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Denitrification Potential of Marsh Soils in Two Natural Saline-alkaline Wetlands

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Abstract: Little information is available on denitrification potential of marsh soils in natural saline-alkaline wetlands. The denitrification potentials of an open wetland in the floodplain (Erbaifangzi wetland) and a closed wetland (Fulaowenpao wetland) in backwater areas in Jilin Province of Northeast China were monitored by an anaerobic incubation at 30℃ for 25 days. Our results showed that the relative denitrification index (RDI) increased gradually with incubation time, and showed a rapid increase in the first 5 days of incubation. The RDI values declined quickly from surface soils to subsurface soils and then kept a small change in deeper soils along soil profiles over the incubation time. Denitrification proceeded much faster in the top 20 cm soils of open wetland than in the closed wetland, whereas no significant differences in RDI values were observed in deeper soils between both wetlands. The RDIs were significantly negatively correlated with bulk density and sand content, while a significantly positive correlation with clay content, soil organic matter, total nitrogen and phosphorous. The maximum net NO_3^--N loss through denitrification in 1 m depth were higher in the open wetland than the closed wetland with higher soil pH values. Future research should be focused on understanding the influencing mechanisms of soil alkalinity.

Keywords: relative denitrification index; potential net denitrification rate; marsh soil; saline-alkaline wetland

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1 Introduction

Denitrification is defined as the biological reduction of nitrate or nitrite to gaseous end products (e.g., nitrous oxide and nitrogen gas) (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) (Madigan *et al*., 1997; Sirivedhin and Gray, 2006). Wetland soils have a large potential for nitrogen removal by denitrification which occurs only in anaerobic regions (Reddy and DeLaune, 2008). Denitrification is a sink of NO_3^- (Howarth *et al*., 1996), which has ecological and geochemical consequences in both freshwater and coastal marine ecosystems (Seitzinger, 1988). The process of denitrification can potentially reduce a large amount of nitrogen transported to water bodies such as rivers or lakes and can regulate plant primary production in aquatic ecosystems (Reddy and DeLaune, 2008). Therefore, wetlands can play an important role in purifying water quality of rivers or lakes.

Most researchers have demonstrated that nitrate and carbon are used as electron acceptor and donor for anaerobic respiration in the process of denitrification, respectively (Madigan *et al*., 1997; Reddy and DeLaune, 2008). Higher nitrate concentration will promote denitrification rate in wetlands (Sartoris *et al*., 2000; Sirive-

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dhin and Gray, 2006). Shao *et al*. (2011) presented that denitrification exhibited zero-order reaction kinetics when nitrate concentration was higher than 4 mmol/L. Meanwhile, carbon sources can potentially support denitrification (Onnis-Hayden and Gu, 2008). The addition of carbon sources would enhance denitrification process in wetlands (Davidsson and Stahl, 2000), whereas the addition of different carbon sources showed significant differences in denitrification rates (Her and Huang, 1995). Soil O_2 controls the rates and end products of denitrification (Burgin and Groffman, 2012). Zhu *et al*. (2013) observed that heterotrophic denitrification was responsible for all N_2O production at 0% O_2. Additionally, soil pH values can regulate denitrification rate. Šimek and Cooper (2002) concluded that denitrification was most rapid in slightly alkaline soils (pH 7–8) compared to acid soils, but Drury *et al*. (1991) found no significant correlation between soil pH and either background or potential dentritrication rates in their studies of 13 soils. Although most researchers have focused on denitrification rate in freshwater or coastal wetlands (Seitzinger, 1988; Madigan *et al*., 1997; Sartoris *et al*., 2000; Sirivedhin and Gray, 2006; Reddy and DeLaune, 2008), few studies on denitrification potential along soil profiles have been carried out in inland saline-alkaline wetlands.

