice daily. Vegetation was dominated by marsh elder (*Iva frutescens*L.), sea lavender (*Limonium nashii* Small), and seaside goldenrod (*Solidago sempervirens*L.) in the transition zone, the short form of smooth cordgrass (*Spartina alterniflora* Loiseleur) in the high marsh, and the tall form of smooth cordgrass in the low marsh.

SITE INSTRUMENTATION AND CHARACTERIZATION

All groundwater (saturated porewater) samples were collected by piezometers or water table wells located in the saturated zone of the subterranean estuary below the capillary fringe. Water in these sampling devices was below the water table under positive fluid pressure. To evaluate groundwater flow paths through the salt marsh, a network of 15 drive-point piezometers (Model 615, 1.9 cm o.d., 15.2 cm screen length; Solinst, Georgetown, Ontario, Canada) was established. These piezometers were placed in a triangulated network along a series of transects from the upland to the estuary at depths ranging from 85 to 530 cm.

For in situ groundwater denitrification capacity testing and ambient groundwater characterization, we installed a network of mini-piezometers (0.8 cm o.d.; 2 cm screen length; AMS, American Falls, Idaho) connected to gas-impermeable Teflon tubing (0.7 cm o.d.) that extended above the ground surface. Three replicate mini-piezometers were placed at depths of 125, 200, and 300 cm in the transition zone, 125 and 200 cm in the high marsh, and 125 cm in the low marsh (Fig. 1). We selected 125 cm depths so that we could always collect groundwater from the sandy mineral soil below the water table; peat in the high and low marshes and a deep water table in the transition zone restricted shallower sampling. The deeper depths were arbitrarily chosen so that we would be sampling from an environment possibly disconnected from surficial influences. Mini-piezometers at a given depth were at least 2.5 m apart laterally. Groundwater was pumped with a Masterflex L/S portable peristaltic pump (Cole Parmer, Vernon Hills, Illinois). Water table wells were also installed within each zone.

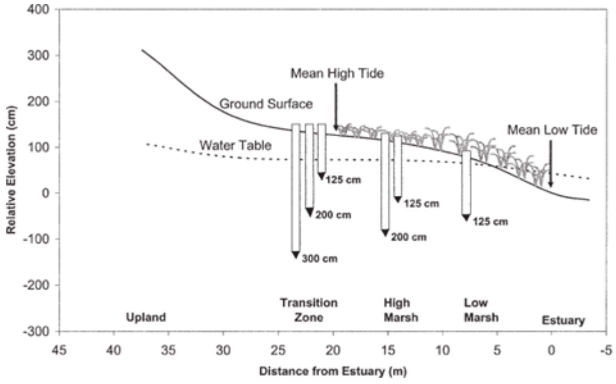

Fig. 1. Location of push-pull mini-piezometers (three replicates per location) in the transition zone, high marsh, and low marsh.

In October 2000, soil pits were dug into the subterranean estuary below the water table within each zone of the study area to a depth of 150–200 cm in an area adjacent to piezometers and wells, but at least 3 m from any sampling piezometer. Groundwater was pumped from the pits with a high-volume pump to enable soil characterization and sampling below the water table. The pit soils were described and characterized into soil horizons (Soil Survey Staff 1999). Bulk soil samples were taken from each soil horizon at low tide. Particle size distribution, soil organic carbon (SOC), and root biomass were determined on samples from each soil horizon.

DETERMINATION OF GROUNDWATER FLOW PATHS

Piezometric heads were measured on September 27, 2001, at low tide, when tidal pumping was at a minimum and the hydraulic gradient through the subterranean estuary is expected to favor groundwater movement toward the estuary (Gardner et al. 2002). A water level meter (Solinst Model 101, Georgetown, Ontario, Canada) was used to measure the height of the water in the piezometers. Piezometric heads were also measured in select piezometers in May 2002 at low tide; vandalism at our site prohibited us from collecting a full set of measurements at this time. Water level elevations were converted to freshwater hydraulic head measurements by correcting for water density (Drexler and Ewel 2001). Water table contour maps and equal-potential lines were determined by triangulation (Freeze and Cherry 1979). In situ K was measured by a slug test (Freeze and Cherry 1979) in the mini-piezometers. In the low marsh, we installed additional mini-piezometers at 50 and 75 cm depths in the marsh soil to assess K. Due to the difficulty in observing water levels in the narrow mini-piezometers, we added an extension of tubing mounted to a frame above the ground surface where we could easily monitor the change in water level during the slug test.

IN SITU DENITRIFICATION PUSH-PULL STUDIES

Groundwater denitrification was measured with the in situ push-pull method at each mini-piezometer (Istok et al. 1997; Addy et al. 2002). This push-pull method uses a single mini-piezometer to push (i.e., inject) and pull (i.e., extract) a groundwater plume containing ^{15}N-enriched nitrate and sulfur hexafluoride (SF_6), a conservative gas tracer. Plume extraction occurs after a preset incubation period, and the groundwater samples are analyzed for select ^{15}N-enriched denitrification gases (N_2O and N_2). This push-pull method does not assess ambient denitrification rates. With this method, denitrification was not N limited. The rates obtained represented groundwater denitrification capacity, how much groundwater denitrification could occur if ample groundwater nitrate were present under current site conditions, including electron donor abundance and soil-groundwater characteristics.

