Patterns of Denitrification Potential in Tidal Freshwater Forested Wetlands

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Abstract

Limited evidence for spatial patterns of denitrification in tidal freshwater forested wetlands (TFFWs), seemingly due to high spatial variability in the process, is surprising considering the various spatial gradients of its biogeochemical and hydrogeomorphic controls in these ecosystems. Because certain physical environmental gradients may be useful for the prediction of denitrification in TFFWs, we measured denitrification and ecosystem attributes in hummock-hollow microtopography of TFFWs along longitudinal riverine positions (upper, middle, and lower tidal river sites, and nearby upstream nontidal forested floodplains) of the adjoining Pamunkey and Mattaponi Rivers, Virginia. We tested differences by river, site, and plot in denitrification enzyme activity (DEA) and substrate limitations of denitrification potential (DP). The Pamunkey River carries greater river nitrate concentrations, and we found less nitrate limitation of DP and greater soil nitrate in hollows of this river. DEA in tidal hummocks was positively correlated with soil organic matter, nitrogen, and carbon, with the highest rates in lower tidal sites. Hummocks also promoted greater oxygen-controlled substrate limitation of DP, whereby experimental aeration stimulated DP under subsequent inundation more in hummocks than hollows. Additionally, tidal sites had greater DEA than nontidal sites, inferred to be caused by a combination of higher moisture, organic, and nutrient content. Our results indicate that the increasing nitrogen concentrations in these rivers will increase denitrification more on the Mattaponi River by alleviating its greater nitrogen limitation compared to the Pamunkey River, and modification to sedimentation, inundation, or microtopography from sea level rise may alter denitrification gradients in TFFWs and upstream low-elevation nontidal floodplains.

Keywords Denitrification enzyme activity · Denitrification potential · Nitrate · Nontidal · Soil biogeochemistry

Introduction

Tidal freshwater forested wetlands (TFFWs), commonly found along tidal rivers at the upstream end of tidal influence, provide important biogeochemical functions that regulate downstream water quality in estuaries. For the case of nitrogen (N) pollution, TFFWs serve as sinks to sequester N through

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mechanical and biochemical means, with the microbially mediated anaerobic process of denitrification as the predominant mechanism of nitrate removal (Ensign et al. 2008; Scott et al. 2008; Tzortziou et al. 2011; Morris et al. 2016; Zheng et al. 2016). With anticipated increasing nutrient enrichment to rivers from intensification of land use in watersheds, and the resulting hydrologic (e.g., tidal fluctuations), geomorphic (e.g., surface accretion), and ecological modifications (e.g., vegetation community shifts) of accelerated sea level rise, estuaries are currently vulnerable to the progressive modification of these functions in TFFWs (Ozalp et al. 2007; Ensign et al. 2014; Paerl et al. 2014; Stagg et al. 2016; Middleton 2016). To help predict how future environmental changes to TFFWs will impact N cycling, studying processes contemporaneously across landscape gradients might provide inferences into future rates of N processes (i.e., denitrification) (Ensign et al. 2008; Marton et al. 2012; von Korff et al. 2014; McCluney et al. 2014; Ensign and Noe 2018).

Hydrologic, geomorphic, and vegetation community processes in TFFWs, which primarily control soil

biogeochemical cycling (Anderson and Lockaby 2007; Groffman 2012; Krauss et al. 2012; Noe et al. 2013), exhibit longitudinal (i.e., downriver) patterns in tidal rivers (e.g., Czwartacki 2013). One well-studied pattern in estuaries is the change in salinity that directly and indirectly impacts biogeochemical N cycling (Larsen et al. 2010; Krauss and Whitbeck 2012; Cormier et al. 2013; Noe et al. 2013). Yet relative distance from the head and mouth of the estuary also affects the balance between fluvial and tidal hydrology in a TFFW: less tidal influence at the most upriver locations affects water table levels, inundation, and the composition of sediment in these wetlands (Czwartacki 2013; Ensign et al. 2013). Other patterns occur with vegetation responses to inundation, such as the mediating effect of autogenic soil building processes or hummock-hollow formations on tidal influence (Courtwright and Findlay 2011; Krauss et al. 2012; Stagg et al. 2016). Variability in spatial positioning is further found in the species composition of forested communities as influenced by a mix of hydrological patterns, salinity, and soil characteristics (Rheinhardt 1992; Anderson and Lockaby 2011; Johnson and Simenstad 2015). These vegetation feedbacks on hydrogeomorphic controls are related, in part, to greater sedimentation and to greater rates of nutrient processing in downriver tidal forest-to-marsh transition zones (Noe et al. 2013; Ensign et al. 2014; Noe et al. 2016).

In addition to longitudinal gradients in hydrogeomorphic and ecological processes, watershed differences in upland land use and landscape physiography create a second dimension of landscape-scale variability (Thoms and Parsons 2002). For floodplains of nontidal rivers, intensity of anthropogenic land use is associated with greater nutrient delivery to streams and increased potential for denitrification (Korol et al. 2018). Coastal rivers display similar gradients in nutrient concentrations, implicating contributions from both upriver and proximal land uses, and are even more hydrologically connected to their floodplains than nontidal rivers. Because nutrient delivery limits biogeochemical cycling in TFFWs (Ozalp et al. 2007), variation in nutrient loading to TFFWs may likewise cause predictable variation in denitrification (Seitzinger 1988; Hopfensperger et al. 2009).

Within TFFWs, ecogeomorphic feedbacks to tidal stress elevate trees above low tide in hummock-hollow microtopography and change the balance of coupled aerobic and anaerobic processes that promote denitrification (Verhoeven et al. 2001; Liu et al. 2017). This defining local characteristic of TFFWs exerts strong control on the magnitude of material transfer from river channel to wetland soil and might impact our understanding of landscape-scale gradients within TFFWs. Hummocks are naturally less exposed to flooding than are hollows, limiting floodwater influence on soils, but the consistent saturation of hollows also impedes soil nitrate penetration from incomplete porewater turnover (Neubauer et al. 2005a; Day et al. 2007). Lower rates of in

situ production of nitrate in hollows of TFFWs (Noe et al. 2013), due to soil anoxia from slow porewater turnover (Grimm et al. 2003; Palmer et al. 2009), likely increase nitrate demand by denitrifiers relative to hummocks. Generally, greater nitrification in TFFW soils reduces the potential for river nitrate uptake by denitrification (McKellar et al. 2007; Koop-Jakobsen and Giblin 2010). How denitrification varies across gradients in resource availability, and particularly nitrate availability, is not well studied for TFFWs.

The purpose of our study was to determine patterns in denitrification and how hydrogeomorphic processes and nutrient availability control denitrification in TFFWs. To do this, we studied denitrification along a longitudinal river gradient in adjoining rivers differing in nutrient supply, and investigated both the potential for denitrification and its nutrient limitation as influenced by tidal fluctuations. Some research has shown patterns of denitrification across rivers that may be positively linked to soil organic matter (Marton et al. 2012), but the various known gradients of biogeochemical and hydrogeomorphic processes, as discussed above, suggest that additional controls of TFFW denitrification exist at landscape scales (e.g., controls consistent across rivers). Further, effects of tidal inundation on denitrification in floodplain forested wetlands along tidal rivers are modeled to be strong (Ensign et al. 2013), but have not been validated with empirical results (Verhoeven et al. 2001). Spatial variation in denitrification reflects both momentary and prolonged abilities to denitrify associated with soil biogeochemical variation over short-term and long-term periods (Wallenstein et al. 2006; Xiong et al. 2015). Exposure to cyclical fluctuations of hydrogeomorphic processes (e.g., frequency of inundation) might affect rates of denitrification through sub-daily changes in microbial activity, while those same hydrogeomorphic processes (e.g., saturation and nutrient delivery) might eventually impact the potential for denitrification through changes to microbial structure (Morrissey and Franklin 2015). Thus, in addition to spatial patterns in denitrification potential, which would be indicative of the long-term potential to denitrify, we investigated nitrate delivery from flood water at high tide and the aerobic process of nitrification at low tide that influence short-term nitrate availability and limitation of denitrification.

For this study, we focused on spatial patterns in TFFWs at or across landscape scales (Fig. 1) and studied denitrification following four objectives: (1) to determine the effect of differences in river N concentrations on denitrification, making use of a natural experiment between two tributaries of the York River estuary in Virginia; (2) to determine the effect of longitudinal riverine-estuarine gradients on variations in denitrification, including nontidal forested floodplain wetlands upstream of tidal influence; (3) to determine if denitrification differs across hummock-hollow microtopography prevalent across TFFWs; and (4) to evaluate denitrification associations with other soil physicochemical properties (e.g., bulk density
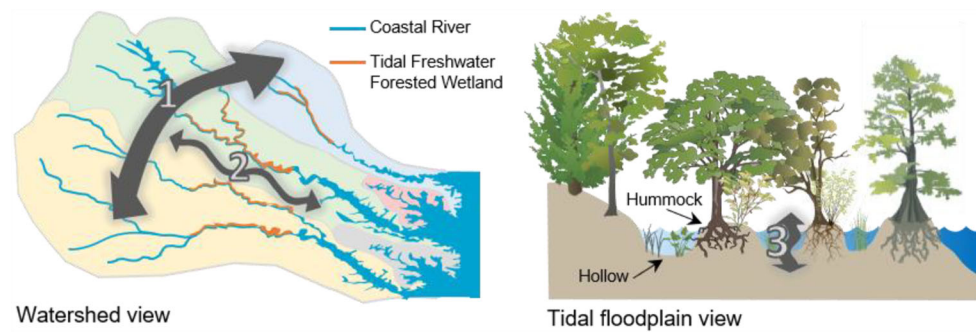
Fig. 1 Hydrogeomorphic, ecological, and biochemical gradients (1) across rivers, (2) in a longitudinal direction, and (3) among hummocks and hollows, hypothesized to impact soil denitrification in tidal freshwater forested wetlands. Images were courtesy of the Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/symbols/)

or potential carbon (C) mineralization) along these TFFW gradients. We hypothesized that denitrification potential would increase with greater river N load, and would increase downstream along a longitudinal gradient due to greater nutrient pools and soil anoxia. We also hypothesized that the greater oxygen availability in hummocks, which inhibits denitrification directly, would nonetheless stimulate the potential for denitrification through greater aerobic nutrient processing at low tide. Because the substrates nitrate and organic labile C directly regulate denitrification, we anticipated that our measurements of nutrient limitation of denitrification would likely be important for understanding gradients in denitrification potential.