Long-term drought and human disturbance have seriously degraded (especially elevated alkalinity) wetlands of the western part of the Songnen Plain, Northeast China with a decrease in wetland productivity (Bai *et al*., 2010). However, the effects of soil alkalinity on denitrification potential or nitrogen loss in inland wetlands are still kept unknown. The objective of this study is to compare denitrification potentials of two alkaline wetland soils (soil pH ranging from 8 to 10) with different properties.

2 Materials and Methods

2.1 Study area

The Xianghai wetland (44°55′–45°09′N, 122°05′–122°31′E) is located in the Xianghai National Nature Reserve (XHNNR), the western part of Jilin Province of Northeast China (Fig. 1). It has the continental monsoon climate with the mean annual temperature of 5.1℃. The mean annual evaporation is 1945 mm and the mean annual precipitation is 408.2 mm, which concentrates in the period from June to September. However, the regional climate has become more and more drier in recent years, with mean annual precipitation decreased by approximately 100 mm (Bai *et al*., 2010). The dominant vegetation is Phragmites australis in this region and soil type is dominantly marsh soil. More detailed information on the study area has been provided in Bai *et al*. (2010).

Erbaifangzi (EBFZ) and Fulaowenpao (FLWP) wetlands were chosen as typical study areas. The Erbaifangzi wetland is located in the downstream floodplain, while the Fulaowenpao wetland is in the backwater area. The Erbaifangzi wetland soils contained lower salt con-

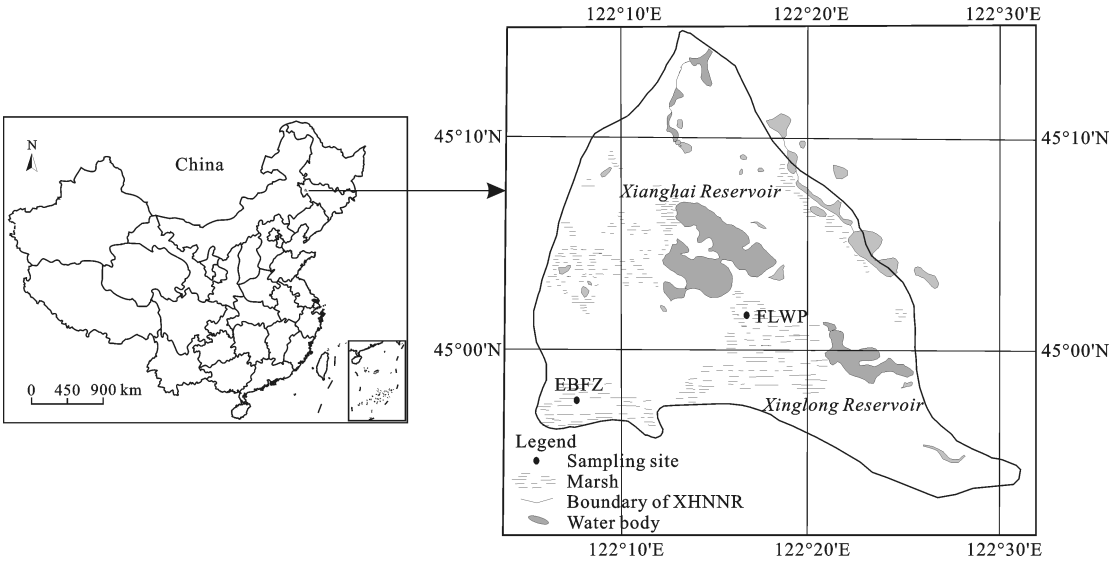

Fig. 1 Location of study area. EBFZ: Erbaifangzi wetland; FLWP: Fulaowenpao wetland. XHNNR: Xianghai National Nature Reserve

tent (0.38%) compared to the Fulaowenpao wetland soils (0.51%) due to different hydrological conditions (Bai *et al*., 2005). Recent drought stress has led to serious alkalization (pH ranging from 8.73 to 9.68) in both wetlands, especially in the Fulaowenpao wetland with pH more than 9.39 (Bai *et al*., 2010).

