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Denitrification rates in tidal marsh soils: The roles of soil texture, salinity, and nitrogen enrichment

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Running title: *Denitrification rates in tidal marsh soils*

Summary

The denitrification rates of freshwater and oligohaline tidal marsh soils with different textures (loam and sandy soils) in a subtropical estuary, and their responses to nitrogen (N) loading, were investigated. In both marshes, the denitrification rates varied significantly with the season only in loam soil. The denitrification rates were highest in oligohaline marsh loam soil and lowest in freshwater marsh sand soil. NH_4NO_3 addition significantly increased the denitrification rates of all the marsh soils. Our findings suggest that soil texture, soil organic matter (SOM) content and low-level increases in salinity all had large effects on denitrification, indicating that the dynamics of denitrification rates in estuarine marshes with low-level salinity were controlled by the interaction of salinity and soil texture but mainly depended on SOM content. We propose that denitrification in tidal marshes plays an important role in regulating current and future N loading into estuary and inshore coastal waters, especially for tidal freshwater marshes, which introduces great uncertainty to the N dynamics of estuaries under global changes.

Keywords: soil texture, salinity, nitrogen enrichment, SOM, denitrification, estuarine tidal marsh

Highlights:

- Denitrification rates were higher in loam than sandy soil, independent of salinity.
- Denitrification rates were higher in oligohaline marsh loam than freshwater marsh loam.
- Exogenous N input enhanced denitrification in estuarine marsh soil.
- Loam with high-N enhanced N removal, especially in freshwater environments.

Introduction

Denitrification is the dominant natural pathway for N-cycling transformation in wetlands. However, the factors that control this pathway are multifaceted and interactive (Day *et al.*, 2018; Neubauer *et al.*, 2019; Zhang *et al.*, 2019). Estuarine marshes play a vital role in denitrification because they consist of a combination of anaerobic environments and include carbonaceous substrates in the soil (Marks *et al.*, 2016). Previous studies confirmed that denitrification in estuaries is variable and potentially regulated by various factors, such as the soil texture, salinity and N loading (Lee *et al.*, 2017; Wang *et al.*, 2017; Hinshaw *et al.*, 2019). Owing to the influence of upstream runoff and tidal saltwater intrusion, the salinity and soil texture change significantly in estuary systems, and, at the same time, large amounts of exogenous N derived from fertilization are introduced into estuaries from runoff, tides and deposition (Hu *et al.*, 2019; Neubauer *et al.*, 2019). However, few studies have explicitly linked potential denitrification with the above factors simultaneously, and this knowledge gap currently limits the understanding of the geochemical processes that drive N cycling and the associated environmental responses.

The Min River estuary tidal marsh undergoes a clear shift from a freshwater to an oligohaline environment (Tong *et al.*, 2017) and has a high N input from agricultural and industrial activities (Hu *et al.*, 2019). Such an estuarine system provides an ideal environment for studying the responses of soil denitrification to the roles of soil texture, salinity and N enrichment. Herein, we conducted a seasonal incubation experiment to assess the interactive effects of soil texture, salinity and N loading on the denitrification of marsh soils. We hypothesized (1) that the denitrification rate of freshwater marshes is higher than that of oligohaline marshes, and (2) in both freshwater and oligohaline marshes, the denitrification rate is higher in loamy than in sandy soils, especially in the soils with additional N.

Materials and methods

Soils were collected from a subtropical estuarine marsh in the Min River estuary in southeastern China (Figure S1). We selected two tidal marshes: the freshwater Longxiangdao Marsh (26°1.8′52.8″ N, 119°18′17.8″ E) and the oligohaline Shanyutan Marsh (26°1.8′13″ N, 119°37′46″ E), with average salinities of 0.08±0.02 and 2.70±0.12 ppt, respectively. In the Longxiangdao marsh, we collected soil samples with different textures from stands of the same plant species (*Cyperus malaccensis*). In the Shanyutan Marsh, the loam soil samples were also collected in *C. malaccensis* stands, but the sandy soil samples were only collected in *Spartina alterniflora* stands near the sea. The biomass of *C. malaccensis* was not significantly different between the two marshes (Wang *et al.*, 2017; Luo *et al.*, 2019). In the Shanyutan marsh, the total above- and belowground biomasses were 4230 g m^{-2} for the *C. malaccensis* stand and 4620 g m^{-2} for the *S. alterniflora* stand, which were nearly identical. Two soils (0–15 cm; in triplicate) of contrasting textures were selected at each marsh, namely sandy (coarse-textured) and loam (fine-textured) soils. The soils were sieved to 2 mm, homogenized, and then divided into two subsamples for measuring the denitrification rate and physicochemical properties. Tidal water was also collected simultaneously for incubation and analysis. The main properties of the soils and tidal water, along with their values, are listed in Table 1.