Methods

Study Rivers, Sites, Plots, and Sampling Scheme

We studied tidal and nontidal forested wetlands along the alluvial Mattaponi and Pamunkey Rivers (Virginia, USA; Fig. 2). The rivers are located within a temperate climate (e.g., humid, hot summers, and mild winters) in the Coastal Plain physiographic province but drain watersheds that extend into the Piedmont physiographic province. Together, these adjacent rivers flow southeast to comprise the primary tributaries of the York River Estuary beginning at West Point, VA, and eventually join the Chesapeake Bay. The Mattaponi River watershed (2359 km^2) discharges 14.3 m^3 s^{-1} above the head of tide (USGS streamgage 01674500, water years 1997–2016; https://doi.org/10.5066/F7P55KJN) from a mix of 75% natural vegetation, 14% agriculture, and 7% developed land cover; the Pamunkey River watershed (3812 km^2) discharges 26.5 m^3 s^{-1} above the head of tide (USGS streamgage 01673000, water years 1997–2016; https://doi.org/10.5066/F7P55KJN) from a mix of 69% natural vegetation, 20% agriculture, and 6% developed land cover (Ries III et al. 2017). Along with a greater proportion of agriculture, the Pamunkey River watershed has larger areas of row crop

agriculture in the uplands proximal to the river channel along its Coastal Plain reaches near our study sites. Long-term (2006–2015) mean nitrate concentrations above the heads of tide are 56% higher on the Pamunkey River (0.25 mg N/L) than Mattaponi River (0.16 mg N/L), and 22% higher on the Pamunkey River (0.22 mg N/L) than Mattaponi River (0.18 mg N/L) in tidal segments measured near the sampled middle tidal wetland sites (Fig. 2; Chesapeake Bay Program 2012).

Four forested floodplain wetland sites were established on each river along a freshwater estuarine gradient including a nontidal forested floodplain wetland close to the fluvial-tidal wetland transition, an upper (upriver) TFFW and middle TFFW, and a lower (downriver) TFFW that is converting to marsh possibly due to low-level salinization (Fig. 2). All measurements were made at nontidal wetlands and the upper and middle tidal wetlands, and a subset of measurements was made at the lower tidal wetlands of both rivers. Hummock-hollow microtopography was present at all tidal wetland sites with nascent formation also present at the Pamunkey nontidal wetland site. Hummocks were discrete higher elevation patches in the wetland comprised of dense mats of soil, moss, and roots from woody and herbaceous vegetation, which resisted compression if walked upon, while hollows comprised the lower elevations around the hummocks where predominantly herbaceous vegetation was observed to grow and soil was saturated and easily compressible. While sampling at low tide, all or part of hollows were inundated while hummocks were generally exposed to air and soils were quick to drain following high tide inundation (many hummocks were observed inundated at higher tide). Hummocks are generally elevated above hollows by 10–60 cm in TFFWs (Kroes et al. 2007; Duberstein and Conner 2009; Courtwright and Findlay 2011) and typically 10–20 cm in this study.

Six 1-m^2 plots were established at each site, at least 3 m apart, capturing three hummocks and three hollows for tidal wetlands; for nontidal wetlands, three plots were placed in either natural levees or nascent hummocks when present, and three plots in depressions or sloughs. Plots were within
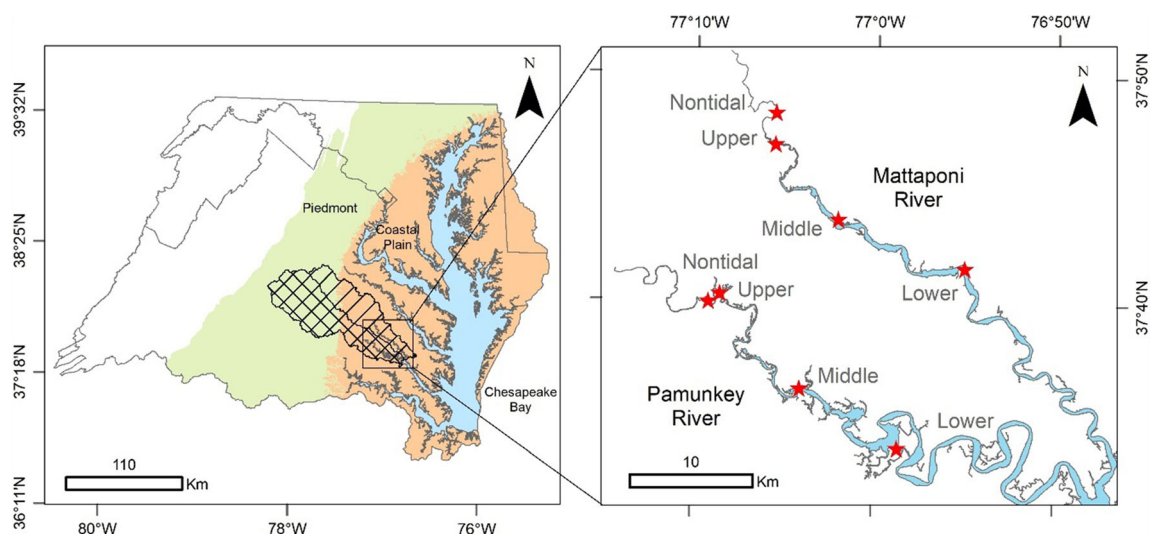
Fig. 2 Map of tidal (upper, middle, and lower) and nontidal forested wetland study sites on the Pamunkey and Mattaponi Rivers, their watersheds spanning the Piedmont and Coastal Plain physiographic provinces, and their location in Virginia within the lower Chesapeake Bay watershed, USA

100 m of stream edge. We visited the sites during the growing season in September 2016 for soil collection and deployment of long-term soil oxygen sensors. Soils for laboratory analyses of denitrification and soil biogeochemical properties were collected during periods of low tide and placed on ice until subsequent refrigeration later the same day. Either one large soil core (7.6 cm diameter) or two smaller cores (4.8 cm diameter) as a composite were retrieved to 5 cm depth, per soil measurement procedure as outlined below. The surficial 5 cm of soil was expected to support the highest nitrification and oxygen fluctuations affecting denitrification in the tidal environments. One replicate measurement per plot was used for all biogeochemical processes and properties, unless otherwise indicated, for a sample size of six per site.

Limitation Experiment of Denitrification Potential

We experimentally assessed the effects of different tidal phases affecting wetland hydrology, specifically inundation and drawdown, on denitrification potential (DP) (Groffman et al. 1999). Following experimental treatments, DP was measured in saturated soils with minimized disturbance to soil structure, an anoxic headspace, nitrate amendment in specified treatments, and an 8-h incubation that allotted sufficient time for denitrifier activity to reach full potential. Because DP measures the ability of denitrifiers to actively acquire/use nutrients during the respiratory process, our DP measurements were sensitive to the local nitrate and labile organic C limitations in the soil that can fluctuate over short (e.g., sub-daily) periods of time (Groffman et al. 1999).

We assessed rates of DP in upper and middle tidal wetland sites only to avoid the confounding effects of salinity in the lower tidal wetlands. We used a 2×2 factorial experiment (factor 1: aeration status, factor 2: nitrate availability) with

four final treatments comprised of a pre-inundation, dry incubation in an either anoxic or oxic environment (factor 1) followed by a wet incubation under saturated, anoxic conditions with or without nitrate amendment (factor 2) during which denitrification measurement took place. For the dry incubation, we exposed ~ 30 g of field moist soil, collected at low tide, to a simulated low tide for 6 h in either ambient air or a headspace comprised of N_2 in the dark. Soils were mixed coarsely from a dedicated larger core, or were cut into small segments (which were held together by roots) for hummocks. To incubate soils anoxically, flasks were capped with rubber stoppers and flushed with N_2 at high flow for 5 min and left slightly pressurized. At the end of the dry treatment, soil cores were amended with 30 mL of media (wet treatment), but not mixed to a slurry to preserve macropore structure, and incubated for another 4 h in the dark for a simulated high tide. Media for the second treatment consisted of either deionized water or river-specific concentrations of long-term mean nitrate (in the form of KNO_3). After addition of the media, the headspace above soils and media was flushed with N_2 for 3 min, then brought to atmospheric pressure, and finally slightly pressurized with 10 mL of acetylene to start the incubation. Gas samples (2 mL into glass vials with aluminum crimp-top caps and butyl rubber septa) were taken at 2 and 4 h after mixing the headspace with a syringe. Gas samples were quantified for N_2O within 48 h of collection using a Shimadzu GC 8-A gas chromatograph with electron capture detection and a packed column (Hayesep Q 80/100). Rates of N_2O production were expressed per dry mass of soil using concurrently measured soil gravimetric moisture (method: dried 4 days to constant mass at 60 °C).