2.2 Soil sampling and analysis

Soil cores from 0 cm to 100 cm depth with three replicates were collected in each wetland and were sectioned at the intervals of 0–10 cm, 10–20 cm, 20–40 cm, 40–60 cm, 60–80 cm and 80–100 cm. Soils from the same layer were homogenized for each wetland. All soil samples were brought to the laboratory, and air-dried for three weeks for incubation experiment. The air-dried soils were sieved through 2-mm sieve to remove recognizable plant litters, coarse root materials, and stones. Another soil core (100 cm^3) from each soil depth was collected for the determination of bulk density.

Soil organic matter (SOM) was determined by the method of Walkley and Black (1934), and total nitrogen (TN) was determined by the Kjeldahl method. Nitrate nitrogen (NO_3^--N) and ammonium nitrogen (NH_4^+-N) were measured in the 2 mol/L KCl extracts on an Astoria Analyzer 300 system (Astoria Pacific International, Taiwan). Total phosphorous (TP) was extracted from soils with 1 N HCl after ignition at 550℃ (Asplia *et al*., 1976). Soil pH (H_2O) was measured by using electrical conductivity method (soil/water = 1∶5). Soil particle size analysis was carried out with Particle Size Analyzer (RS-1000, made in Japan). Soil cores were oven dried at 105℃ for 24 hours and then weighed. Bulk density was calculated for each soil layer on a dry weight basis.

2.3 Incubation experiment

The incubation experiments were carried out under anaerobic conditions in an oven with constant temperature after adding certain KNO_3 solution (Reddy *et al*., 1980). Soil samples (10 g dry weight) for each layer were placed in 100 mL polypropylene containers. Among these samples, 180 soil samples (15 replicates for each soil layer) were amended dropwise 1 mL 4.55 mg/mL NO_3^--N (as KNO_3) at first and then 24 mL deionized water was again added to each soil sample; 36 samples (three replicates for each soil layer) were not amended by NO_3^--N, while 25 mL deionized water was added. All the containers were sealed with rubber plugs and were pre-incubated at 30℃ in the dark for 2 days. After 2 days of incubation, 36 NO_3^--unamended samples were collected and extracted using 25 mL 2.5 mol KCl and filtered through #42Whatman filter paper after 1 h shaking to determine initial NO_3^--N contents in these soils. Similarly, three NO_3^--amended samples of each soil layer were collected at days 2, 5, 10, 17 and 25, respectively. They underwent similar proceeding as above mentioned to get the extracts for the determination of NO_3^--N. The extracted samples were refrigerated at 4℃ for subsequent colorimetric analysis on an Astoria Analyzer 300 system (Astoria Pacific International, Taiwan).

2.4 Relative denitrification index and potential net denitrification rate

The relative denitrification index (RDI) was defined as the ratio of the NO_3^--N loss (the difference between initial and final NO_3^--N) to the initial NO_3^--N content. It can be calculated by using the following equation:

$$RDI = \frac{NO_3^-\text{-}N_{initial} - NO_3^-\text{-}N_{final}}{NO_3^-\text{-}N_{initial}} \quad (1)$$

Potential net denitrification rate after 25 days of incubation (Denitr25) were calculated by dividing the total NO_3^--N loss (the difference between initial and final NO_3^--N) by the incubation time (i.e., 25 days).

2.5 Statistical analysis

One-way ANOVA was used to analyze the difference in NO_3^--N contents between both sites or among soil layers. Pearson correlation coefficients were calculated to identify the relationships among denitrification potential and selected soil properties. Statistical analysis was conducted by using the SPSS 10.0 and Origin 6.0 software packages, and differences were considered to be significant if $p < 0.05$.