The denitrification potential was measured using the modified chloramphenicol-amended acetylene (C_2H_2) inhibition technique (Magalhães *et al.*, 2005; Marton & Craft, 2012; Ballantine *et al.*, 2014). Although the acetylene inhibition technique may result in an underestimated ambient denitrification rate due to an inhibitory effect on nitrification (Rudolph *et al.*, 2006; McCrackin & Elser, 2010; Palta *et al.*, 2016), this technique has nevertheless been employed by many previous studies (Ullah & Zinati, 2006; McCrackin & Elser, 2010; Marton & Craft, 2012; Lishawa *et al.*, 2014;

Tomasek *et al.*, 2017) to compare denitrification rates among different sites and treatments. Briefly, 30 g of fresh soil was transferred to 140 mL flasks; chloramphenicol (0.3 mg) and glucose (1.2 mg) were added as substrate and enzyme inhibitor, respectively. Then, 30 mL of the treatment solutions were injected into each flask, in which N was added as an NH_4NO_3 solution (0.5 $mg \cdot g^{-1}$ dry soil); tidal water was added instead of the NH_4NO_3 solution in the control samples (six replicates for each treatment). Each treatment sample was sealed and made anoxic by filling with pure N_2 for 5 min. Then, the samples were divided into two subgroups (triplicate): with and without acetylene (10% vol: vol). All samples were then incubated at four temperatures (21, 11, 17 and 28 °C representing the seasonal conditions of the autumn, winter, spring and summer samples, respectively) in a dark shaking incubator for 6 h. Gas samples were taken after 0.5, 1.5, 3.5 and 6 h of incubation, and the N_2O concentrations were analyzed using a gas chromatograph (Shimadzu Corporation, Kyoto, Japan) equipped with an electron-capture detector. The denitrification rates were calculated as the difference between the N_2O produced with and without acetylene (Magalhães *et al.*, 2005) and were expressed in $\mu g\ N \cdot kg^{-1} \cdot h^{-1}$ based on their dry weight. The physicochemical properties of soils and tidal waters were measured as described in our previous study (Tong *et al.*, 2017; Hu *et al.*, 2019).

When necessary, data (i.e., denitrification rates and environment variables) were log-transformed to meet the analysis of variance (ANOVA) assumption of normality and homoscedasticity. The differences in denitrification rates and environment variables between the different seasons and soils were tested using a one-way ANOVA. In cases where significant fixed effects were detected, pairwise comparisons among groups were conducted via Tukey’s post hoc test. A two-way ANOVA was used to identify the effects of soil texture and season on the denitrification rates using R version 3.5.1 (R Development Core Team, 2008). Overall distributions and variations in soil denitrification rates and environmental parameters among the study sites were summarized using a principal components analysis (PCA) in R platform.

Results and discussion

The pH of the soils varied significantly but irregularly with the season (Table S1). Soil ammonium-N (NH_4^+-N) concentrations varied substantially with the season but were less variable in response to salinity and texture. Soil nitrate-N (NO_3^--N) concentrations had similar seasonal patterns among the sites and were larger in the loam than the sandy soil. First, these larger nitrate concentrations should drive increased nitrification in the fine-texture soils because they continue to have larger nitrate concentrations despite having greater nitrate losses due to increased denitrification rates. Second, the larger total C and N contents in the loam soils together with the higher soil C/N ratios (Table 1) clearly indicates less mineralization of organic N, allowing more substrates for nitrification (Janssen, 1996). The concentrations of dissolved organic carbon (DOC) and organic matter (OM) in the oligohaline marsh with loam soil were the largest and had clear seasonal patterns (Table S1). The high DOC and OM concentrations in the loam soil were assumed to be caused by the physical protection and large surface area of the bigger fine fraction in the loam compared to that of the sand, which protected OM against decomposition and improved adhesion (Perryman *et al.*, 2011).