Prior to the denitrification studies, an in situ conservative tracer pretest was conducted in at least one mini-piezometer at each depth within a zone, allowing us to determine a suitable incubation time to achieve at least 70% tracer recovery. To create a pretest solution, SF_6 (100 μl l^{-1} SF_6, balanced in helium; Matheson Trigas, Gloucester, Massachusetts) was bubbled into 10 l of previously collected groundwater. The pretest solution was pushed into the mini-piezometer over a period of 1 h at an approximate injection rate of 160 ml min^{-1}. The saturated K of the sandy media at each mini-piezometer location was relatively high, allowing the mini-piezometers to accommodate this injection rate. After an incubation period of 5 h, groundwater was pulled from the same mini-piezometer. Incubation times were adjusted for the nitrate push-pull test based on tracer recovery and were 4–5 h at all zones and depths. Push-pull pretests were started approximately 3 h before low tide. Following the conservative tracer pretests, we removed two to three times the push volume and post tested the mini-piezometers to ensure the SF_6 concentrations returned to negligible levels before conducting the nitrate push-pull test.

The nitrate push-pull tests were conducted from 2000 to 2002. Shortly before the commencement of nitrate push-pull tests, enough groundwater was collected from each zone and depth to be tested in order that we used the same groundwater throughout a season at a particular location. All groundwater was stored at 4°C until the nitrate push-pull test. Immediately prior to the push-pulls, we sampled each mini-piezometer for ambient groundwater dissolved oxygen (DO), temperature, and salinity and collected samples for later analysis of ambient pH, DOC, NO_3^--N, and N_2, N_2O, and SF_6 gases. Ten liters of groundwater was amended with 32 mg l^{-1} NO_3^--N as KNO_3 enriched with 20 atom % ^{15}N and SF_6. SF_6 (100 μl l^{-1} SF_6, balanced in helium; Matheson Trigas, Gloucester, Massachusetts) was bubbled into the amended groundwater to serve as the gaseous tracer and to adjust the DO to ambient concentrations per each mini-piezometer location. The solution was injected into the dosing mini-piezometers approximately 3 h before low tide.

We used temperature of the shallow, groundwater in the subterranean estuary to categorize sampling seasons that reflected potential differences in biological transformation rates. Groundwater temperature follows a well-described geothermal gradient with groundwater at the surface responding to air temperature to a greater extent than deep groundwater (Freeze and Cherry 1979; Goni and Gardner 2003). The warm season was defined as times when the groundwater temperature was >12°C in the summer and fall. The cool season was defined as times when the groundwater temperature was <10.5°C in the spring. Mini-piezometers at 125 cm in all zones and 200 cm in the transition zone were tested in both the cool and warm seasons. Mini-piezometers at 300 cm in the transition zone and 200 cm in the high marsh were only assessed in the warm season (after we noted minimal groundwater denitrification in the cool season at shallower depths in these zones). During each season of assessment, the mini-piezometers being studied were subjected to a nitrate push-pull test twice, with a 4-wk interval between trials. After the first nitrate push-pull test, we extracted two to three times the push volume and measured SF_6 to ensure that it had returned to ambient levels. If necessary, we pumped additional water out of the mini-piezometer until ambient SF_6 concentrations were reached. The first push-pull test was performed for the possibility that the subsurface microbial community might require priming to reach denitrification capacity (Groffman 1994; Aelion and Shaw 2000). Data presented here are based on the second push-pull test to minimize priming issues.

In October 2002, 4 wk after the second nitrate push-pull test, we conducted a third set of push-pull tests in the transition zone 200-cm and low marsh 125-cm mini-piezometers to determine whether carbon limitation affected denitrification. The groundwater in these trials was amended with 145 mg C l^{-1} as sodium acetate in addition to the 32 mg l^{-1} NO_3^--N as KNO_3 enriched with 20 atom % ^{15}N and SF_6, as previously described. Four and two days prior to this C-enriched push-pull test, we also injected 10 l of groundwater amended with 145 mg C l^{-1} as sodium acetate into the mini-piezometer to allow for microbial priming.

Samples of the dosing solution were taken for dissolved gas analysis (N_2O, N_2, $^{15}N_2O$, $^{15}N_2$, and SF_6) during each push phase. The dosing solutions were pushed into mini-piezometers over a period of 1 h at an injection rate of 160 ml min^{-1}. After an incubation period of 4–5 h, samples were obtained from each mini-piezometer during the pull phase. The pumping rate during pull phase sampling was between 100 and 200 ml min^{-1}. Sampling was restricted to the first 3 l where tracer recovery is highest, the core of the plume, as follows: at 0.5-l intervals for dissolved gases (N_2, N_2O, $^{15}N_2$, $^{15}N_2O$, and SF_6), and 1-l intervals for NO_3^--N, resulting in 6 gas samples and 3 liquid samples from each mini-piezometer's core of the plume. We then sampled for groundwater DO, temperature, and salinity. All groundwater samples were stored on ice in coolers in the field and at 4°C in the laboratory.

