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Denitrification of soil nitrogen in coastal and inland salt marshes with different flooding frequencies

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ABSTRACT

Denitrification is an important process for removing nitrogen in wetlands, and it is influenced by many environmental factors. However, little information is available on the relationship between hydrologic conditions and denitrification. In this study three typical sampling sites with different flooding frequencies, including short-term flooding wetlands (STFW), seasonal-flooding wetlands (SFW) and tidal flooding wetlands (TFW) were chosen as the study sites in the Yellow River Delta. In contrast, five typical sampling sites with different flooding frequencies, including 100-year floodplain (H), 10-year floodplain (T), 5-year floodplain (F), 1-year floodplain (O) and permanently flooded floodplain (B) were chosen as the study sites in Xianghai wetlands. This study reflected that the denitrification rates decreased with depth along soil profiles in both inland and coastal salt marsh soils. Flooding periods, soil depth and their interaction showed significant effects on the denitrification processes. Generally, higher flooding frequencies will cause higher denitrification rates in salt marshes. Moreover, the denitrification rates were significantly positively correlated with soil moisture content in both wetlands. Additionally, the denitrification rates were significantly positively correlated with organic matter and NO_3^--N content while negatively correlated with soil pH and salinity in inland salt marshes. Therefore, the changes in soil properties (e.g. SOM, TN, pH and salinity) can become an important way to control NO_3^- levels in inland salt marshes.

1. Introduction

Denitrification is defined as the biological reduction of nitrate or nitrite to gaseous end products (Reddy and Delaune, 2008), which is strictly an anoxic process including two steps such as the reduction of nitrate (NO_3^-) to nitrite (NO_2^-) and the reduction of NO_2^- to ammonia or nitric oxide (NO), nitrous oxide (N_2O) and dinitrogen (N_2) (Sirivedhin and Gray, 2006). Riickauf et al. (2004) reported that denitrification was the main nitrogen transformation process in the wetland soil under the condition of flooding. Therefore, wetlands have been used worldwide for the removal or reduction of excessive nitrogen from polluted surface and subsurface waters through denitrification (Jordan et al., 2011), and the purification of polluted water flowing through wetlands has been mainly attributed to denitrification due to anaerobic conditions in wetlands (Teele et al., 2014).

Most researchers have observed the effects of organic matter and nitrogen contents on denitrification (Sirivedhin and Gray, 2006; Onnis-Hayden and Gu, 2008). Denitrification was considered to be a process which requiring organic C as energy sources (Shiau et al., 2016). When the carbon source was the limiting factor, adding effective carbon can increase the availability of C and NO_3^-, then promote the denitrification significantly (Hill and Cardaci, 2004; Hernandez and Mitsch, 2007b). Davidsson and Leonardson (1996) found that when organic material was added to the soil, the soil denitrification rate increased by increasing the microbial quantity and diversity. However, the activity of denitrification was only determined by the concentration of NO_3^- in the favorable environmental conditions, such as adequate effective carbon and low oxygen partial pressure (Weier et al.,1993; Bouwman, 1990). Additionally, the denitrification in wetlands can also be influenced by soil type (Dandie et al., 2011), pH (Simek and Cooper, 2002), temperature (Sheibley et al., 2003), soil moisture (Aulakh et al., 1992) and salinity (Craft et al., 2009).

Wetland hydrology such as flooding patterns can regulate denitrification rate (Hernandez and Mitsch, 2007a). The

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hydrological regime determined the oxygen availability and the exchange of nutrients between water and sediment affecting the overall rate of denitrification in systems with hydrological fluctuations (Baldwin and Mitchell, 2000; Rassam et al., 2006). Pinay et al. (2007) reported that hydrological regime can directly control duration of oxic and anoxic phases in soil, thus affecting the denitrification. Groffman and Hanson (1997) presented that the soil with poor drainage had a higher denitrification rate. A large number of literatures have focused on the important factors influencing the denitrification, however, the differences in the denitrification rates and their influencing factors between inland and coastal salt marshes is kept little known. Moreover, little information is available about the effects of flooding frequencies on denitrification in inland and coastal salt marshes.