The denitrification rates of both freshwater and oligohaline marsh soils varied significantly with the season (F=12.59, P<0.01, and F=17.23, P<0.01, respectively; Figure 1; Table S2), which is consistent with the results of a previous study in which optimum temperatures triggered high denitrification rates by stimulating substrate availability and denitrification potential (Wang *et al.*, 2017). The denitrification rates were higher during the winter than during the spring and autumn, probably owing to the increased NO_3^--N availability in the winter (Table S1), which can supply more N substrates to denitrifiers. The denitrification rates were significantly higher in the loam soil than in the sandy soil (Figure 1). These findings may be mainly attributed to a variety of circumstances. First, the lower aeration in the fine-textured loam soil, due to the high level of soil moisture and the low bulk density (Table 1), favours denitrification processes by reducing redox potentials during anaerobic incubation. Second, the larger concentrations of NO_3^--N, DOC and OM in the loam soil than in the sand (Table S1) accelerate denitrifier growth and enzyme synthesis by supplying organic substrates and inorganic electron acceptors (Gu *et al.*, 2013; Palta *et al.*, 2016), thereby enhancing soil denitrification capacity. Soil OM provides not only electrons for NO_3^--N denitrification through mineralization, but also decreases soil redox potential, thus resulting in high substrate availability and suitable environment for the growth and activity of denitrifiers (McLain & Martens, 2006; Xu & Cai, 2007). Moreover, NO_3^--N leaching, which is necessary for microbial growth, is less in loam soil, which contributes to denitrification (Lee *et al.*, 2014).

The denitrification rate of the loam soil was significantly higher in the oligohaline marsh than the freshwater marsh (F=52.01, P<0.001; Figure 1). This finding is inconsistent with earlier studies where denitrification decreased strongly as salinity increased (Osborne *et al.*, 2015; Marks *et al.*, 2016). This is most likely because the small variation of salinity in this estuary (0-3‰) cannot substantially affect the activity of denitrifiers, which in turn also implies that the denitrifiers were acclimated to the soils with low-level increases in salinity. Moreover, the concentrations of NO_3^--N, DOC and OM in the loam soil were greater in the oligohaline than the freshwater marsh (Table S1), which provides sufficient substrate for denitrifiers. A review of the literature also indicated that denitrification rate patterns change with fluctuations in salinity (salinity range 0‰–30‰; Table S3), suggesting that the effect of salinity is site-dependent. The overall PCA indicated that the groups of samples of each soil type are clearly separated in the 2-dimension layout generated by the two main axes (Figure 2). Soil denitrification rates consistently correlate positively with soil N, DOC, OM and pH and negatively with bulk density. Overall, our data indicate that the variable salinity and texture, and the interaction between them, have a significant effect on denitrification (F=21.92, 271.54, and 5.27; P<0.001, <0.001, and <0.05, respectively).

The addition of NH_4NO_3 substantially changed the denitrification rates, but the impact varied with the soil texture and salinity (Figure 3). Specifically, NH_4NO_3 addition significantly increased the denitrification rate relative to the control in the sandy soil (F=9.58, P<0.05) and loam soil (F=7.33, P<0.05) of the freshwater marsh, and in the loam soil (F=1.99, P<0.01) of the oligohaline marsh by 212, 102, and 125%, respectively. Nitrogen is often the limiting nutrient in this estuary system (Wang *et al.*, 2014); therefore, NH_4NO_3 addition provided abundant NO_3^--N as the substrate for direct denitrification (Figure S2b) and also contributed to nitrification by increasing NH_4^+-N availability (Figure S2a), which can lead to high rates of coupled nitrification-denitrification (Gu *et al.*, 2013).

Our findings initially indicated that soil texture might be a critical factor controlling N cycling in wetland systems, regardless of salinity. They suggested that fine-textured soils with low porosity and large contents of NO_3^--N and OM would fuel the denitrification process and would be accompanied by the removal of N. Further, different soil textures in the estuary system are formed at different sedimentation times due to sea-land interactions (Wallace *et al.*, 2005). The longer the sedimentation time, the finer the soil texture, and the associated increases in the content of substrates such as OM eventually leads to increases in denitrification capacity. Our data also clearly demonstrated that an increased N loading could potentially promote the denitrification rates of soils with contrasting textures and salinities. These results indicate that denitrification could be an important pathway to regulate current and future N loading into estuarine and inshore coastal waters, which reduces the negative effects of exogenous N on water eutrophication and acidification but, on the other hand, may increase the risk of global warming. Further, we conclude that tidal marshes with fine-textured loam soil had relatively higher N removal potential, especially for high N-enrichment environments. This partly supports our hypothesis that much uncertainty is introduced in the N dynamics of estuaries under longer-term, climate change-mediated sea level rise and N deposition. Thus, the detailed mechanisms and processes that control estuarine denitrification and, subsequently, N cycling need further consideration.