Groundwater to be analyzed for dissolved gases (N_2, N_2O, $^{15}N_2$, $^{15}N_2O$, SF_6) was collected using a syringe attached to a port in a bucket filled with deionized water; by sampling under water, we minimized atmospheric contamination. Groundwater for SF_6 analysis was kept in the syringe under water iced or refrigerated at 4°C until analysis. Groundwater for all other gas analyses was injected into a previously evacuated serum bottle capped with a rubber septum. The headspace was then filled with high-purity helium gas. We used the phase equilibration headspace extraction technique (Lemon 1981; Davidson and Firestone 1988), storing samples at 4°C overnight, shaking, and sampling the bottle headspace with a syringe. Samples to be analyzed for DOC were filtered and collected in 45 ml amber glass bottles, fixed with phosphoric acid at 85% strength, and stored at 4°C.

Denitrification Rate Calculation

We calculated denitrification gas (N_2O-N and N_2) generation rates using the 3 gas samples that had the highest tracer recovery (of 6 within-sample replicates), minimizing error from dilution and dispersion. Because of our relatively brief incubation period combined with low ambient groundwater ammonium concentrations (always <0.3 mg N l^{-1}), we assumed negligible nitrification. All samples used in denitrification capacity calculations contained at least 2 mg l^{-1} NO_3^--N to ensure that our denitrification rate estimates were not nitrate-limited (Schipper and Vojvodic-Vukovic 1998). To calculate masses of N_2O-N and N_2 gases in headspace extraction samples, we used equations and constants provided by Tiedje (1982) and Mosier and Klemedtsson (1994). The mass was then transformed to the mass of $^{15}N_2O$-N or $^{15}N_2$ by multiplying it by the respective ^{15}N sample enrichment. The masses of $^{15}N_2O$-N or $^{15}N_2$ generated during the incubation period were calculated as the mass present in the pulled sample minus the mass present in the pushed sample. The total masses of N_2O-N and N_2 generated during the incubation period were then calculated by dividing the masses of $^{15}N_2O$-N and $^{15}N_2$ by the pushed NO_3^--N atom %.

Gas production rates (N_2O-N + N_2) were expressed as μg N l^{-1} water d^{-1}. The incubation period was defined as the length of time between the end of the push phase and the start of the pull phase to reflect the incubation of the core of the plume. Denitrification rates may be slightly underestimated since we did not measure NO_2^- and NO, other intermediates of the denitrification process, although these forms of N do not usually account for a substantial portion of denitrification products.

Analytical Methods

Dissolved oxygen and temperature were measured with a YSI DO-temperature model 55 meter (YSI, Yellow Springs, Ohio) in groundwater as it was continuously pumped into and overflowed a 150 ml sample bottle to minimize atmospheric contamination. Groundwater samples were analyzed for NO_3^--N using the SM 4500 NO_3 F automated cadmium reduction method (APHA 1998) on an Alpkem RFA 300 Rapid Flow Autoanalyzer (O.I. Analytical, Wilsonville, Oregon), DOC by infrared analysis using an O.I. Corporation Model 1010 Carbon Analyzer (College Station, Texas), pH on an Accumet Model 925 pH meter (Fisher Scientific, Pittsburgh, Pennsylvania), and salinity on a YSI salinity-conductivity-temperature model 30 meter (YSI, Yellow Springs, Ohio). Concentrations and isotopic composition of N_2 and N_2O gases were determined on a PDZ Europa 20-20 continuous flow isotope ratio mass spectrometer coupled to a PDZ Europa TGII trace gas analyzer (Sercon Ltd., Cheshire, U.K.) at the Stable Isotope Facility, University of California Davis, Davis, California. Concentrations of SF_6 were measured using a gas stripping-trapping system connected to a Hewlett Packard Model 5890A gas chromatograph with a 2 m Poropak Q column and electron capture detector at 350°C. Concentrations of headspace equilibrated N_2O gases were analyzed by electron-capture gas chromotography on a Tracor Model 540 (Thermo-Finnigan, Austin, Texas).

Samples taken from the soil pits were analyzed for SOC on a Carlo Erba NA 1500 Series 2 CN Analyzer (CE Instruments [now ThermoFinnigan Italia], Milan, Italy). In one undisturbed soil subsample (minimum 800 g of soil) from each soil horizon, we extracted all visible roots and rhizomes with forceps. We did not distinguish between live and dead roots or rhizomes. Roots and rhizomes were washed using a 2% sodium hexametaphosphate dispersing agent (Kilmer and Alexander 1949), dried in a 65°C oven for 6 h, and weighed. Total root biomass (g m^{-2}) combines root and rhizome biomass.