Xianghai wetlands and Yellow River Delta (YRD) wetlands are one of the typical inland and coastal salt marshes in China, respectively. Most salt marshes in both regions are impacted by flooding frequencies and showed alkaline conditions. These salt marshes are typical nitrogen-limiting ecosystems and thus a little change in nitrogen level would greatly impact the structure and functions of these salt marshes. Therefore, the primary objectives of this study were to: (1) investigate the changes in denitrification rates in soil profiles in inland (Xianghai) and coastal (Yellow River Delta) salt marshes with different flooding frequencies; (2) identify the effects of flooding frequencies and soil properties on denitrification rates by using correlation analysis and two-way variance analysis.

2. Materials and methods

2.1. Site description

The YRD coastal salt marsh is located in the Yellow River Delta Natural Reserve (37°35′-38°12′N, 118°33′-119°20′E), the northern part of Shandong Province. It has a temperate, continental monsoon climate, distinct seasons with the annual mean air temperature of 11.9 °C. The annual mean rainfall is 640 mm and approximately 70% of rainfall is mainly allocated in summer. The annual mean evaporation is 1962 mm (Gao et al., 2012). The Xianghai wetland is located in the Xianghai National Natural Reserve (44°55′-45°09′N, 122°05′-122°31′E), the western part of Jilin Province. It has the continental monsoon climate with the annual mean air temperature of 5.1 °C. The annual mean rainfall is 408.2 mm, which concentrates in the period from June to September. The annual mean evaporation is 1945 mm (Bai et al., 2014).

2.2. Sample collection and analysis

In October of 2007, three typical sampling sites with different flooding frequencies, including short-term flooding wetlands (STFW, approximately one-month flooding per year due to flow-sediment regulation), seasonal-flooding wetlands (SFW, approximately three-month flooding per year due to flow-sediment regulation) and tidal flooding wetlands (TFW, twice one day due to tidal cycles) were chosen as the study sites in the Yellow River Delta. Soil cores with three replicates were collected to a depth of 50 cm in each sampling site and sectioned at the intervals of 0–10 cm, 10–20 cm, 20–30 cm, and 30–50 cm. In contrast, five typical sampling sites with different flooding frequencies, including 100-year floodplain (H), 10-year floodplain (T), 5-year floodplain (F), 1-year floodplain (O) and permanently flooded floodplain (B) were chosen as the study sites in Xianghai wetlands. Soil cores with three replicates were collected to a depth of 80 cm in each sampling site and sectioned at the intervals of 0–10 cm, 10–20 cm, 20–40 cm, 40–60 cm, and 60–80 cm. All these sampling sites were dominately covered by *Phragmites australis*.

All soil samples were brought to the laboratory, and divided into two parts. One part was placed and dried in the natural state, and the other was reserved in the refrigerator to determine the initial nitrate nitrogen and ammonium nitrogen in soil. The air-dried soils were sieved through 0.149 mm sieve for the determination of soil physical and chemical properties.

Soil organic matter (SOM) was determined by Walkley and Black (1934) method; Total nitrogen (TN) and total carbon (TC) were measured on a C and N Elemental Analyzer (Vario EI, Elmentar, Germany); Soil pH and salinity were measured with a pH meter and an electricity conduction meter (soil: water = 1: 5); NO_3^--N was determined in the 2 mol/L KCl extracts on an Astoria Analyzer 300 system (made in Taiwan).

2.3. Incubation experiment

The incubation experiment was conducted according to the method by Vernimmen et al. (2007). The incubation process in this study was described as follows. Soil samples (20 g fresh weight) were placed into the test tubes, and each tube was added to 100 mL mixed solution of 0.2 mmol KH_2PO_4, $CaCl_2$ and $MgSO_4$ respectively. After 24 h of pre-incubation at room temperature, 5 mL supernatant was extracted to determine initial NO_3^--N content after 1 h shaking in these samples. And 5 mL 420 mg/mL nitrate solution was respectively added to these samples, and then all these samples were incubated for 24 h at 25 °C, after that 5 mL supernatant was extracted from these incubated tubes after 1 h shaking to determine NO_3^--N levels. The difference in NO_3^--N levels between pre and post incubation was calculated, and the potential denitrification rate of soil nitrogen was calculated by the nitrogen denitrified and the incubation time.