Differences among the experimental treatments of DP were used to test mechanisms of nitrate limitation and oxygen-controlled substrate limitation in TFFWs (Online Resource

1). Regarding nitrate limitation, tidal delivery of nitrate to wetland soils can alleviate nitrate limitation of denitrification. Thus, nitrate limitation of DP was calculated by relating experimental treatments with and without nitrate amendment. This calculated difference in treatments was also applied across both levels of the first experimental factor to determine whether limitation was conditional on soil aeration status before inundation. Regarding oxygen-controlled substrate limitation, persistent anoxia in wetlands can slow biogeochemical processing in wetlands compared to conditions permitting intermittent oxygen penetration into the soil (Liu et al. 2017): insufficient oxygen limits organic matter mineralization and the resulting release of labile C and inorganic N, as well as the conversion of ammonium to nitrate. Thus, we calculated the primary substrate (i.e., nitrate and organic C) limitation of denitrification caused by persistent low soil oxygen levels by measuring the effect of soil aeration prior to inundation on DP. This calculated effect of oxygen was applied across both levels of the second experimental factor to determine oxygen-controlled substrate limitation of DP given varying concentrations of river nitrate.

Surveys of Soil Denitrification Enzyme Activity

Denitrification enzyme activity (DEA) was measured on soils from all sites: nontidal, upper, middle, and lower tidal sites. Unlike DP, DEA was measured in a mixed slurry of soil and media, with both nitrate and organic C amendment, and within a shorter < 2-h incubation, but like DP, still with saturated soils and an anoxic headspace (Groffman et al. 1999). In this way, DEA captured denitrifying activity at the time of sampling, rather than full potential activity, making DEA more reflective of denitrifiers' long-term potential to denitrify (Groffman et al. 1999). In 125-mL flasks, slurries were formed with 30 g of soil, from a dedicated core, and 30 mL of media containing KNO_3 (1.01 g/L), dextrose (1.80 g/L), and chloramphenicol (0.1 g/L). We flushed slurries with N_2 prior to capping the flasks with rubber stoppers and twice pressurizing and depressurizing the headspace. The start of the incubation was marked by injecting 10 mL of acid-scrubbed acetylene into the headspace, slightly pressuring it, and placing flasks on a shaker table. Gas samples (2 mL) were retrieved after 45 and 105 min. The rest of the procedure was identical to methods used to sample, quantify, and express DP.

Surveys of the Change in Soil Net Nitrification Potential and Soil Nitrate Concentration

To assess the potential for nitrate production during aeration of tidal soils at low tide, as well as post-flood aeration in nontidal soils, we measured the effect of aeration on net nitrification potential during 7-day laboratory incubations (Hart et al. 1994; Roy and White 2013). Soils from dedicated soil cores, collected at both tidal (upper and middle) and nontidal wetland sites, were incubated under oxic conditions to determine the potential for nitrate production, and were paired with soils incubated under anoxic conditions as a control. Change in net nitrification potential with aeration was calculated as the difference between the net nitrification potential rates incubated oxically and anoxically. Measurements from the non-incubated soils also indicated ambient soil extractable nitrate concentrations. Soils were held refrigerated for ~ 10 days before analysis. We placed 4 g wet mass-equivalent samples of homogenized soils into flasks for each treatment: extraction at time zero, and an extraction after a 7-day incubation. Soils for initial extractions were mixed well with 50 mL of a 2 M KCl solution and placed on a shaker table for an hour. Soils were then left to gravity separate for 30 min and then 20 mL were syringe-filtered to 0.45 μm (Acrodisc Supor membrane). On the same day, soils for incubation were prepared similarly and incubated in the dark. Soils incubated oxically were left partially open to ambient air, and evaporated water was replenished by mass after 4 days. Soils incubated anoxically were flushed for 5 min with N_2 and left slightly pressurized with rubber stoppers; we repeated the flushing procedure after 4 days to reduce pressure build-up and maintain more consistent headspace concentration across soils; soils maintained pressure throughout the incubation and thus oxygen penetration was negligible. On day 7, we extracted soil nitrate as we had done with the day-0 initial soils. Extracts were measured for $NO_3 + NO_2$ on a discrete analyzer (SEAL analytical, AQ2). Discrete analyzer runs included 2 M KCl blanks and an external standard (ERA, Arvada, Colorado, USA) evaluated with a 10% tolerance. We used the mean of two replicates for time zero soils, and one replicate for day-7 soils, for each of the oxic and anoxic treatments, yielding $N = 1$ per sampling location.

Surveys of Soil Physicochemical Properties

Soil physicochemical properties were measured at all tidal and nontidal sites. We collected soils for bulk density using a slide hammer soil sampler with a cylindrical metal retaining liner (5 cm length × 4.8 cm diameter). Soils for bulk density and separate soil samples for gravimetric moisture were dried to constant weight at 60 °C to measure mass loss on drying. We chose a low temperature to allow time for water evaporation while reducing the potential for N volatilization.

We measured soil %C and %N in an element analyzer (Perkin Elmer 2400 Series II) and %organic matter in a furnace. Collected soils were dried for 3 days at 55 °C and then laid to air dry further after being ground to 2 mm and separated from large roots. Immediately prior to analyses, 1–3 g of soils were completely dried at 105 °C for 2 h. Soil for C and N analysis was placed into capsules for dry combustion and quantification by gas chromatography, and soil for organic

matter was combusted at 550 °C for 4 h and calculated by mass loss on ignition (Nelson and Sommers 1996).

Potential aerobic C mineralization was measured with a LI-COR 8100A automated soil gas flux system adapted for benchtop analysis (Craft et al. 2003). We followed the procedure by Robertson et al. (1999), which calls for soil moisture to be raised to 60% water-filled pore space (WFPS). For the tidal wetlands, most soils exceeded 60% WFPS so we first brought all soils to a common moisture level by drying them for 3 days at 55 °C (Franzluebbers 1999). We then ground the soils to 2 mm and measured their bulk density. We raised moisture levels on 20 g of this soil using the target of 60% WFPS, new bulk density measurements, and a calculation assuming particle density = 2.65 g cm^{-3}. Soils were incubated in the dark for 21 days, partially covered, with moisture loss replaced weekly. Two replicate measurements of CO_2 flux per plot were measured over 2-min periods and averaged. Rates of flux were expressed per dry mass of soil. Potential C turnover was calculated as potential C mineralization normalized by soil %C.

Long-term in situ soil oxygen was measured in hummocks and hollows to determine the relative effect of tidal drawdown and the resulting availability of soil oxygen for aerobic biogeochemical processing. Two sensors were placed in hummocks, one in both the upper and middle tidal sites, and two sensors were placed similarly for hollows. A fifth sensor was placed in the nontidal site, between a levee and slough plot, as a reference to the tidal sensors. A higher level of soil oxygen was expected to be detected in the nontidal site, as well as the tidal hummocks compared to tidal hollows. Sensors, calibrated in the laboratory, were inserted 3 cm into the ground and recorded oxygen levels above a protective wire mesh cage extending 3.5 cm deeper into the soil. Oxygen sensors (Apogee Instruments SO-110) and data loggers (Pace Scientific XR5-SE-100mV) recorded soil %O_2 levels for roughly 8 weeks until the end of November 2016. Recording intervals and periods slightly varied by site in accordance with dates of deployment and retrieval.

Long-term soil porewater conductivity ($\mu S \ cm^{-1}$) and water level were recorded at 20-min intervals from a sonde (Solinst Levelogger model 3001) installed in a surficial well casing with continuous slotting from 60 cm below to 40 cm above the soil surface, but closed to precipitation, in each of the eight sites. Beginning from installment, which occurred sequentially throughout 2015, measurements were made over 10 months on the Pamunkey River and over 12–18 months on the Mattaponi River that both captured the period of soil sampling. Salinity (ppt) was calculated assuming 1 dS m^{-1} = 640 ppm dissolved ions in water.

Data

All data collected for this study are available in an associated data release (Korol and Noe 2019).

Data Analysis

Statistical tests were used to evaluate variation in denitrification metrics among rivers, longitudinal positions, and microtopography, and to test for associations of denitrification metrics with soil characteristics, at significance level (α) = 0.05. Prior to statistical testing, certain variables were transformed to improve normality or reduce the influence of outliers: DEA, potential C mineralization, nitrate, and change in net nitrification potential under aeration were log$_{10}$ transformed; and oxygen-controlled substrate limitation of DP was square root transformed.

For tidal wetlands, we evaluated the effect of river, longitudinal site position, and hummock/hollow microtopography using factorial analysis of variance (ANOVA) and Type II sums of squares. A factor's main effect was only interpreted in this ANOVA where interactions with that factor were not statistically significant after controlling for the main effects. Where interactions were significant, factors were assessed with post-hoc pairwise comparisons using Tukey's honestly significant difference test. To support our study objectives, only the river*microtopography and site*microtopography interactions were generally reported for the associated soil biogeochemical measurements, though all interactions were statistically evaluated. Two of three bulk density measurements in hollows from the Mattaponi River upper tidal site were eliminated from analyses due to apparent measurement error, identified from the calculation (not reported) of volumetric water content >> 1.0 cm^3/cm^3. For that reason, no site*microtopography interactions were interpreted due to insufficient sample size. To explain these patterns of denitrification measurements by river, site, or microtopography, correlations between DEA and soil physicochemical measurements in tidal wetlands were tested with Spearman Rank correlation.

To compare nontidal and tidal soils, we used a mixed effect multilinear model fit by log likelihood, with site and river as fixed effects and microtopography as a random effect (Field et al. 2012). Nontidal site means were compared to those of each tidal site using non-orthogonal contrasts and planned comparisons. We interpreted only the effect of site from model results.