STATISTICAL ANALYSES

We used the Mann-Whitney U test (Ott 1993) to determine significant differences in groundwater denitrification rates between depths within zones and between nitrate push-pull rates and C-enriched nitrate push-pull rates. Spearman's rank order correlations were performed to determine significant correlations between groundwater denitrification rates, distance from shore, temperature, DO, salinity, DOC, and pH. All statistical analyses were considered significant at the $p < 0.05$ level and were performed on Statistica (StatSoft 2002).

Results

GROUNDWATER FLOW PATHS

The water table of the subterranean estuary dropped with the ebb tide and rose with the flood tide. The greatest depth to the water table was always in the transition zone, followed by the high marsh and the low marsh. Due to the tidal cycle, the water table of the subterranean estuary is in constant flux, and we did not monitor the water table wells continuously. Over the course of this study, we noted that the transition zone flooded on a few occasions, but the water table depth typically ranged from 60 to 110 cm below the surface. The high and low marsh locations flooded twice daily. The maximum water table depth observed was 54 and 16 cm below the surface in the high marsh and low marsh, respectively.

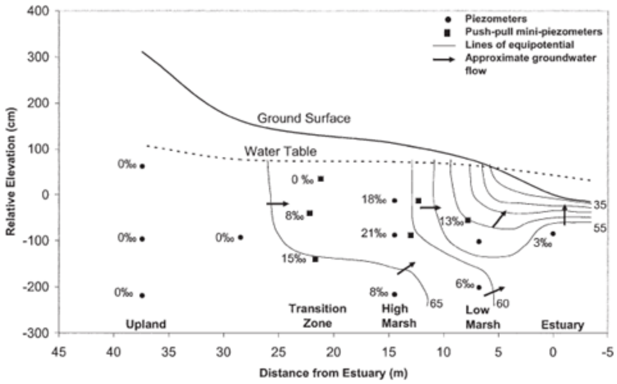

Fig. 2. Approximate groundwater flow paths through the subterranean estuary at low tide in September 2001. Salinity measurements are in ‰.

Groundwater flow on September 27, 2001, at low tide was generally horizontal (Fig. 2). As groundwater approached the high marsh, low marsh, and estuary, the hydraulic gradient increased and deep groundwater tended to upwell. In May 2002, where we were able to measure piezometric heads, we found a similar pattern in hydraulic gradients at low tide. K measurements in all transition zone and the 125-cm high marsh mini-piezometers were relatively uniform, ranging from 14.2 to 18.4 m d^{-1}. The 200-cm high marsh and 125-cm low marsh mini-piezometers yielded lower K values, ranging from 0.5 to 2.1 m d^{-1}, respectively. Measured K in the marsh at 50 and 75 cm depths was highly variable, ranging from 0 to 11 m d^{-1}. Using the mean K values, porosity (assumed a porosity of 0.38 that is typical for the media found at our site; Freeze and Cherry 1979; Nelson et al. 1995), and the hydraulic gradients, we determined the mean porewater velocity to be 11.9, 12.6, and 9.3 cm d^{-1} in the transition zone, high marsh, and low marsh, respectively.

AMBIENT CHARACTERISTICS

The subterranean estuary displayed substantial differences in ambient groundwater characteristics along the transition gradient from the upland to the ocean (Table 1). From the upland to the low marsh, the water table became shallower, groundwater DO decreased, groundwater pH increased, the thickness of C enriched layers increased (Fig. 3), and total root biomass increased (Fig. 4). As distance to the shore decreased, groundwater DO ($r_s = 0.72$) significantly decreased and groundwater pH ($r_s = -0.82$) significantly increased.

Mean groundwater DO was always less than 2.2 mg l^{-1} in low and high marsh mini-piezometers while it fluctuated between 2.6 to 8.6 mg l^{-1} in transition zone mini-piezometers. Salt water mixed with freshwater in all groundwater samples except

TABLE 1. Ambient groundwater characteristics. Values are mean (SE) from three replicate mini-piezometers. Samples were taken immediately prior to the push phase of the nitrate push-pull test, unless otherwise noted. nd = no data.