2.4. Statistical analysis

Least-significant difference (LSD) was used to compare the denitrification rates of NO_3^--N levels between flooding frequencies and soil depth. Two-way ANOVA analysis was used to determine the effects of soil depth and flooding frequency and the interaction of the two factors on the soil denitrification rates. Pearson correlation analysis were performed to identify the relationship between denitrification rates and soil properties. Statistical analysis was carried out by using the SPSS 14.0 and Origin 6.1 software packages.

3. Results and discussion

3.1. Changes in denitrification rates along soil profiles in the coastal salt marshes

Table 1 showed the average denitrification rates in soil profiles in coastal and inland wetlands. The average denitrification rate of the TFW soils was higher than those of the STFW and SFW soils (Table 1). This was associated with the different flooding periods in three wetlands in the YRD. Jacinthe et al. (2012) presented that floods in riparian zones could result in pulses of denitrification and the magnitude of this response varied with flood duration. Tides cycles might increase the amount of the effective C and N, thus promoting denitrification in TFW soils, because the multiple drying-wetting cycles can promote the soil C and N mineralization by increasing the quantity of microorganism (Patten et al., 1980). Ensign et al. (2013) indicated that the high frequency of tidal inundation might enhance their abilities to remove nitrogen via denitrification. The denitrification rates in three wetland soils of the YRD wetlands generally decreased with depth along soil

Table 1
The average denitrification rates (mg/kg/d) in soil profiles from inland and coastal salt marshes with different flooding periods.

Coastal salt marshes	STFW		SFW		TFW
	139.49 ± 8.51		146.49 ± 11.22		153.92 ± 7.54
Inland salt marshes	H	T	F	O	B
	18.60 ± 5.74	51.75 ± 45.36	90.15 ± 35.54	143.16 ± 48.47	145.10 ± 21.92

Note: STW, SFW, and TFW represent short-term flooding wetlands, seasonal flooding wetlands, and tidal flooding wetlands, respectively; H,T,F,O and B represent 100-year floodplain,10-year floodplain,5-year floodplain,1-year floodplain and permanently flooded floodplain, respectively.

profiles (Fig. 1). This was consistent with the results by Liu et al. (2013) and Sun et al. (2010), who presented higher denitrification rates in upper soils in wetlands. ANOVA analysis also showed that soil depth had a significant effect on the denitrification rates in the coastal salt marshes (Table 2). The possible explanation for higher denitrification rates in upper soils might be related to higher denitrification activities. Oscar et al. (2001) presented that the upper soils exhibited significantly higher activities of the denitrification enzyme in soil profiles compared with the deeper soils.

3.2. Changes in denitrification rates along soil profiles in the inland salt marshes

The average denitrification rates increased with the increasing flood frequencies in Xianghai inland wetlands (Table 1). This was in