3 Results

3.1 Soil characterization

Figure 2 shows profile distributions of selected soil properties in both FLWP and EBFZ wetlands. Some properties such as SOM, TN, pH and bulk density exhibited similar distribution with depth along soil profiles in both wetlands. Generally, the SOM and TN gradually decreased with depth along soil profiles in both wetlands. The surface soils contained higher SOM and TN

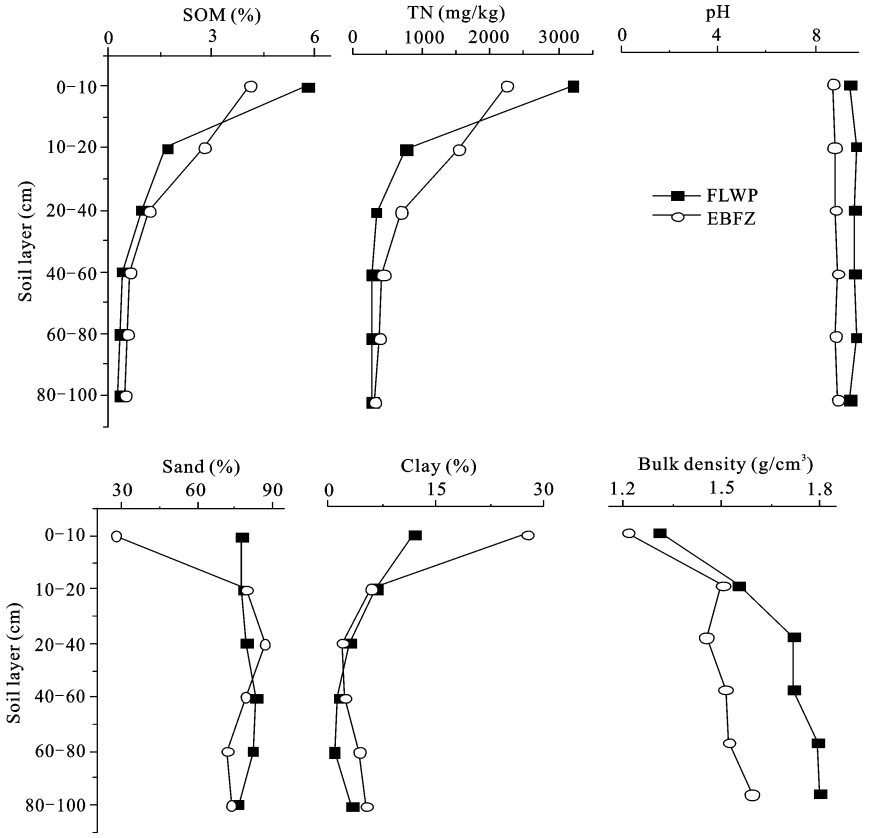

Fig. 2 Changes in soil properties with depth along soil profiles. SOM, soil organic matter; TN, total nitrogen; EBFZ, Erbaifangzi wetland; FLWP, Fulaowenpao wetland

contents in FLWP wetland compared to EBFZ wetland, whereas they showed similar contents in deeper soils in both wetlands. Soil pH values are higher in FLWP wetland than EBFZ wetland and they kept constant trend in profiles of both wetlands. Soil bulk densities increased with increasing depth, and they were lower in EBFZ wetland than FLWP wetland. Higher sand and lower clay contents were observed in the surface soils of FLWP wetland compared to EBFZ wetland, whereas no significant differences were found in deeper soils.

3.2 Profile distribution of relative denitrification index (RDI)

Figure 3 shows spatio-temporal variations in the RDI of nitrate nitrogen in marsh soils from two natural saline-alkaline wetlands. Generally, RDI values for each soil layer increased gradually with increasing incubation time in both wetlands. RDI values increased quickly before the first 5 days of incubation for each layer in both wetlands. However, after 5 days of incubation, they increased slightly in deeper soils compared to upper soils. At the end of the incubation experiment, RDI values for surface soils in EBFZ and FLWP wetland soils reached about 90% and 80%, respectively, whereas all subsoils in both wetlands reached up to about 50%.