Zone	Depth[a] (cm)	Date	Water table[ab] (cm)	Temperature (°C)	DO (mg l⁻¹)	Salinity (‰)	DOC (mg l⁻¹)	pH
Warm season								
Low marsh	125	September 2002	3.0[c]	20.5 (0.6)	0.6 (0.1)	12.8 (2.6)	2.2 (0.7)[d]	6.6 (0.1)
High marsh	125	October 2000	36.3 (1.7)	14.8 (1.1)	0.9 (0.2)	17.9 (1.4)	7.0 (0.5)	nd
High marsh	200	August 2001	40.0[e]	21.4 (0.5)	0.7 (0.1)	21.4 (0.5)	2.9 (1.1)	6.4 (0.1)
Transition zone	125	November 2000	88.2 (1.2)	12.0 (0.2)	2.6 (0.1)	0.1 (0.0)	7.4 (3.3)	nd
Transition zone	200	September 2002	92.7 (3.8)	18.6 (0.3)	3.5 (0.5)	7.7 (4.0)	1.2 (0.3)	4.6 (0.1)
Transition zone	300	August 2001	103.3[e]	15.3 (1.4)	5.0 (0.3)	15.3 (0.1)	0.9 (0.1)	5.5 (0.2)
Upland	300	October 2002[f]	nd	13.5 (0.7)	4.3 (0.5)	0.2 (0.2)	0.6 (0.1)	4.9 (0.0)
Cool season								
Low marsh	125	April 2001	−1.0 (1.0)	5.6 (0.1)	1.4 (0.5)	6.7 (1.7)	0.6 (0.1)	nd
High marsh	125	April 2001	14.7 (2.0)	6.1 (0.3)	2.2 (0.3)	7.0 (1.8)	0.8 (0.3)	nd
High marsh	200	Not sampled						
Transition zone	125	April 2001	84.0 (0.0)	10.4 (0.1)	8.6 (0.5)	10.4 (0.1)	0.7 (0.1)	nd
Transition zone	200	May 2001	85.3 (0.7)	9.9 (0.3)	5.1 (0.8)	3.1 (3.1)	0.6 (0.0)	nd
Transition zone	300	Not sampled						
Upland	300	May 2001[f]	nd	9.4 (0.0)	5.1 (0.2)	0.0 (0.0)	0.6 (0.1)	4.6 (0.3)

[a] Depth below ground's surface.
[b] Water table measured in water table well within applicable zone at the end of the push phase.
[c] Water table was measured 4 d later at comparable time in tidal cycle.
[d] n = 2.
[e] Water table was not measured in August 2001 due to field error; this value represents the mean August 2000 and 2002 water table.
[f] Groundwater denitrification capacity not determined in this zone.

in that extracted from transition zone 125 cm mini-piezometers in the warm season. Mean salinity in the subterranean estuary ranged from 3.1‰ to 21.4‰. The salinity in the adjoining estuary ranged from 26‰ to 30‰. Groundwater pH was not consistently measured during the push-pull investigations, but it was higher in the marsh zones (mean range: 6.4–6.6) than the transition zone (mean range: 4.6–5.5). Ambient groundwater NO_3^--N was always less than 0.3 mg l^{-1} in all zones.

SOC (Fig. 3) and total root biomass (Fig. 4) decreased with depth in all zones; the pattern of this decrease also reflected the transition gradation from upland to the open water. SOC in the transition zone was elevated for only the upper 4 cm of the soil; whereas elevated SOC levels persisted to a 75 cm depth in the low marsh (Fig. 3). The high marsh was intermediate between the other two zones–elevated SOC contents were also evidenced down to 75 cm, but the SOC levels were less than that observed in the low marsh. The same pattern was reflected in total root biomass (Fig. 4). Minimal roots and rhizomes were found throughout the soil in the transition zone; elevated root biomass extended to 75 cm in the high and low marshes. Where we were able to sample, SOC (mean range: 0.04–0.11%) and total root biomass (mean range: 3.3–39.6 g m^{-2}) were low at all mini-piezometer locations.

Groundwater temperature, water table depth, groundwater salinity, and groundwater DOC differed seasonally (Table 1). The mean groundwater temperature ranged from 5.6°C to 10.4°C during our cool season nitrate push-pull tests and from 12.0°C to 21.4°C during our warm season nitrate push-pull tests. The mean water table depth at low tide was shallower in the cool season than the warm season within all zones. During our cool season nitrate push-pull tests, groundwater salinity was

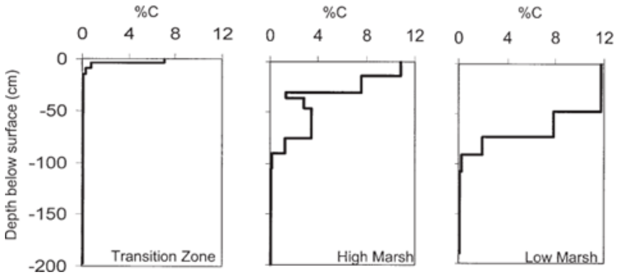

Fig. 3. Soil organic carbon (SOC) with depth in the transition zone, high marsh, and low marsh.

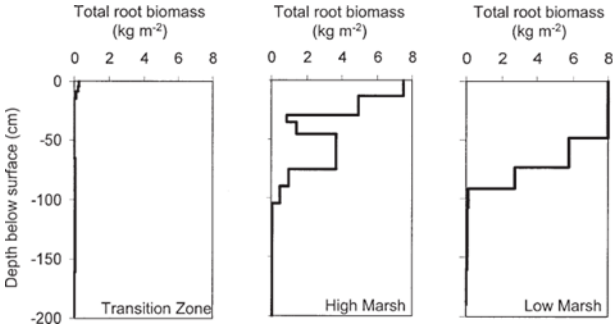

Fig. 4. Root biomass in the transition zone, high marsh, and low marsh.