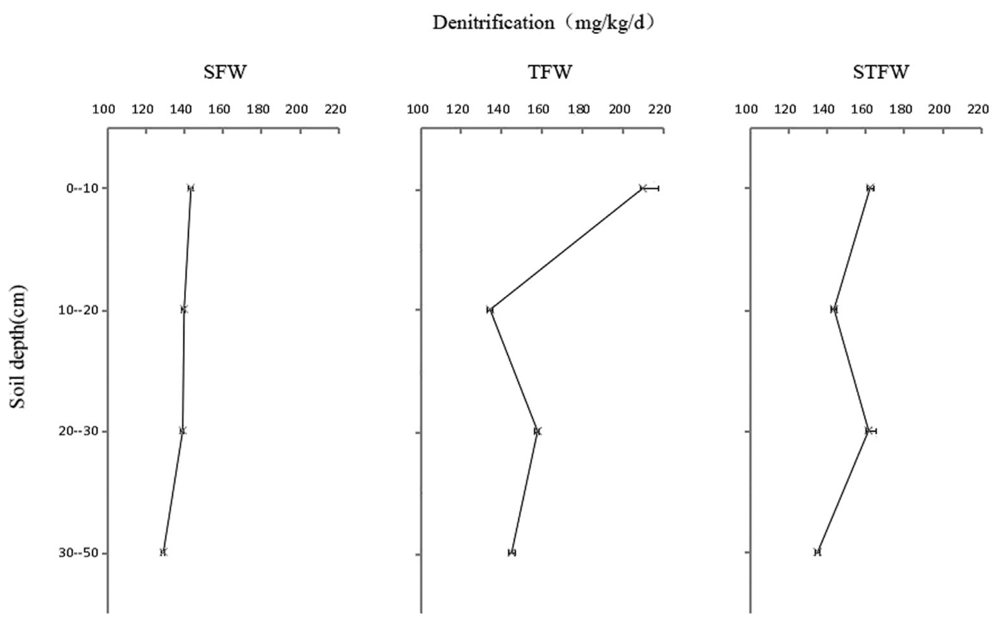

Fig. 1. The changes in denitrification rates with depth in coastal salt marshes with different flooding periods in the Yellow River Delta.

Table 2
Two-way variance analysis of soil denitrification rate in different flooding periods and depth of soil in the coastal and inland salt marshes.

Source	Sum of squares	Degree of freedom	Mean square	F	Sig.
Coastal salt marshes					
Corrected model	3228.295[a]	11	293.481	46.160	0.000
Intercept	740256.710	1	740256.710	116,431	0.000
Flooding periods	1258.823	2	629.411	98.997	0.000
Soil depth	1287.516	3	429.172	67.502	0.000
Flooding periods × Soil depth	693.780	6	115.630	18.187	0.000
Error	146.232	23	6.358		
Total	756424.961	35			
Corrected Total	3374.527	34			
Inland salt marshes					
Corrected model	320032.142[b]	24	13334.673	37.598	0.000
Intercept	675759.091	1	675759.091	1905.342	0.000
Flooding periods	217751.266	4	54437.816	153.491	0.000
Soil depth	32704.355	4	8176.089	23.053	0.000
Flooding periods × Soil depth	92903.966	16	5806.498	16.372	0.000
Error	15959.948	45	354.666		
Total	1040367.634	70			
Corrected Total	335992.090	69			

[a] R Squared = 0.957 (Adjusted R Squared = 0.936).
[b] R Squared = 0.952 (Adjusted R Squared = 0.927).

agreement with the results by Batson et al. (2012), who presented higher annual denitrification rates in permanently flooded open water area compared with intermittently flooded transitient areas. Groffman and Hanson (1997) presented that the poor drainage could promote denitrification processes in soil and this was probably because the flooding frequencies could affect the proportion of the denitrification genes assemblage of the soil denitrification bacteria due to their different preferences with respect to oxygen conditions in the environment (Teele et al., 2014). Along with the increasing flooding frequencies, the denitrification rates generally showed an increase in upper soil layers in these soil profiles from Xianghai wetlands (Fig. 2). ANOVA analysis also showed that soil depth had a significant effect on the denitrification rates in the coastal salt marshes (Table 2). At Sites B, O and F with shorter flooding frequencies, the denitrification rates decreased along soil profiles, whereas they showed an increase in the denitrification rates in the soil layers of 20–40 cm and 40–60 cm of Site T. Comparatively, the denitrification rates generally kept stable in soil profiles at Site H. The possible explanation might be related to the fact that the initial NO_3^- content in the 20–40 cm and 40–60 cm soil layers of Site T was almost 4–5 times higher than those in other soils layers, and soil organic matter content was also relatively high in both soil layers. Most researchers presented that the increased availability of C and NO_3^- could significantly promote the denitrification (Hill and Cardaci, 2004; Hernandez and Mitsch, 2007a). Compared with other soil layers of Site T, the lower denitrification rates in the soil layers of 10–20 cm and 20–40 cm was attributed to higher salinity in both two layers. This was consistent with the results by Craft et al. (2009), who reported the denitrification rates decreased with increasing salinity in Georgia, USA, and the reason might be explained by the fact that higher soil salinity could limit some denitrification microorganisms (Larsen et al., 2010).