Results

Limitation of Denitrification Potential by Nitrate and Oxygen

Denitrification potential was generally nutrient limited in tidal soils over the simulated tidal cycle. Nitrate amendment to river concentrations raised denitrification potential by 151.9 μg-N kg^{-1} h^{-1} compared to the nitrate-free treatment groups during the laboratory inundation, while a 6-h soil exposure to oxygen during the pre-inundation laboratory drawdown boosted

denitrification potential by 35.9 μg-N kg^{-1} h^{-1} compared to the non-aerated treatment groups. Both factors raised DP independently of the other (Online Resource 2): nitrate limitation of DP did not differ by aeration treatment groups (oxic vs. anoxic treatment, $t = -0.49$, df = 45.8, $P = 0.626$); nor did oxygen-controlled substrate limitation differ by the nitrate-amendment treatment groups (unamended vs. amended treatment, $t = -0.45$, df = 29.6, $P = 0.655$). We therefore pooled the two treatments testing nitrate limitation as well as the two treatments testing oxygen-controlled substrate limitation for presentation and subsequent statistical analyses of the effects of river, longitudinal site position, and microtopography.

Effects of River N Differences: Tidal Mattaponi vs. Pamunkey Rivers

Tidal wetlands of the two rivers had different soil nitrate concentrations and nitrate limitations of DP that were dependent on microtopography (Table 1) and that we attribute to differences in river N availability (Pamunkey > Mattaponi). Hollows in Pamunkey River tidal wetlands had greater soil nitrate concentrations and less nitrate limitation of DP (less increase in DP with nitrate amendment) compared to hollows along the Mattaponi River (Table 1; Fig. 3). In contrast, hummocks had less soil nitrate in Pamunkey River tidal wetlands than on the Mattaponi River (Fig. 3). River had no effect on tidal DEA, change in net nitrification potentials with aeration, or oxygen-controlled substrate limitation of DP (Table 1).

Longitudinal Patterns Across Tidal and Nontidal Wetlands

Among tidal wetlands, hummock microtopography mediated longitudinal effects on soil nitrate concentrations and nitrate limitation of DP (Table 1). Middle tidal wetland hummocks had greater nitrate concentrations than upper tidal wetland hummocks (Fig. 3), and nitrate limitation of DP was lower in middle tidal wetland hummocks than upper tidal wetland hummocks (Fig. 3). Neither DEA, change in net nitrification potentials with aeration, nor oxygen-controlled substrate limitation of DP differed longitudinally among tidal wetland longitudinal positions (Table 1; Fig. 3); however, the highest DEA rates occurred in the middle and lower tidal wetland soils.

Tidal wetlands had greater soil DEA than nontidal wetlands (Table 2; Fig. 3). Tidal and nontidal wetlands did not differ in soil nitrate concentrations nor the change in net nitrification potentials with aeration (Table 2; Fig. 3). For soil nitrate, concentrations trended higher in tidal wetlands than nontidal wetlands on the Mattaponi River but not the Pamunkey River (Fig. 3). For the change in net nitrification potential with aeration, highest rates occurred at the Pamunkey River nontidal wetland site and in middle tidal wetland hummocks of both rivers.

Effects of Hummocks and Hollows of Tidal Wetlands

Hummock-hollow microtopography impacted oxygen-controlled substrate limitation of DP across tidal wetlands, in addition to mediating effects of river and longitudinal position on soil nitrate and nitrate limitation of DP as reported above (Table 1). Pre-inundation, laboratory aeration stimulated DP more in hummocks than hollows (i.e., oxygen-controlled substrate limitation of DP was higher in hummocks), with a greater relative difference on the Mattaponi River (Fig. 3). Microtopography had no effects on DEA or the change in net nitrification potentials with aeration (Table 1).

Spatial Patterns of Soil Physicochemical Properties

Other soil physical and biogeochemical properties in tidal wetlands did not differ across the two rivers (all $P > 0.05$)

Table 1 Factorial ANOVA for tidal wetland soil measurements with river, site, and microtopography (MT) as main effects

Response variable[a]	Statistic	River	Site	MT	River*Site	River*MT	Site*MT	River*Site*MT
DEA[b]	F	0.19	2.01	1.84	0.69	0.61	1.76	0.33
	P	0.665	0.156	0.187	0.513	0.441	0.191	0.720
DP$_{nitrate}$	F	1.48	3.28	0.17	2.84	8.36	10.4	2.34
	P	0.230	0.078	0.684	0.100	**0.006**	**0.002**	0.134
DP$_{oxygen}$	F	1.13	1.40	5.21	4.16	2.31	0.00	0.03
	P	0.295	0.244	**0.028**	**0.048**	0.137	0.994	0.873
Nitrate	F	13.7	5.84	6.87	3.03	18.7	15.7	1.89
	P	**0.002**	**0.028**	**0.019**	0.101	**< 0.001**	**0.001**	0.188
adjNNP	F	1.13	1.82	1.39	0.18	0.02	2.44	0.54
	P	0.304	0.196	0.255	0.674	0.895	0.138	0.473

P values in boldface are statistically significant ($P < 0.05$)

[a] *DEA* denitrification enzyme activity, *DP$_{nitrate}$* nitrate limitation of denitrification potential, *DP$_{oxygen}$* oxygen limitation of denitrification potential, *nitrate* extractable soil NO_3^- concentration, *adjNNP* change in net nitrification potential with aeration

[b] Additionally measured at lower tidal site

Fig. 3 Mean (± standard error) measurements of soil denitrification enzyme activity, denitrification potential (DP), nitrate, and net nitrification potential (NNP) change by river, by site (NT: nontidal, UT: upper tidal, MT: middle tidal, LT: lower tidal), and where present, by microtopography (i.e., UT, MT, and LT only)
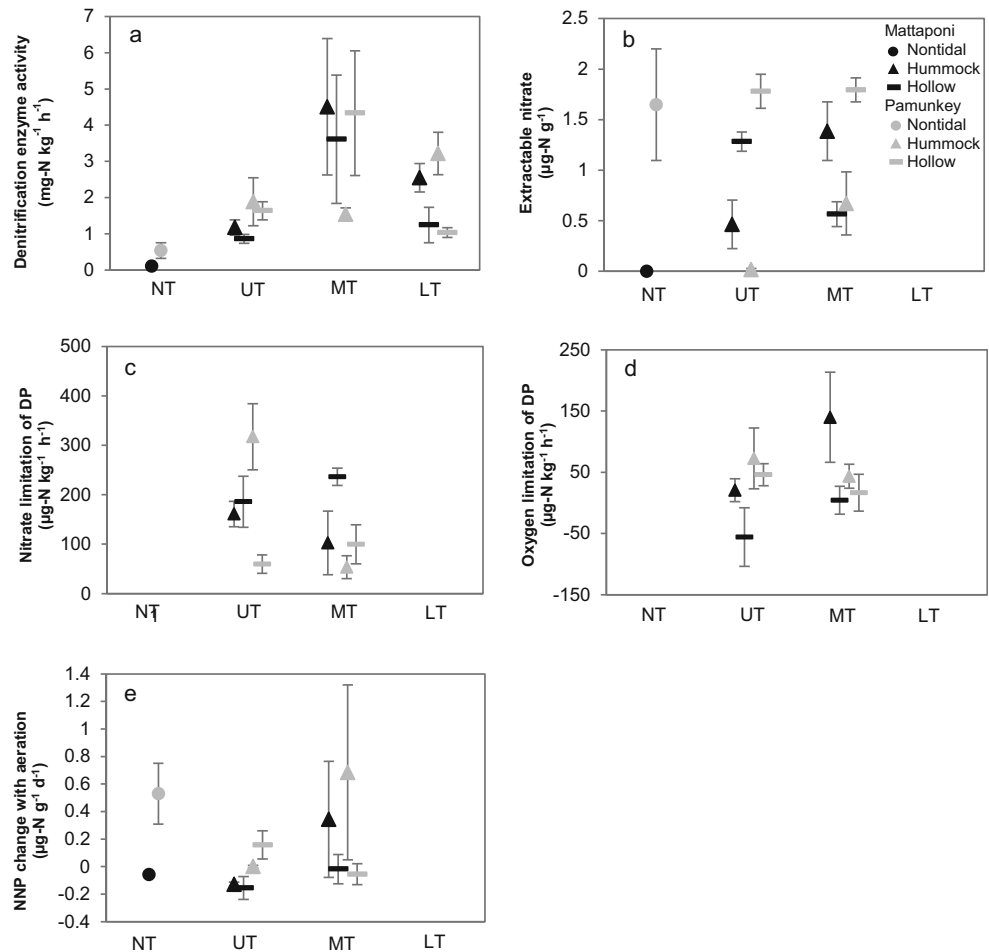
(Table 3), but many properties exhibited gradients longitudinally and by microtopography. Compared to tidal soils, all nontidal soils were lower in gravimetric moisture levels ($P = 0.043$) and higher in bulk densities ($P < 0.001$) (Table 4). Nontidal wetlands also had lower N, C, and organic matter concentrations than middle and lower tidal wetland sites ($P < 0.009$), and nontidal wetlands had lower potential C

mineralization rates than middle tidal sites only ($P = 0.024$; Table 4). Nontidal and tidal soils did not differ for rates of potential C turnover ($P = 0.133$) (Table 4).