TABLE 2. Ambient groundwater conditions in mini-piezometers undergoing a nitrate push-pull test and a subsequent C-enriched nitrate push-pull test. Values are mean (SE) from three replicate mini piezometers. Samples were taken immediately prior to the push phase of the push-pull test, unless otherwise noted.

Characteristic	Transition zone (200 cm)		Low marsh (125 cm)	
	^{15}N-nitrate push-pull test	C-enriched ^{15}N-nitrate push-pull test	^{15}N-nitrate push-pull test	C-enriched ^{15}N-nitrate push-pull test
Date	September 2002	October 2002	September 2002	October 2002
Water table (cm)[ab]	92.7 (3.8)	112 (0.0)	3.0[c]	7.0 (0.0)
Temperature (°C)	18.6 (0.3)	16.2 (0.5)	20.5 (0.6)	20.5 (0.5)
DO (mg l^{-1})	3.5 (0.5)	3.7 (1.0)	0.6 (0.1)	0.8 (0.1)
Salinity (‰)	7.7 (4.0)	1.7 (3.5)	12.8 (2.6)	14.2 (0.8)
DOC (mg l^{-1})	1.2 (0.3)	5.4 (3.3)	2.2 (0.7)[d]	37.4 (19.5)
pH	4.6 (0.1)	5.0 (0.1)	6.6 (0.1)	6.6 (0.1)

[a] Depth below ground's surface.
[b] Water table measured in water table well within applicable zone at the end of the push phase.
[c] Water table was measured 4 d later at comparable time in tidal cycle.
[d] n = 2.

<10‰ and DOC was <0.9 mg l^{-1} throughout the various zones of the subterranean estuary, whereas both characteristics displayed substantial variability during the warm season push-pull tests (salinity range: 0.1–21.4‰; DOC range: 0.9 to 7.4 mg l^{-1}). Groundwater salinity was weakly correlated with groundwater DOC ($r_s = 0.38$) and temperature ($r_s = 0.59$). Groundwater DOC was also weakly correlated with temperature ($r_s = 0.56$).

Ambient groundwater conditions immediately prior to the nitrate push-pull tests (September 2002) and the C-enriched nitrate push-pull tests (October 2002) in the low marsh and transition zone are compared in Table 2. The greatest difference in ambient conditions is the ambient groundwater DOC content, which is elevated immediately prior to the C-enriched nitrate push-pull test. This increase is due to the DOC priming injections 4 and 2 d prior to the C-enriched nitrate push-pull test. All other ambient groundwater characteristics remained relatively stable between these push-pull tests.

RATES OF GROUNDWATER DENITRIFICATION CAPACITY

In all nitrate push-pull tests, virtually all of the denitrification gases generated were N_2. In the warm season, elevated groundwater denitrification capacity was observed in at least one depth within each zone (Table 3). The warm season groundwater denitrification rate measured in the low marsh at 125 cm (mean: 538 μg N l^{-1} water d^{-1}; SE: 131) was significantly higher than all other zones and depths. Warm season groundwater denitrification rates within the high marsh did not vary significantly by depth (range: 108–319 μg N l^{-1} water d^{-1}). Within the transition zone, there was significant variation in warm season groundwater denitrification with depth (range: 0–371 μg N l^{-1} water d^{-1}). In the cool season (Table 3), the only zone with elevated groundwater denitrification capacity was the low marsh (mean: 263 μg N l^{-1} water d^{-1}; SE: 112). In all other zones and depths, cool season groundwater denitrification rates ranged from 0 to 71 μg N l^{-1} water d^{-1}. Over all the nitrate push-pull tests, groundwater denitrification capacity was weakly correlated with distance from shore ($r_s = -0.48$), temperature ($r_s = 0.48$), DO ($r_s = -0.56$), and salinity ($r_s = 0.41$).

Our C-enriched nitrate push-pull test did not result in significantly different rates of groundwater denitrification compared to the nitrate push-pull tests conducted at the same zones and depths (Table 4).

Discussion

The gradient in ambient characteristics observed from the upland to the low marsh reflected the zonation of the salt marsh environment. Closer to

TABLE 3. Groundwater denitrification rates at multiple depths in the upland-marsh transition zone, high marsh, and low marsh in the warm season and cool season. Values are mean (SE) of three replicate mini-piezometers. Different letters within a column indicate significant differences. nd = no data.