RDI values declined quickly from surface soils to subsurface soils and then kept a small change in deeper soils along soil profiles over the incubation time. Moreover, RDI values for surface soils (0–10 cm) were significantly higher than deeper soils in both wetlands ($p < 0.01$), while no significant differences were observed among deeper soils ($p > 0.05$). Compared to EBFZ wetland, RDI values for the top 20 cm soils were much lower in FLWP wetland over the incubation time ($p < 0.01$), while no significant differences were observed between deeper soils in both wetlands ($p > 0.05$).

3.3 Relationships between RDIs and soil properties

The results of correlation analysis showed that RDIs were significantly positively correlated with SOM, TN and TP ($p < 0.01$, Table 1). However, ammonium nitrogen did not exhibit significant correlation with dentrification poten- tial. Meanwhile, RDIs were significantly negatively correlated with soil bulk density and sand

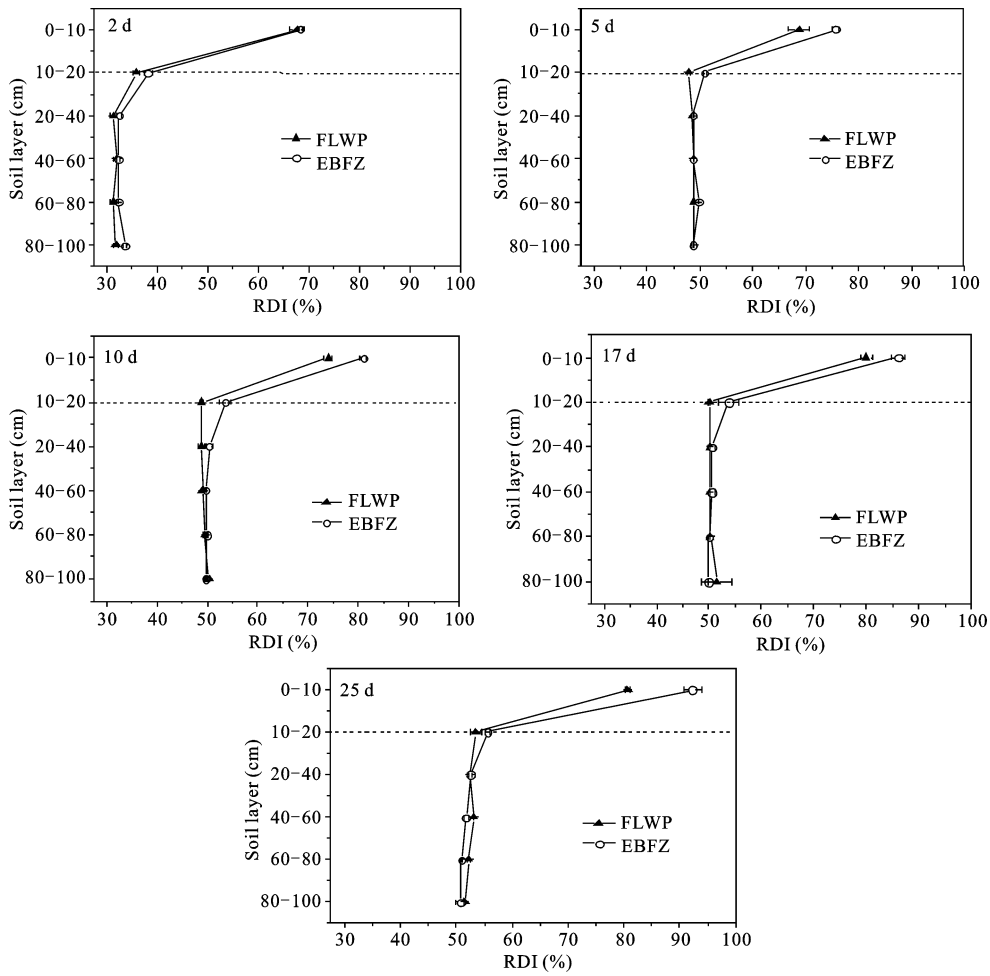

Fig. 3 Spatio-temporal variation in relative denitrification index (RDI) of nitrate nitrogen in marsh soils in two natural saline-alkaline wetlands. Data point represents the mean value ± SD