With the exception of gravimetric moisture levels ($P > 0.05$), all soil physicochemical characteristics exhibited longitudinal patterns within tidal wetlands (all $P < 0.05$). Middle and lower tidal soils differed from upper tidal soils with higher

Table 2 Factorial mixed effect ANOVA for nontidal and tidal wetland soil measurements with microtopography as random effect

Responsevariable[a]	Statistic	Site	River[b]	Site*River[b]	Fixed factors coefficient of determination	Full model c oefficient of determination
DEA[c]	L. Ratio	16.79	4.26	10.37	68.8%	72.2%
	P	**< 0.001**	**0.039**	**0.016**		
nitrate	L. Ratio	1.45	4.65	11.18	9.7%	29.2%
	P	0.485	**0.031**	**0.004**		
adjNNP	L. Ratio	7.72	5.55	2.33	16.2%	16.6%
	P	0.257	**0.019**	0.312		

P values in boldface are statistically significant ($P < 0.05$); *L. Ratio* likelihood ratio

[a] *DEA* denitrification enzyme activity, *nitrate* extractable soil NO_3^- concentration, *adjNNP* change in net nitrification potential with aeration

[b] Effect of river and interaction not interpreted for this study

cAdditionally measured at lower tidal site

Table 3 Mean (± standard error) soil physicochemical properties of rivers by microtopography pooled across sites

Microtopography	River	OM (%)	N (%)	C (%)	BD (g cm^{-3})	GM (g g^{-1})	Pot. C min. (mmol-C kg^{-1} day^{-1})	Pot. C turnover (mol-C mol-C^{-1} day^{-1})
nontidal Plots	Mattaponi	10.9 ± 0.38	0.23 ± 0.02	4.02 ± 0.31	0.77 ± 0.06	0.85 ± 0.24	2.84 ± 0.13	0.0009 ± 0.0000
	Pamunkey	13.8 ± 1.91	0.38 ± 0.07	5.54 ± 1.15	0.65 ± 0.09	0.81 ± 0.12	5.30 ± 0.84	0.0012 ± 0.0001
TIDAL hummocks	Mattaponi	43.7 ± 7.50	0.22 ± 0.02	3.20 ± 0.36	0.29 ± 0.04	3.20 ± 0.36	20.0 ± 5.79	0.0008 ± 0.0000
	Pamunkey	34.4 ± 5.21	0.38 ± 0.11	2.50 ± 0.33	0.28 ± 0.03	2.50 ± 0.33	16.7 ± 3.65	0.0013 ± 0.0001
TIDAL hollows	Mattaponi	42.9 ± 7.34	0.25 ± 0.03	5.02 ± 0.45	0.18 ± 0.04	5.02 ± 0.45	7.24 ± 1.17	0.0009 ± 0.0001
	Pamunkey	33.1 ± 3.68	0.38 ± 0.10	4.25 ± 0.45	0.21 ± 0.02	4.25 ± 0.45	8.04 ± 2.05	0.0011 ± 0.0002

Soil organic matter (OM), total nitrogen (N), total carbon (C), bulk density (BD), gravimetric moisture (GM), potential aerobic C mineralization (Pot. C min.), potential aerobic C turnover (Pot. C turnover)

soil organic matter, N, and C concentrations, and lower tidal soils were lower in bulk density compared to upper tidal soils (Table 4). Longitudinal patterns in organic matter concentrations were further dependent on microtopography: in hummocks, organic matter concentrations increased along a down-river gradient by site (upper < middle < lower tidal wetlands); in hollows, upper tidal soils had the lowest organic matter concentrations (upper < middle and lower tidal wetlands), while middle tidal soils had the highest organic matter concentrations (middle > upper and lower tidal wetlands) (Table 4). Potential aerobic C mineralization rates were higher in lower tidal soils compared to upper tidal soils (Table 4). Middle tidal soils had lower potential C turnover rates than lower tidal soils, and neither differed from upper tidal soils (Table 4).

Hummocks and hollows across sites and rivers varied by multiple physicochemical properties. Compared to hollows, hummock soils had lower gravimetric moisture levels, greater bulk densities, greater rates of potential C mineralization, and greater rates of potential C turnover (all $P < 0.05$) (Tables 3 and 4). Hummock and hollow microtopography did not impact organic matter, N, or C concentrations (all $P > 0.05$) (Tables 3 and 4).

Hummocks also supported higher oxygen levels than hollows. The two sensors in tidal wetland hollows were deployed at low tide with standing water present and almost immediately and consistently recorded 0% for the monitoring period presumably due to undrained soils during low tides. The sensors in the tidal wetland hummocks were not deployed directly under water, and were assumed to be inundated less frequently than the sensors in the hollows and to rapidly drain at low tide. The sensor in the lower tidal hummock remained at < 1% with slight variability indicative of high microbial oxygen demand, while the sensor in the higher tidal hummock revealed higher long-term soil oxygen levels (> 10%) that fluctuated with watershed-driven storm flooding occurring from 9/20/16 to 10/15/16 (Fig. 4). By comparison, near-atmospheric oxygen levels were recorded by the one sensor in the nontidal wetland site outside of the period affected by the watershed-driven storm flooding.

Porewater salinity and water level trends differed by river and longitudinal position. Median soil porewater salinities on the Mattaponi River were higher than the Pamunkey River for the nontidal, upper tidal, and middle tidal sites, with little overlap in range (Fig. 5). For all sites, median porewater salinities were below 0.5 ppt (Fig. 5). Over the same measurement periods, median water levels in nontidal and upper tidal wetlands were both higher on the Mattaponi River (above soil surface) than the Pamunkey River (below the soil surface, Fig. 5). In the tidal wetlands, the Mattaponi upper tidal site had the

Table 4 Mean (± standard error) soil physicochemical properties of nontidal and tidal sites by microtopography pooled across rivers

Microtopography	Site	OM (%)	N (%)	C (%)	BD (g cm^{-3})	GM (g g^{-1})	Pot. C min. (mmol-C kg^{-1} day^{-1})	Pot. C turnover (mol-C mol-C^{-1} day^{-1})
Nontidal plots	Nontidal	12.3 ± 1.02	0.31 ± 0.04	4.78 ± 0.61	0.71 ± 0.06	0.83 ± 0.13	4.07 ± 0.55	0.0011 ± 0.0001
Tidal hummocks	Upper	16.3 ± 1.61	0.37 ± 0.06	5.67 ± 0.79	0.38 ± 0.03	1.86 ± 0.34	5.56 ± 0.98	0.0012 ± 0.0002
	Middle	47.5 ± 6.56	1.37 ± 0.18	23.4 ± 3.64	0.28 ± 0.03	3.11 ± 0.40	16.1 ± 2.62	0.0009 ± 0.0001
	Lower	53.4 ± 2.83	1.55 ± 0.08	27.1 ± 2.02	0.19 ± 0.01	3.58 ± 0.22	33.4 ± 5.22	0.0015 ± 0.0001
Tidal hollows	Upper	19.2 ± 0.83	0.49 ± 0.07	7.33 ± 0.90	0.30 ± 0.03	3.58 ± 0.46	3.66 ± 0.45	0.0006 ± 0.0000
	Middle	50.4 ± 7.43	1.36 ± 0.19	25.6 ± 4.24	0.17 ± 0.03	5.05 ± 0.57	7.09 ± 0.65	0.0004 ± 0.0000
	Lower	44.4 ± 3.20	1.43 ± 0.10	23.6 ± 1.52	0.16 ± 0.01	5.28 ± 0.45	12.2 ± 2.34	0.0006 ± 0.0001

Soil organic matter (OM), total nitrogen (N), total carbon (C), bulk density (BD), gravimetric moisture (GM), potential aerobic C mineralization (Pot. C min.), potential aerobic C turnover (Pot. C turnover)
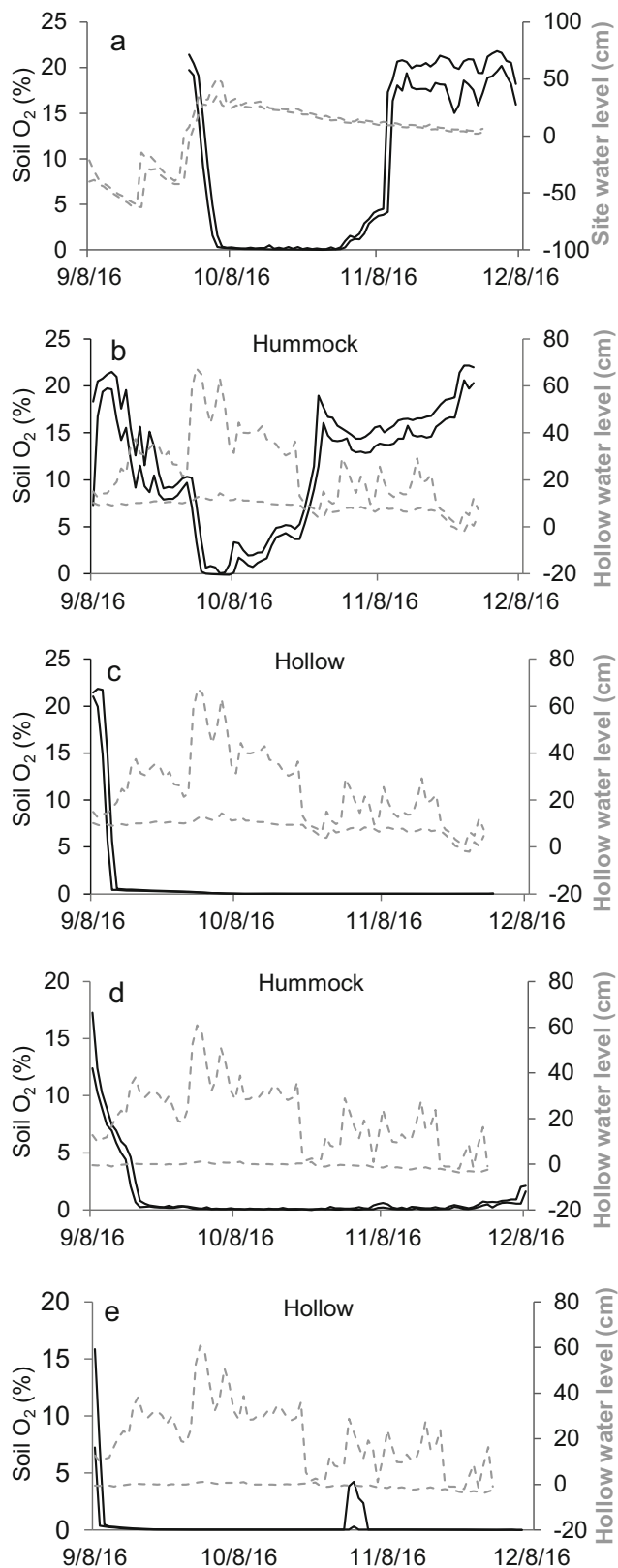
Fig. 4 Daily minimum and maximum soil oxygen levels (black, solid) of the Mattaponi River measured at the nontidal site (a), upper tidal site (b, c), and middle tidal site (d, e) juxtaposed with daily minimum and maximum water levels relative to soil surface of hollows (gray, dashed)

highest median standing water level (9 cm), associated with the highest minimum water level, while the Pamunkey upper tidal site had the lowest median water level (− 3 cm) (Fig. 5). Low-tide water levels dropped farther below ground in the wetlands along the Pamunkey River than on the Mattaponi River for each set of tidal sites (Fig. 5). In the 2 weeks leading up to sampling, water levels in nontidal wetlands were generally lower than middle and lower tidal wetlands (Fig. 5).