3.3. Effects of flooding periods and soil depth on the denitrification rates

The effects of flooding period and soil depth and their interaction on the denitrification rates were significant in the coastal and inland salt marshes ($p < 0.01$; Table 2). Hernandez and Mitsch (2007a) presented that wetland hydrology could considerably regulate denitrification rates through controlling the duration of oxic and anoxic phases in soil (Pinay et al., 2007). Rassam et al. (2006) also reported that hydrological fluctuations would affect the denitrification rates due to the changes in the availability of oxygen and nutrient. This was because the flooding frequency, duration and period would greatly influence the nutrient allocation and the dynamics of ecosystem in wetlands (Day et al., 1998). Generally, the soil-depth effects on the denitrification rates could be associated with the profile distribution of denitrifiers and soil nutrients in the coastal and inland salt marshes. The mechanism of the interaction effects on the denitrification rates might be attributed to the change in soil physical and chemical properties in soil profiles under different flooding conditions (Bai et al., 2005), consequently affecting the underlying bacterial community and enzyme responsible for the denitrification (Teele et al., 2014).

3.4. Effects of soil properties on the denitrification rates

Table 3 showed the correlation coefficient matrix between soil properties and the denitrification rates in coastal and inland salt marshes. Generally, the denitrification rates were only significantly positively correlated with soil water content in coastal salt marshes ($p < 0.05$). In inland salt marshes, the denitrification rates showed a significant positive correlation with soil organic matter and water content ($p < 0.01$) and a significant negative correlation with soil pH and salinity ($p < 0.01$). Soil moisture was an important factor influencing denitrification processes in both wetlands. Vinther (1992) stated that even a small changes of the soil moisture content could cause the rapid increase in the denitrification rates.

The reason why soil nutrients did not show the significant relationships with the denitrification rates in the coastal salt marshes ($p > 0.05$) was probably because the lower NO_3^--N and organic C levels influenced by hydrological regime compared to Xianghai inland wetland. The mechanism of how soil pH affect the denitrification remains uncertain due to different findings in different soils (Kandeler et al., 2006; Philippot et al., 2009; Enwall et al., 2010). In this study the denitrification rates were only significantly and negatively correlated with soil pH (ranging from 7.06 to 9.11) in Xianghai inland wetlands. This might be associated with the longer pH span in Xianghai inland wetlands compared with coastal salt marshes (pH ranged from 8.06 to 8.59). This also implies that higher pH values (at least higher than 8.59) could prohibit the denitrification processes. Dorsch et al. (2012) presented that the denitrifiers might have a community-dependent response to the