Acknowledgments

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest

The authors declare that they have no conflicts of interest in this work.

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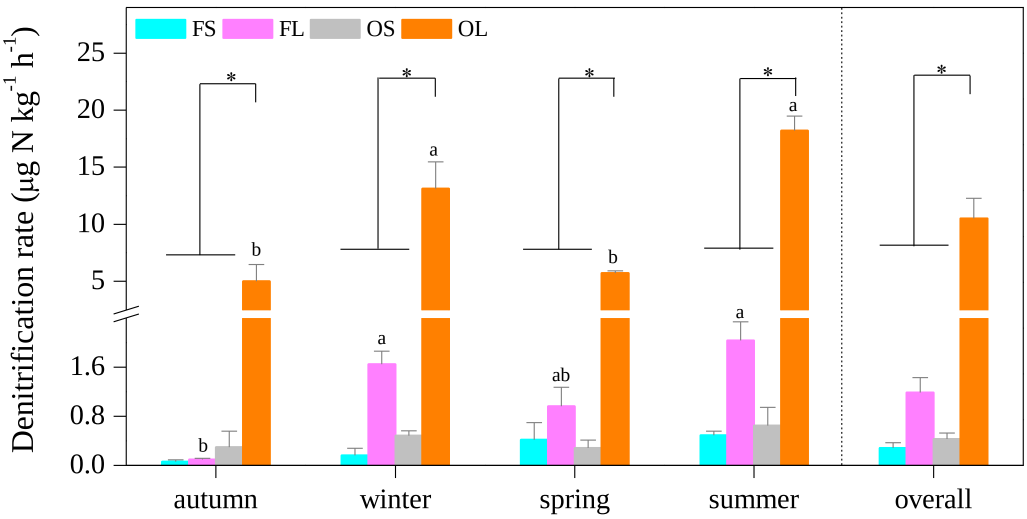
Figure captions

Figure 1 Changes in potential denitrification rates from different soil textures and salinities based on dry weights. Different lowercase letters on the bars indicate significant differences between seasons ($P<0.05$); the absence of letters indicates an absence of significant differences; the asterisks (*) indicate significant differences between the soil types ($P<0.05$). FS and FL represent sand and loam soil in the freshwater marsh, respectively; OS and OL represent sandy and loam soil in the oligohaline marsh, respectively.

Figure 2 Principal component analysis of soil denitrification rates and main environmental parameters in different soil texture and salinity conditions. The means of PC scores of each one of the four soil types are depicted by small circles and the corresponding area around these circles depicts confidence intervals at 95%. BD: bulk density; EC: electrical conductivity; NH_4^+-N: ammonium nitrogen; NO_3^--N: nitrate nitrogen; DOC: dissolved organic carbon; SM: soil moisture; OM: organic matter. TC: total carbon; TN: total nitrogen. FS and FL represent sandy and loam soil in the freshwater marsh, respectively; OS and OL represent sandy and loam soil in the oligohaline marsh, respectively.

Figure 3 Responses of the potential denitrification rate to nitrogen addition in soils with different textures and salinities. Different letters on the bars indicate significant differences between soils ($P<0.05$). Asterisks indicate significant differences between the control and N treatments (*$P<0.05$ and **$P<0.01$). C: control; N: NH_4NO_3 addition. FS and FL represent sand and loam soil in the freshwater marsh, respectively; OS and OL represent sandy and loam soil in the oligohaline marsh, respectively.