Correlations of DEA and Nutrient Limitation of DP with Soil Physicochemical Properties Within Tidal Wetlands

We correlated denitrification metrics with additional soil characteristics to explore explanations for spatial patterns in
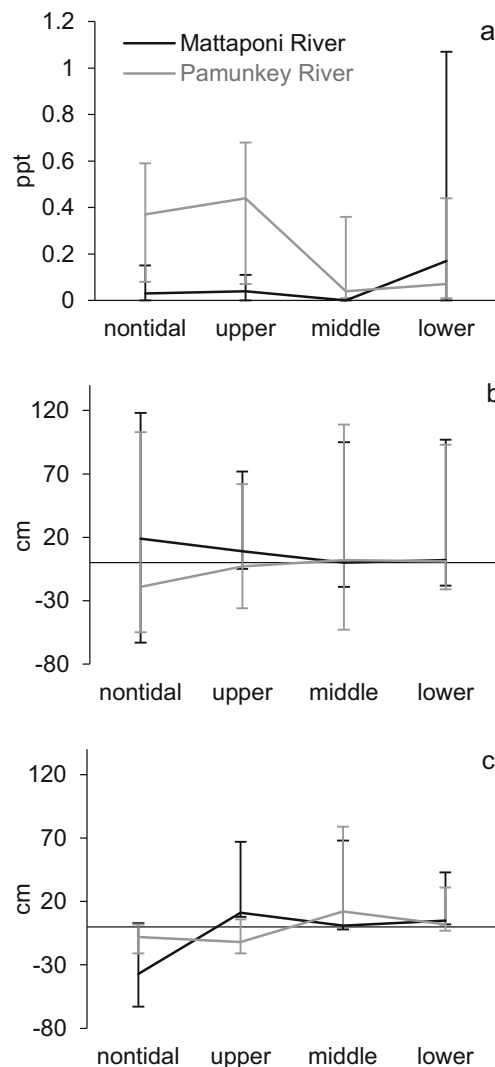

Fig. 5 Median and range of soil porewater salinity (a), long-term water level (b), and water level before sampling (c) for study sites. Long-term water level was measured for 10 months on the Pamunkey River and for 12–18 months on the Mattaponi River. As a subset of long-term water levels, water levels before sampling captured the 2-week span prior to each site's sampling date

denitrification. Three sets of correlations were calculated: two for soils in hummocks and hollows to assess differences in river and site by microtopography, as well for all tidal soils combined to assess trends at the site and river level. DEA was positively correlated with total N, C, and organic matter concentrations, and negatively correlated with bulk densities in hummocks (all $P < 0.05$, Fig. 6). DEA was also correlated with total N in all plots ($P = 0.024$), but this trend was apparently driven by the relation with hummocks and not hollows. No other correlations with DEA were found, including none in hollows (all $P > 0.05$). The lack of correlation between DEA and either potential C mineralization or potential C turnover rates ($P > 0.05$) in hummocks indicates that the correlations between DEA and organic matter or C concentrations were not related to C availability and aerobic microbial activity. Nitrate limitation of DP was negatively correlated with potential C mineralization in hummocks and with bulk densities in hollows (both $P < 0.05$, Fig. 6), but not with any other soil physicochemical property or process across microtopography (all $P > 0.05$). Oxygen limitation of DP was not correlated to the soil physicochemical properties (all $P > 0.05$). Relationships between the denitrification measurements and moisture levels were not assessed because moisture was used to adjust their calculated rates.

Discussion

This study investigated spatial patterns in denitrification and its controlling factors that inform our understanding of N cycling and water quality functions in TFFWs. Across three spatial dimensions—across two rivers (and associated watershed land-use differences), along a longitudinal river gradient, and between hummock and hollow microtopography—we expected differences in N concentrations and soil nutrient availability to affect denitrification. By river, we found less nitrate limitation of DP in the nitrate-enriched hollows of the Pamunkey River that carries greater nitrate concentrations than the Mattaponi River. Along a longitudinal gradient, we found that DEA was elevated in tidal wetlands and particularly in the two most downriver tidal wetlands compared to nontidal wetlands, reflecting long-term changes in the capacity for denitrification in these forested wetlands. Finally, denitrification was affected by eco-hydrogeomorphic processes associated with hummock-hollow microtopography, particularly where oxygen stimulated substrate availability for DP more in hummocks than hollows, but also where hummock-hollow microtopography mediated the patterns of denitrification by river or longitudinal gradient.

Effect of Tidal River N on Potential Denitrification

Experimental nitrate limitation of denitrification using nitrate amendment is commonly demonstrated in tidal wetlands, as are relationships of denitrification with nitrate availability (Koop-Jakobsen and Giblin 2010; Morrissey and Franklin 2015; Pastore et al. 2016). In line with previous findings, almost all of our denitrification potential rates (> 90% of measurements) were nitrate limited (stimulated by nitrate amendment). Nitrate concentrations and loads in both the Pamunkey and Mattaponi Rivers indicate a relatively moderate influx of anthropogenic N (Langland et al. 1995; Shields et al. 2008), which should increase N processing in tidal wetland soils. Yet, both the Pamunkey and Mattaponi Rivers are relatively low (below the 25th percentile) in anthropogenic N yield (kg year^{-1} km^{-2}) compared to other rivers in the Chesapeake Bay watershed (Chanat et al. 2015). For that reason, concentrations in both rivers are within a range where denitrification rates are still nitrate limited (Inwood et al. 2005; Arango et al. 2007) and where denitrifiers may still rely predominantly on in situ nitrification (Seitzinger et al. 2006; Hopfensperger et al. 2009; Jäntii et al. 2011).

River effects on wetland soil nitrate concentrations depended on hummock-hollow microtopography. For hollows, soil nitrate concentrations were consistently greater on the Pamunkey River where similar concentrations were found across tidal wetland sites. The shallow soils of hollows are strongly influenced by dissolved and particulate matter of floodwater due to tidal pumping (Zheng et al. 2016), and thus hollows should reflect river nitrate concentrations where nitrate demand by the ecosystem has been met. In contrast, hollows of the Mattaponi River had lower nitrate concentrations that varied among sites, suggesting that nitrate concentrations on this river were not meeting demand. Nitrate concentrations in hummocks showed the opposite pattern than hollows, with greater nitrate concentrations on the Mattaponi River than Pamunkey River. We provide the following two observations that suggest site-specific, rather than river-specific, phenomena caused the observed difference in hummocks across the two rivers. For the upper tidal wetland site on the Pamunkey River, hummocks likely received less dissolved nitrate from the river than hummocks on the Mattaponi River due to lower water levels (Fig. 5). For the middle tidal wetland site on the Pamunkey River, lower nitrate concentrations in hummocks reflected the lower soil moisture levels and lower organic matter, C, and N concentrations (all $P < 0.001$) than the Mattaponi River (middle tidal soils had greater concentrations of organic matter, C, and N than upper tidal soils); middle tidal hummocks on the Mattaponi River more closely resembled lower tidal hummocks in organic matter concentrations than the Pamunkey River middle tidal hummocks. In conclusion, there was strong evidence that differences in river N loads affected N pools in tidal hollows, but only weak
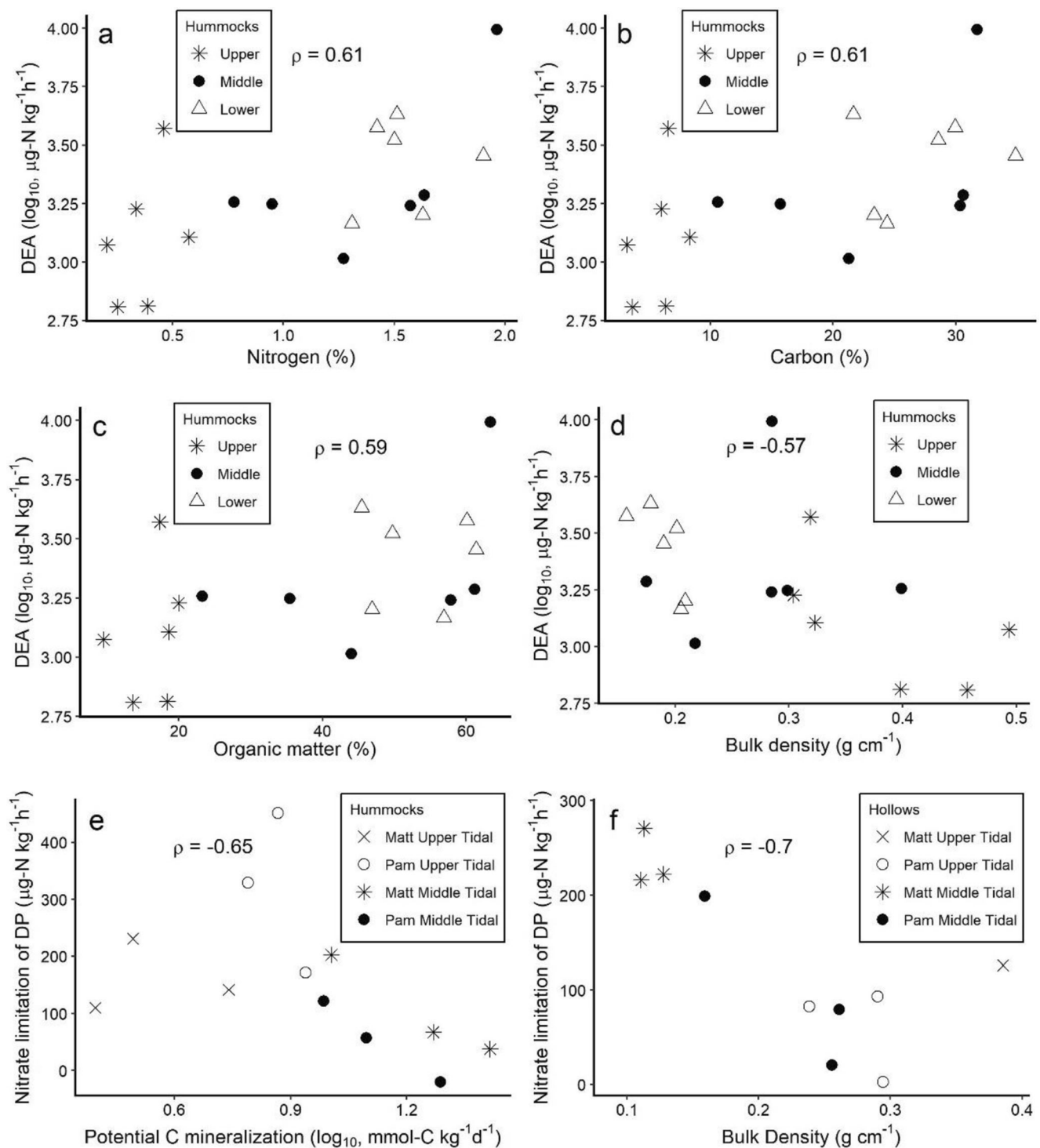
Fig. 6 Scatterplots showing statistically significant associations of denitrification enzyme activity (DEA) (**a**–**d**) and nitrate limitation of denitrification potential (DP) (**e**, **f**) with soil biogeochemical properties and processes by microtopography on the Mattaponi River (Matt) and Pamunkey River (Pam)