Table 1 Relationship between relative denitrification index (RDI) (after 25 days of incubation) and selected soil properties

	Bulk density (g/cm^3)	Sand (%)	Clay (%)	pH	SOM (%)	TN (mg/kg)	NH_4^+-N (mg/kg)	NO_3^--N (mg/kg)	TP (mg/kg)
RDI	–0.774**	–0.770**	0.930**	–0.228	0.867**	0.874**	0.398	0.403	0.942**

Notes: *, represents significant correlation at the level of $p < 0.05$; **, represents significant correlation at the level of $p < 0.01$

content ($p < 0.01$), and significantly positively correlated with clay content ($p < 0.01$). However, no significant correlation was observed between RDI and pH value ($p > 0.05$).

3.4 Profile distribution of potential net denitrification rates

As shown in Table 2, the potential net denitrification rates after 25 days of incubation (Denitr25) were higher in surface soils than those in deeper soils ($p < 0.05$) in both wetlands. Compared to EBFZ wetland, surface soils in FLWP wetland exhibited lower Denitr25 values. Expressed on unit area, the maximum potential net NO_3^--N loss through denitrification process in 1 m depth reached 168.48 g N/(m^2·d) and 149.51 g N/(m^2·d) in EFBZ and FLWP wetlands, respectively.

Table 2 Net denitrification rates (Denitr25) of marsh soils in Xianghai wetland after 25 days of incubation

Site	Denitr25 (mg N/(kg·d))					
	0–10 cm	10–20 cm	20–40 cm	40–60 cm	60–80 cm	80–100 cm
FLWP	147.37±17.39	97.43±41.56	95.67±8.96	96.85±2.88	95.13±9.60	93.87±7.40
EBFZ	168.55±59.92	101.35±17.36	95.96±29.76	94.32±18.84	92.76±2.64	92.46±34.5

Note: Results are expressed on dry soil basis

4 Discussion

4.1 Relative denitrification index (RDI)

The surface soils (0–10 cm) exhibited much higher RDI values than deeper soils in both wetlands ($p < 0.01$), which was caused by higher net NO_3^--N loss in surface soils. This is consistent with the results of Williams *et al*. (1999). Higher RDI values in surface soils were probably associated with higher soil nutrients contents (i.e., SOM and TN) (Fig. 1). The results of correlation analysis also showed that RDIs were significantly positively correlated with SOM, TN and TP ($p < 0.01$) (Table 1). Reddy and DeLaune (2008) presented that higher SOM contents in top soils could improve denetrification as carbon can serve as an essential electron donor for the denitrification processes. The RDIs proceeded fast in the first two days, which was also associated with higher SOM contents during this period. With the increasing incubation time, the SOM was greatly consumed and could not provide enough carbon donors, and thus the denitrification process was inhibited. The RDIs showed significant correlations with soil bulk density, clay and sand contents ($p < 0.01$). This was because heavy soil texture contributed to forming anaerobic environment under flooding conditions, which favored dentrificiaton processes. Additionally, higher denitrification enzyme activity in upper soils than those in deeper soils in wetlands also contributed to higher dentrification potential (Flite *et al*., 2001).