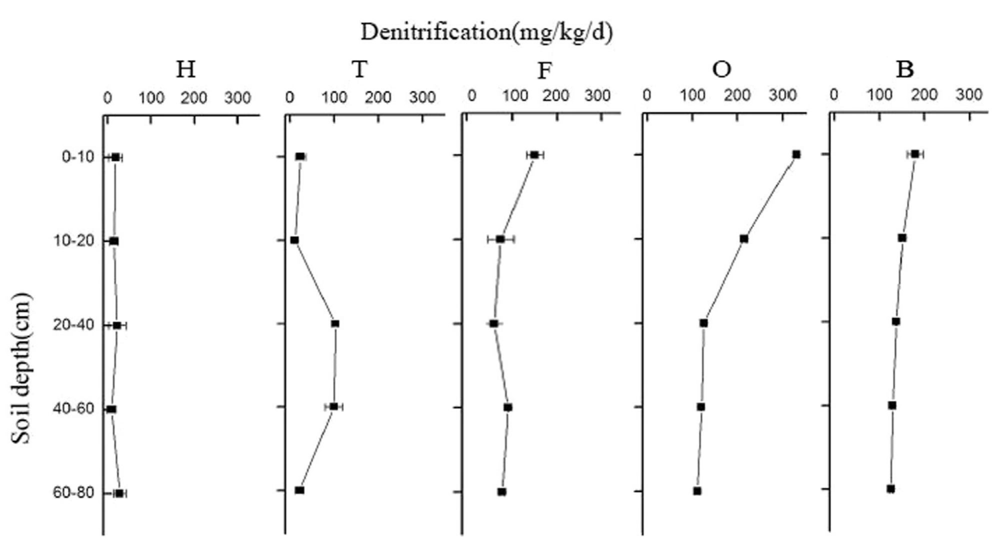

Fig. 2. The changes in denitrification rates with depth in inland salt marshes with different flooding periods in Xianghai Wetland.

Table 3
Correlation between soil properties and denitrification rates in the coastal and inland salt marshes.

Item	Water content	pH	Salinity (‰)	SOM (g/kg)	NO_3^-N (mg/kg)	Denitrification rate (mg/kg/d)
Coastal salt marshes						
Moisture content	1.0000	0.3563	−0.2915	0.5458	0.0243	0.6942*
pH	–	1.0000	0.0757	0.1571	−0.1419	−0.0895
Salinity (‰)	–	–	1.0000	0.3020	0.2942	−0.4863
SOM(g/kg)	–	–	–	1.0000	0.6682*	0.4264
NO_3^--N content (mg/kg)	–	–	–	–	1.0000	0.2285
Denitrification Rate(mg/kg/d)	–	–	–	–	–	1.0000
Inland salt marshes						
Moisture content	1.0000	−0.6483**	−0.4127*	0.8296**	0.5653**	0.7437**
pH	–	1.0000	0.7831**	−0.6307	−0.3037	−0.7488**
Salinity (‰)	–	–	1.0000	−0.2845	−0.0536	−0.6955**
Organic matter (g/kg)	–	–	–	1.0000	0.8291**	0.6852**
NO_3^--N content (mg/kg)	–	–	–	–	1.0000	0.4434
Denitrification rate (mg/kg/d)	–	–	–	–	–	1.0000

Notes: *represents significant correlation at the level of $P < 0.05$.
**represents significant correlation at the level of $P < 0.01$.

changes in soil pH because soil pH could influence the gene proportion and abundance of microorganisms (Teele et al., 2014). The negative correlation between salinity and denitrification in the inland salt marsh soils was in agreement with the results in Tamsui estuary (average salinity was 3.6 psμ) (Shiau et al., 2016). The increasing salinity might cause a physiological stress to microbial activity, consequently leading to the decrease of denitrification rates (Larsen et al., 2010). However, the correlation was not significant in the coastal salt marshes. This might be because the salinity in the three sampling sites were not distinctive enough to affect microbial community distribution.

4. Conclusions

This study demonstrated that the denitrification rates decreased with depth along soil profiles in both inland and coastal salt marsh soils. Flooding periods, soil depth and their interaction showed significant effects on the denitrification processes. Generally, higher flooding frequencies would cause higher denitrification rates in salt marshes. Moreover, the denitrification rates were significantly positively correlated with soil moisture contents in both wetlands. We can modify the flooding periods and soil moisture to adjust denitrificaiton rates to remove or retain NO_3^- in wetlands. Additionally, the denitrification rates were significantly and positively correlated with soil organic matter and NO_3^--N contents while negatively correlated with soil pH and salinity in inland salt marshes. Therefore, the changes in soil properties (e.g. SOM, TN, pH and salinity) can become an important way to control NO_3^- levels in inland salt marshes. However, further study on the microbial mechanisms on the denitrification rates in these salt marshes are still needed.

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