evidence for hummocks. The soil nitrate differences in hummocks we observed by river appeared to be confounded by longitudinal gradients in hydrogeomorphic processes that affected the degree of internal control over N processing.

Along with greater soil nitrate concentrations, the Pamunkey River hollows also had less nitrate limitation of

DP compared to the Mattaponi River. We found that DP was less stimulated by nitrate amendment on the Pamunkey River indicating less demand in these wetlands. Intensifying river nitrate loads to these rivers are expected to boost high-tide denitrification rates more in TFFW hollows on the Mattaponi River, and as an extension, to boost TFFW

denitrification rates more in rivers where anthropogenic N loads are relatively low. Though greater nitrate delivery on the Pamunkey River theoretically stimulates greater overall rates of denitrification, and N fertilization to tidal freshwater wetlands has been shown to positively affect denitrification potentials through shifts in microbial community composition (Morrissey and Franklin 2015), we found no differences in DEA rates by river for either hummocks or hollows. Long-term excess nitrate could lead to additional demands for organic C and cause greater regulation of denitrification by temperature and pH (the other mechanistic controls of the process) (e.g., Reddy et al. 1982; Wallenstein et al. 2006). For instance, tidal wetlands are known to export dissolved organic C to estuaries (Tzortziou et al. 2011), and thus labile C (the electron donor in denitrification) may also not accumulate in large quantities in shallow soils due to tidal flushing (Neubauer et al. 2005b).

An alternative explanation for the lack of stimulation of DEA on the Pamunkey River relates to the consequences of long-term N and phosphorus (P) loading, which include soil acidification, lower soil cation exchange capacity, and changes to plant diversity in terrestrial environments (Bowman et al. 2008). In addition to greater river N, the Pamunkey River has higher long-term (1985–2012) P and sediment concentrations compared to the Mattaponi River (Chanat et al. 2015), as well as higher wetland soil pore-water salinity (this study). We hypothesize that greater soil acidity (not measured in this study) resulting from a decline in available base cations from the higher anion concentrations, could have contributed to the higher soil pore-water salinity along the Pamunkey River, which would have directly and indirectly reduced soil suitability for denitrification.

Longitudinal Patterns in Denitrification Along a Watershed to Estuary Gradient in Tidal Wetlands

Along both tidal rivers, the physicochemistry of tidal freshwater wetland soils exhibited strong longitudinal gradients (Table 4)—with greater organic matter, C, and N, and lower bulk density in lower tidal soils and sometimes middle tidal soils compared to upper tidal soils—that we attribute to concurrent longitudinal patterns in inundation and sedimentation. Kroes et al. (2007) showed that sedimentation deposits in TFFWs of the Pocomoke River, MD, was composed of greater organic material than the more mineral sediment deposits in upriver nontidal forested floodplains, as a result of less effective transport of watershed mineral (terrigenic) sediment into tidal rivers given more consistent inundation. Applying this mechanism to the gradient of inundation within TFFWs, the ratio of organic matter to minerals in sediment may also be highest in downriver TFFW soils compared to upriver TFFW soils because tidal inundation frequency and recurrence is

highest in downriver zones compared to upriver zones (Ensign et al. 2013). Additionally, our longitudinal patterns in biogeochemical properties of TFFWs along both rivers are consistent with the hypothesis that only TFFWs near the head of tide and not TFFWs in lower tidal rivers accumulate meaningful amounts of terrigenic sediment from watershed floods, which can increase sediment accretion rates (Ensign et al. 2013). Longitudinal patterns along the tidal gradient in soil organic matter, nutrients, and bulk density are noteworthy as they appear to partly explain differences in denitrification metrics and soil nitrate (as discussed below).

Compared to upper tidal wetland hummocks, middle tidal wetland hummocks had higher nitrate concentrations and less nitrate limitation of DP, with trends in DP most dramatic on the Pamunkey River. Though greater denitrification rates in upper tidal wetlands would have reduced soil nitrate concentrations and promoted nitrate limitation of denitrification, our data on potential C mineralization suggest that differences in nitrate concentrations more likely reflected differences in soil organic matter quality and quantity. Less nitrate limitation of DP in middle tidal wetland hummocks was related to greater potential C mineralization, and the greater decomposition often accompanying larger organic matter stocks has been linked to the alleviation of nitrate limitation in denitrification potential (Morrissey and Franklin 2015) through coupled ammonification and nitrification. Nitrate availability might also have been particularly limiting in upper tidal wetland Pamunkey sites because low water levels recorded in the 2 weeks preceding soil sampling suggest less delivery of river nitrate (Fig. 5).

Though mean DEA did not change with statistical significance across tidal wetlands (but where middle and lower tidal sites supported the highest rates), many relationships between DEA and other soil physicochemical properties were mediated by longitudinal position and microtopography. Relations between DEA and soil properties in hummocks followed a positive downstream trend with high rates of DEA in middle and especially lower tidal hummocks that had greater C, N, and organic matter concentrations, and lower bulk density. Because the latter three soil properties relate to soil C concentrations, these relationships together indicate that increasing organic nutrient quantity (in a downstream direction) promoted denitrification. Von Korff et al. (2014) also found positive associations between denitrification and the quantity of organic matter in TFFWs and their channels in North Carolina, though the trends were seasonally dependent and suggestive of mediation by C quality. The longitudinal trends in DEA and DP indicate that the upper reaches of TFFWs will increase denitrification rates most in response to either increasing inundation from sea level rise or increasing river N loading from land-use change. Overall, our mean (and standard error) DEA rate (2.30 ± 0.39 mg-N kg^{-1} h^{-1}) for the tidal soils is higher than the mean DEA rates reported for TFFWs (0.07–0.57 mg-

N kg^{-1} h^{-1}) and tidal freshwater marshes (0.1 ± 0.05 mg-N kg^{-1} h^{-1}) in Georgia (Craft et al. 2009; Marton et al. 2012), but our range in DEA rates (0.31–9.8 mg-N kg^{-1} h^{-1}) overlaps largely with rates in tidal freshwater marshes found in Virginia (Hopsfensperger et al. 2009), New York (Alldred et al. 2016), and Connecticut (Findlay et al. 2003).

While we found no statistical differences in DEA between middle and lower tidal wetlands, we had expected salinity and/or sedimentation to stimulate denitrification in lower tidal sites and note that consistently elevated DEA rates occurred in the lower tidal wetland hummocks relative to middle and upper tidal wetland hummocks. Lower TFFW sites are thought to be distinct from middle and upper tidal wetlands by greater nutrient and sediment deposition that result from greater river sediment availability near the oligohaline zone and greater wetland herbaceous biomass (Ensign et al. 2014; Noe et al. 2016). To the extent that sedimentation delivers labile nutrients to soils, this sedimentation in forest-to-marsh transition zones would promote DEA. As evidence of this feedback, soil organic matter concentrations in hummocks were positively correlated with DEA rates and were highest in lower tidal wetlands. Lower tidal wetlands have also likely endured more frequent storm- and drought-related saltwater surges than upriver tidal wetlands (Fig. 5) that elevate soil salinities and cause stress and mortality for woody species (Cormier et al. 2013; Anderson and Lockaby 2012). Higher salinities may indirectly benefit denitrification from greater plant N inputs into the soil from early leaf senescence (Brinson et al. 1985) or to greater labile biomass from decomposing herbaceous plants, which has been attributed to the higher net rates of ammonification in salt-stressed forested wetlands (Noe et al. 2013). In lower tidal wetland hummocks, four of the six highest rates of DEA coincided with relatively high rates of potential aerobic C mineralization. Higher salinities within forested or herbaceous freshwater wetlands have been associated with greater denitrification potential and gene abundance (Marton et al. 2012; Franklin et al. 2017). Inhibition of denitrification due to salinity is not expected in TFFW hummocks due to soil aeration during tidal drawdown (Liu et al. 2017), though inhibition may occur in hollows (see discussion below).