Zone	Depth below surface (cm)	Warm season denitrification rate (μg N l^{-1} water d^{-1})	Cool season denitrification rate (μg N l^{-1} water d^{-1})
Low marsh	125	538 (131)[a]	263 (112)[a]
High marsh	125	219 (50)[bc]	9 (3)[bc]
High marsh	200	163 (44)[bc]	nd
Transition zone	125	21 (14)[d]	34 (21)[ac]
Transition zone	200	304 (38)[b]	3 (3)[bc]
Transition zone	300	90 (58)[dc]	nd

TABLE 4. Groundwater denitrification rates from nitrate push-pull test and a subsequent C-enriched push-pull test. Tests were conducted during the warm season. Values are mean (SE) of three replicate mini-piezometers. Different letters within the table indicate significant differences.

Zone	Depth below surface (cm)	Nitrate push-pull denitrification rate (μg N l^{-1} water d^{-1})	Nitrate, C-enriched push-pull denitrification rate (μg N l^{-1} water d^{-1})
Low marsh	125	538 (131)^{a}	451 (33)^{a}
Transition zone	200	304 (38)^{b}	327 (23)^{b}

the low marsh, the influence by estuarine processes became more evident in marsh and groundwater characteristics. Groundwater salinity and water table measurements suggest that groundwater flux through the subterranean estuary appears to be greater during the cool season than the warm season. In the cool season, groundwater salinity was consistently low throughout the marsh regardless of time in the tidal cycle. In the warm season, groundwater salinity varied markedly, most likely a result of the combined effects of evapotranspiration, rainfall, and tidal flooding (Gardner et al. 2002). The weak correlation between groundwater salinity and groundwater DOC raises the possibility that subterranean estuary DOC dynamics are driven by both estuarine and terrestrial processes. Goni and Gardner (2003) found a similar relationship between salinity and DOC but point out that this relationship is not simple due to the complex nature of the water fluxes, i.e., tidal recharge, evapotranspiration, and upwelling of groundwater (Ford and Naiman 1989; Gardner et al. 2002; Gardner and Reeves 2002) in the subterranean estuary. DOC and salinity may covary with other groundwater conditions, especially temperature.

Estuarine effects were also observed in the SOC distributions with depth; the highest SOC and deepest penetration of elevated SOC was in the low marsh. Total root biomass found in the upper soil horizons was comparable to that found by several salt marsh researchers (Hackney and Cruz 1986; Gross et al. 1991; Turner et al. 2004). Elevated total root biomass extended to about 75 cm into the subsurface in the high and low marshes, which is substantially deeper than the assumed extent of root penetration (15–25 cm) in salt marshes (Valiela and Teal 1974), and at the riparian sites examined by Kellogg et al. (2005) where it was limited to the upper 10 cm. Turner et al. (2004) found live *S. alterniflora* roots down to 1 m depth. This extensive rooting zone has the potential to contribute labile C to the subsoil.

Since we observed high variability in K values at 50 and 75 cm in the low marsh, this area was not an aquiclude. Tidal water is certainly transmitted through these surface soil materials, and there may be opportunities for substantial water flux through the organically enriched media. The presence of macropores due to fiddler crab (*Uca pugnax*) tunnels may explain some of the variability in K (Shultz and Ruppel 2002). In the subsoil below marsh surface layers, the lower K values deeper and closer to the estuary indicated a different depositional environment. This area was also where we observed steep hydraulic gradients, creating a zone of upwelling groundwater as also observed by Thibodeau et al. (1998); the groundwater flux throughout the site remained relatively constant. The porewater velocities, based on K, porosity, and hydraulic gradients in the subterranean estuary, were comparable to values expected for saturated sandy soils (Freeze and Cherry 1979; Nelson et al. 1995; Kellogg 2005) and slightly higher than those observed in a subterranean estuary composed primarily of saturated fine to medium sands (Thibodeau et al. 1998). Coupling these values with travel paths through each zone, the approximate residence times of groundwater in the subterranean estuary are 34, 88, and 112 d in the transition zone, high marsh, and low marsh, respectively.

In the warm season, we found evidence of substantial denitrification in the subterranean estuary below each zone of the salt marsh. We recognize that several confounding factors restrict the robustness of our statistical tests in assessing the differences in groundwater denitrification with depth or between zones. For instance, there is enough difference in groundwater temperatures in the warm season that some depth and zone differences may be obscured. We would like to emphasize the importance of finding substantial denitrification capacity in many locations through the subterranean estuary especially since it is often assumed that groundwater nitrate can move through sandy aquifers with minimal transformations and can represent a significant source of N to estuaries (Giblin and Gaines 1990; Valiela et al. 1990, 1992). The subterranean estuary below the low marsh may be a year-round sink for groundwater nitrate.

The relatively long incubation periods coupled with the high denitrification capacity observed in the subterranean estuary, especially below the low marsh, argues for its significance in annual watershed scale N budgets. The groundwater flow paths, measured at low tide to minimize tidal pumping (Gardner et al. 2002), converged toward the low marsh. Combining our rates with incubation times, 29 (cool season) to 60 (warm season) mg N could be removed from 1 l of water flowing through the subterranean estuary below the low marsh portion of the site. These substantial rates of groundwater denitrification could have a considerable effect on the groundwater nitrate flux through the subterranean estuary. We estimated groundwater flux based on hydraulic gradients in the early fall when water tables and groundwater flux are at their annual low in New England (Winter et al. 1999). Groundwater flux is expected to be higher in the spring, as evidenced by lower groundwater salinity in our cool season samples, due to an increase in hydraulic gradient (Tobias et al. 2001b), which might slightly lower the N removal function of the subterranean estuary in the cool season.