Figure 1


FS
FL
OS
OL
Denitrification rate (μg N kg^{-1} h^{-1})
25
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15
10
5
1.6
0.8
0.0
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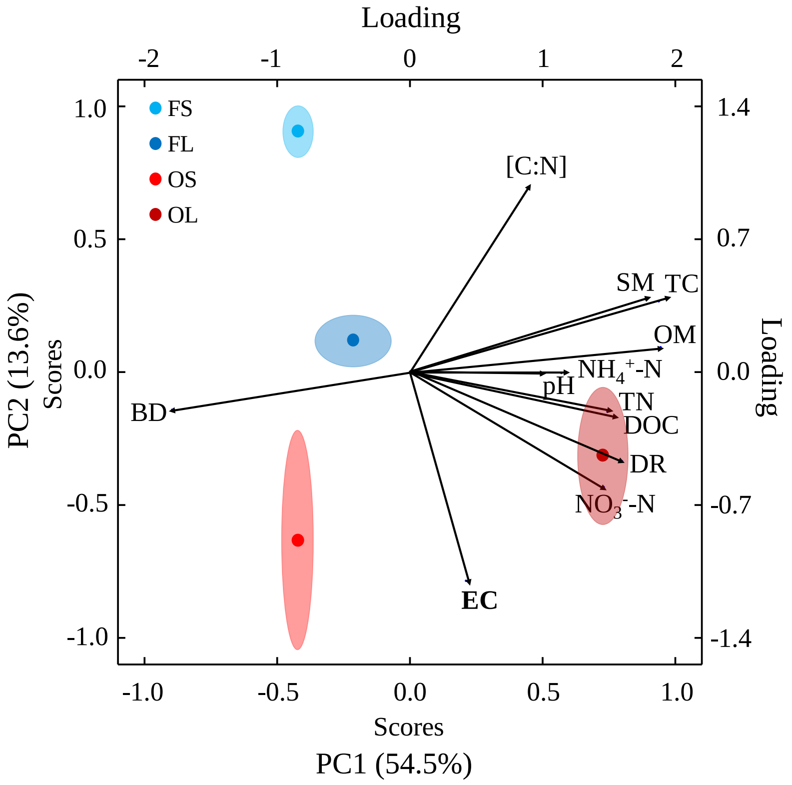
a
a
autumn
winter
spring
summer
overall

Figure 2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-1
0
1
2
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0.5
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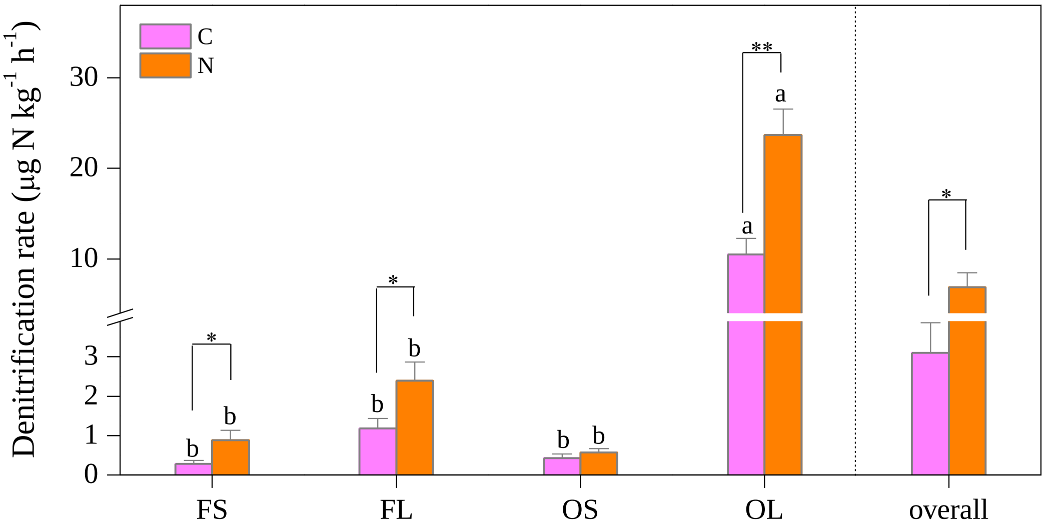
-0.7
-1.4
FS
FL
OS
OL
[C:N]
SM
TC
OM
NH_4^+-N
pH
TN
DOC
DR
NO_3^--N
BD
EC
PC2 (13.6%)
Scores
Loading
-1.0
-0.5
0.0
0.5
1.0
Scores
PC1 (54.5%)

Figure 3


Denitrification rate (μg N kg^{-1} h^{-1})
C
N
30
20
10
3
2
1
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a
a
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b
b
b
b
b
b
FS
FL
OS
OL
overall