The strong longitudinal gradients in soil organic matter content, linked to watershed floods, salinity, and sedimentation, could affect denitrifiers in hummocks by promoting gradients in microbial metabolism and oxidation reduction potentials. Middle tidal hummocks had over four times more soil C, roughly three times more soil N and organic matter, and less nitrate limitation of DP than upper tidal hummocks (Table 4). Velinsky et al. (2013) reported positive relationships between N$_2$ production and soil oxygen demand in tidal wetlands.

In contrast to hummocks, DEA was unrelated to the measured soil physicochemical properties in hollows and was further markedly lower in hollows than in hummocks at the salt-

impacted, lower TFFW sites (Fig. 3). To put these results in context, Arango et al. (2007) found a positive association between denitrification and substratum organic matter in freshwater streams over the same wide range of organic matter as found in this study. One reason for the lack of relationships between DEA and soil physicochemical properties in hollows may be due to greater vertical sediment accretion in hollows than in hummocks (Ensign et al. 2014), the long-term implications of which are not understood. Recently buried plant litter with greater sediment accumulation constitutes low-quality C that provides less stimulatory effect on denitrification compared to more humified material (Morrissey and Franklin 2015); high C:N ratios of undecomposed plant litter can increase competition for ammonium and suppress nitrification (Strauss and Lamberti 2000; Starry et al. 2005; Megonigal and Neubauer 2009). A second reason for the lack of denitrification correlations in hollows might be the longer period of interaction with salinity compared to higher-elevation hummocks in the lower TFFW sites (e.g., Neubauer et al. 2018). For instance, Liu et al. (2017) found that gaseous N$_2$O release from the soil was inhibited by low levels of salinity (5 ppt) under constantly flooded conditions but not under fluctuating soil moisture conditions; specifically, hydrogen sulfide (H$_2$S) can directly inhibit NO and N$_2$O reduction in the multi-step process of denitrification (Sorenson et al. 1980). The adverse effects of H$_2$S on nitrification (Joye and Hollibaugh 1995; Dollhopf et al. 2005) would also be substantial for consistently inundated hollows of TFFW. Our data show that the highest rates for the change in net nitrification potential (given aeration) were not in tidal hollows but tidal hummocks and nontidal floodplains. It should be noted, however, that others have found positive effects of, or associations between, salinity and nitrification in tidal streams or forested wetlands (Magalhães et al. 2005; Noe et al. 2013). Third, higher concentrations of sulfate in the lower tidal hollows, if present, could stimulate C mineralization through sulfate reduction (Weston et al. 2011), which would reduce labile C stores to the detriment of denitrifiers.

Patterns of Denitrification in Nontidal vs. Tidal Wetlands

Tidal wetlands had greater DEA than nontidal wetlands, a pattern that was more pronounced on the Mattaponi River than on the Pamunkey River. Soil oxygen levels measured from one sensor at the nontidal Mattaponi River floodplain wetland were above 15% O$_2$ during base-flow conditions, which would have an inhibitory effect on denitrification (Burgin et al. 2010; Duncan et al. 2013). On the Pamunkey River, lower DEA rates in the nontidal wetland occurred despite relatively high NO$_3$ concentrations and the change in net nitrification potential, providing novel evidence that tidal inundation promotes denitrification in forested wetlands along

coastal rivers (see Verhoeven et al. 2001). This finding suggests that TFFWs should have an important impact on removing river nitrate. Because all tidal wetland sites had greater soil gravimetric moisture and lower bulk density than nontidal wetland sites, tidal inundation likely primed DEA consistently in tidal soils through greater saturation and anoxia. Greater differences in nutrient and organic matter pools and microbial activity between the nontidal wetland sites and two sets of downstream (middle and lower) tidal wetland sites also likely contributed to DEA differences.

For the change in net nitrification potential under aerating conditions across nontidal and tidal wetland sites, rates appeared to be positively affected more by nutrient availability than potential differences in oxygen availability—as originally hypothesized. Our highest rates of change in net nitrification potential with aeration were measured in the nontidal wetlands of the Pamunkey River as well as in all middle tidal wetland hummocks, locations with relatively elevated organic matter and potential C mineralization rates. This finding is consistent with evidence that NH_4, a product of organic matter decomposition and a substrate for nitrification, mechanistically controls nitrification (Gribsholt et al. 2005; Magalhães et al. 2005).

Effect of Hummock and Hollow Microtopography on Denitrification

Hummock-hollow microtopography is ubiquitous in TFFWs, where trees are generally restricted to growing on hummocks (Day et al. 2007). Hummocks form when woody plants colonize decaying trees and stumps, which naturally elevates the plants above base flow and mitigates physiological stress caused by continuous inundation. From a comprehensive survey of TFFWs of the Pamunkey River, Rheinhardt (1992) reported that hummocks comprised 35% of the forest floor in ash-black gum communities and roughly 75% of maple-sweetgum communities.

In this study of TFFWs influenced by daily lunar tides, hummocks were elevated 10–20 cm over hollows. Because the median difference between daily high and low tide for the upper and lower tidal Mattaponi River sites, for periods outside of watershed flooding events, were 13 cm and 20 cm, respectively, hummocks were inundated and drained regularly. Along with less sustained inundation in hummocks than hollows, our long-term soil oxygen sensors on the Mattaponi River demonstrated that soil oxygen levels in hummocks were higher and/or showed greater variability than in hollows. In particular, high oxygen levels (> 10%) were measured in the upper tidal wetland hummock at river base flow. These data indicate that aerobic microbial respiration at low tide was likely greater in hummocks than hollows.

We further found that tides influence denitrification more in hummocks than hollows. For all soils, an experimental tidal fluctuation (encompassing a dry aeration of soils prior to inundation with varying nitrate concentrations) generally increased rates of DP relative to no fluctuation, a result consistent with previous studies demonstrating that dry-wet cycles produce greater N_2O flux and microbial respiration in tidal forested wetlands soils (Krauss et al. 2012; Liu et al. 2017). This oxygen-controlled substrate limitation of DP was greater in hummocks than hollows, and thus hummocks stimulate denitrification with aerobic nutrient processing at low tide more than do hollows. Despite the lack of direct correlations, elevated rates of potential C mineralization and C turnover likely contributed to greater potential for denitrification, and thus greater oxygen-controlled substrate limitation of DP in hummocks. Previous studies have found hummocks to have faster decomposition rates and greater phenol oxidase (enzyme) activity than hollows (Courtwright and Findlay 2011; Minick et al. 2018), and tidal influx of organic material and/or primary productivity levels might sustain elevated C mineralization, and in turn, long-term denitrification. This mechanism supporting long-term denitrification activity in hummocks, though, is more at risk from sea level rise than in hollows. Though we did not find microtopography differences in the change in net nitrification potential under aeration, Noe et al. (2013) found that TFFW hummocks in South Carolina and Georgia tidal rivers had higher rates of field-measured net nitrification, as well as greater microbial biomass nutrient (i.e., N, C, and P) content than hollows.

Conclusions

Understanding the existence and causes of spatial patterns of denitrification in TFFWs will improve predictions of ecosystem change from increasing watershed N export and sea level rise. To that end, we investigated landscape patterns in denitrification metrics across two rivers. We found that greater nitrate availability in TFFW hollows was linked to less nitrate limitation of DP, which suggests that TFFWs along rivers with low relative N concentrations will show the strongest increases in denitrification with increasing anthropogenic N inputs. Further, tidal soils had much greater DEA and gravimetric moisture levels, and lower bulk densities than nontidal soils, while middle and/or lower tidal soils had higher organic matter, N, and C pools and higher potential C mineralization rates (also lower in bulk densities) than upriver (upper) tidal soils. These longitudinal gradients in tidal soils were positively related to DEA in hummocks, which have not been demonstrated before in TFFWs. Upstream tidal extension from sea level rise will thus increase denitrification in nontidal soils that convert to tidal freshwater. Sea level rise that affects hydrogeomorphic processes and hummock-hollow pattern, such as sedimentation, inundation, and salinity, will also alter current biogeochemical spatial gradients in TFFWs. Hummock-

hollow microtopography, which controls soil biogeochemistry by regulating tidal inundation, was present across tidal sites. Compared to hollows, hummocks had higher soil oxygen levels and greater oxygen-controlled substrate stimulation of DP from short-term laboratory aeration, the latter of which we attributed to greater potential C mineralization and turnover in hummocks. The mechanism supporting denitrification in hummocks is thus more at risk from sea level rise than in hollows. Influence of this ecogeomorphological feature on ecosystem processes and its vulnerability to sea level rise warrant consideration in future studies of TFFWs. In line with previous findings suggesting either biogeochemical and hydrologic control on denitrification at landscape scale (Marton et al. 2012; Ensign et al. 2013), denitrification in TFFW does indeed have multiple largescale (i.e., river and longitudinal position) and small-scale (i.e., hummock-hollow microtopography) spatial differences in rates and controls that can aid in estimating the ecosystem services of TFFWs and the prediction of future rates.

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