While the correlations were weak and there are many ambient conditions that may covary, the relationship of groundwater denitrification capacity (the denitrification rates obtained under our non-N limiting push-pull conditions) to distance from the estuary (with the higher rates occurring closer to the estuary) and salinity may indicate that tidal interaction increased transport of labile DOC and other electron donors into the subterranean estuary, especially during the warm season, to fuel subsurface denitrification. In freshwater river systems, hyporheic exchange—mixing of surface water and groundwater in the subsurface—has been found to contribute electron donors to deeper near-stream soils (Duff and Triska 2000; Harvey and Wagner 2000).

Our results suggest that groundwater DOC was not a limiting factor for groundwater denitrification in this subterranean estuary. Groundwater DOC concentration was not significantly related to groundwater denitrification. This result is contrary to Tobias et al. (2001a) who found maximum potential denitrification coinciding with peak DOC concentration in a fringing marsh's surface sediments. Our direct C additions did not stimulate groundwater denitrification. Acetate, our C source, has successfully been used to promote denitrification in drinking water and wastewater treatment (Mohseni-Bandpi et al. 1999; Cervantes et al. 2001). Other studies have found higher denitrification potential in soils amended with DOC (DeCatanzaro and Beauchamp 1985; Beauchamp et al. 1989; Bradley et al. 1992; McCarty and Bremner 1992; Clough et al. 1999). Hedin et al. (1998) and Hill et al. (2000) observed increases in nitrate removal rates in response to in situ additions of groundwater DOC to riparian zones. Where the DOC amendment of Hill et al. (2000) did not stimulate groundwater denitrification, they suggested it might reflect a limitation of their short-term injection method. We attempted to overcome this limitation by injecting groundwater amended with acetate into the subterranean estuary prior to the C-enriched nitrate push-pull. Our C : N ratio was 4.5 : 1, which follows Payne's (1981) suggestion that a 3 : 1 or 4 : 1 C : N ratio is optimal for denitrification. Our priming injections raised the ambient groundwater DOC but did not affect the groundwater DO (Table 2) indicating that heterotrophic activity may not have been stimulated. The lack of response to labile C could suggest that an alternative electron donor, in particular reduced sulfur compounds (Gayle et al. 1989; Joye 2002; Korom 1992), may be limiting groundwater denitrification at this site. While we did not measure concentration of sulfur compounds in the subterranean estuary, the groundwater in all locations had a hydrogen sulfide odor. At three salt marsh sites, Koretsky et al. (2003) found bacterial sulfate reduction to sulfide highest in the summer, corresponding with our observation of higher groundwater denitrification rates in the warm season. Several researchers (Gould and McCready 1982; Joye and Hollibaugh 1995; An and Gardner 2002) have found that sulfide can inhibit denitrification and shift nitrate reduction from denitrification to dissimilatory nitrate reduction to ammonium (DNRA). In some salt marsh systems, more nitrate may be reduced by DNRA than by denitrification. The relationship of sulfur to denitrification and DNRA in the subterranean estuary warrants further investigation.

Our data indicate that denitrification in the subterranean estuary we assessed would not be a significant source of N_2O in this landscape. In field and laboratory studies, pH has been shown to influence the end products of denitrification with greater amounts of N_2 produced in more neutral soils (Simek and Cooper 2002). Interaction with more neutral estuarine water may contribute to denitrification proceeding to completion, preventing substantial N_2O emission. Low $N_2O : N_2$ ratios are also common when the demand for electron acceptors is high, which is likely the case in these marshes that have low oxygen and nitrate levels and relatively high C availability (Blicher-Mathiesen and Hoffmann 1999).

Because our study included only one site, there is a strong need to examine other subterranean estuaries, with an explicit focus on variation in groundwater flow paths, to better assess the factors controlling groundwater denitrification in these ecosystems. There is also a need to assess the effects of shoreline alteration by humans that can have important effects on hydrology and denitrification (Valiela et al. 2000; Gold et al. 2001; Bertness et al. 2002). More generally, accelerated nutrient enrichment of estuaries may lead to a shift in salt marsh plant communities, most commonly to a systems dominated by *S. alterniflora* or *Phragmites* (Bertness et al. 2002; Pennings et al. 2002; Wigand et al. 2003). Vegetation shifts can have marked effects on soil nutrient cycling functions, including denitrification (Otto et al. 1999; Windham and Ehrenfeld 2003). Additional research will advance efforts to predict groundwater nitrate removal in subterranean estuaries and N delivery to coastal waters.

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