The EBFZ wetland soils exhibited much higher RDI values for the top 20 cm soils than the FLWP wetland soils ($p < 0.01$), while the RDI values kept similar in deeper soils in both wetlands ($p > 0.05$). It indicates that the FLWP wetland showed lower denitrification potential despite higher mineralization and nitrification potential were observed in this wetland (Bai *et al*., 2005; 2010). The study results of Zak *et al*. (1991) also showed the similar results. Groffman *et al*. (1996) presented a significantly negative correlation between denitrification potential, mineralization and nitrification potential in four wetland soils. Although no significant correlations between pH values and RDI values (Table 1), higher soil pH values in the FLWP wetland might greatly inhibit denitrification potential in top soils compared to the EBFZ wetland. Scholefield *et al*. (1997) found that the estimated nitrogen denitrified greatly decreased when soil pH values were adjusted from 5.1 to 9.4 in grassland soils. Šimek and Cooper (2002) also pointed out the optimal soil pH range is 7–8 for dentrification. However, higher soil pH in deeper soils showed little or no effects on dentrification in both wetlands in this study. Therefore, further studies are still needed on the influencing mechanisms of soil pH in saline-alkaline wetlands.

4.2 Potential net denitrificaiton rate

The potential net denitrification rate after 25 days of incubation (Denitr25) was calculated to represent potential denitrification rate of the tested soils (Table 2) as the net loss of NO_3^--N approached to the maximum after 25 days of incubation for surface soils. Higher Denitr25 values in surface soils compared to deeper soils ($p < 0.05$) in both wetlands were associated with higher SOM contents (Fig. 1). Bonnett *et al*. (2013) found that denitrification rate was the highest in the organic Histosol with higher organic matter. Lin *et al*. (2007) also found that soil denitrification rate was significantly correlated ($p < 0.01$) with the extractable organic carbon and organic matter, as organic matter would control denitrification capacity when moisture and NO_3^--N were not limiting (Johns *et al*., 2004). Higher TP content might be another factor influencing potential net denitrification rate. A significant correlation was also observed between RDI values and TP content (Table 1). White and Reddy (2003) presented that potential denitrification rates were the highest in the surface soils than deeper soils, which was significantly correlated with TP content. It suggests that potential denitrification rates could be increased in wetland soils with higher phosphorous contents. Additionally, Burgin and Groffman (2012) also pointed out that soil O_2 might be an important factor controlling denitritrication rate in wetlands because soil denitrification rate was significantly correlated with the redox potential of wetland soil (Lin *et al*., 2007). Therefore, higher clay contents in the surface soils of the EBFZ wetland would contribute to the denitrification process as soils with heavier texture contained less O_2. Similarly, higher Denitr25 value in 1 m soil depth was observed in the EBFZ wetland compared the FLWP wetland (Fig. 1), implying that heavy alkalinity due to drought in wetland ecosystems would lead to lower denitrifiction rate, though denitrification was most rapid in slightly alkaline soils compared to acid soils (Šimek and Cooper, 2002).

5 Conclusions

We investigated the relative denitrification rates in soil profiles in two natural saline-alkaline wetlands and compared the differences in RDIs between an open wetland and a closed wetland. Compared to the EBFZ wetland soils, higher pH values (more than 9) in the FLWP wetland soils demonstrate heavy alkalinity due to drought in the study area. The RDI can serve as a good indicator to show the denitrification processes. We observe that RDIs is significantly correlated with such soil properties as SOM, TN, TP and soil particle size, indicating that the denitrification process might be affected by soil characteristics. The denitrification potential in all soils gradually increases with increasing incubation time in both wetlands and decreases dramatically from surface soils to deeper soils. Less carbon in deeper soils might be the important factor limiting denitrification potential. Generally, the FLWP wetland soils with higher pH values will inhibit the denitrificaiton processes comparing with the EBFZ wetland soils, implying that heavy alkalinity shows the inhibiting effects on the denitrification potential in inland wetland soils. Therefore, it is necessary to control carbon and pH in wetland soils to enhance nitrate nitrogen removal from wetlands by means of denitrification processes. Further studies are also needed to find the most suitable carbon content and pH range to improve wetland ecological service functions.

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