



# Differences in Soil Nitrogen Availability and Transformation in Relation to Land Use in the Napahai Wetland, Southwest China

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## Abstract

Changes in land use can affect soil nitrogen availability and transformation as well as atmospheric N<sub>2</sub>O concentration and global warming. The total nitrogen (N), NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N, net N mineralization, and denitrification of natural wetland, grazing meadow, and cropland soils in Napahai Wetland were measured to determine whether the conversion of natural wetland to grazing meadow or cropland significantly impacts soil N availability and transformation. The results showed that grazing meadow and cropland soils exhibited significantly lower total N and inorganic N concentrations than those of natural wetland soils. The net N mineralization rate was higher in the natural wetland soils than that in the grazing meadow soils. The net N mineralization rate of cropland soils was highest in spring and summer but was at its lowest in autumn. Soils of natural wetlands had higher denitrification rates than those of grazing meadows, while cropland soils had higher denitrification rates than those of natural wetlands. These data suggest that the conversion of natural wetland to grazing meadow substantially decreases total N and inorganic N concentrations, net N mineralization, net nitrification, and denitrification. Conversions of natural wetland to cropland could also decrease the total N and inorganic N concentrations throughout the growing seasons but increase both N mineralization, in spring and summer, and denitrification rates, in May, September, and November. Natural wetland soils have higher N availability and N transformation rates than those of grazing meadow soils and cropland soils, indicating that natural wetland would have the greater potential effect on climate change.

**Keywords** Nitrogen mineralization · Nitrification · Denitrification · Land use type · Napahai

## 1 Introduction

Nitrogen (N) is a particularly important nutrient for plant growth, but it is often limited in soils (Janja et al. 2009). The availability of N in soils has important consequences for primary productivity, plant species composition, and nutrient retention (Guo et al. 2012a, b; Liu et al. 2017). Shifts in soil N status can be caused by variations in N transformations (Lang et al. 2010; Brunetto et al. 2017).

N transformations in soils are complex. The major transformations include mineralization of organic N (ammonification

and nitrification), denitrification, NH<sub>4</sub> volatilization, and N<sub>2</sub> fixation (Reddy and Patrick 1984). Mineralization of organic N in soils provides the major source of N to plants for uptake and biomass synthesis. In addition, soil N mineralization is of prime importance in ecosystem productivity and in the potential availability and loss of N from ecosystems (Tripathi and Singh 2009). Denitrification is a microbial process that removes nitrate from wetlands by transforming it into the gases N<sub>2</sub>O and N<sub>2</sub> (Mitsch and Gosselink 2007).

Soil N transformations are microbially mediated processes that are influenced by microbial biomass and microbial activity (Uri et al. 2008; Liu et al. 2019). Changes in the type of land use can result in different soil microclimates, which can also affect microbial biomass and activity (Tripathi and Singh, 2009). Moreover, cultivation of a soil that previously supported natural vegetation leads to considerable loss of soil organic matter (Tripathi and Singh, 2009). Thus, the conversion of wetland into grazing meadow or cropland could significantly alter soil N transformation by affecting soil organic matter and microbial activity.

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In recent years, a large area of natural wetland was converted to grazing meadow or cropland in the Napahai Wetland, a Ramsar wetland of international importance. Such conversions can alter a soil's physical and chemical characteristics as well as its microbial community structure and functioning, thereby promoting different pathways of N transformation (Li et al. 2018; Wilson et al. 2011). For instance, wetland soils are typically characterized by high concentrations of organic carbon relative to meadow soils (Guo et al. 2012a, b). Such differences in soil properties are likely to affect microbially mediated processes such as N transformation. However, it is uncertain how this change of land use affects soil N availability and transformation.

The objective of this study is to estimate the impact of three land uses (natural wetland, grazing meadow, and cropland) on soil N availability (inorganic N) and N transformation (net N mineralization, net nitrification, and denitrification). This study was done to provide basic data in quantitative understanding of environment and agriculture implications of land use shift from natural wetland to grazing meadow or cropland.

## 2 Materials and Methods

### 2.1 Site Description

We conducted our field research in the Napahai Wetland region (27°47'~27°55'N, 99°35'~99°43'E; elevation of 3260 m above sea level), southwest China, a Ramsar wetland of international importance. The site is located in the overlap of the Tibet plateau climate zone, subtropical monsoon climate zone, and Indo-China Peninsula monsoon climate zone; the summer is short, warm, and rainy, while the winter is long and cold. The annual average temperature is 5.4 °C, and the valid cumulative temperature is approximately 1529.8 °C. The annual average precipitation is approximately 619.9 mm, and approximately 76% of it occurs from June to September.

The Napahai Wetland presents as a typical seasonal marsh wetland. In recent years, the majority of the natural wetland of Napahai has been converted to grazing meadow or cropland, coincident with climate change and human activity. At present, natural wetland, grazing meadow, and cropland are the three main landscapes in the Napahai region. The three land use types are located on different topographical gradients. The soils are predominantly bog soil, humus soil, meadow soil, and cultivated soil. The natural wetland flora is composed of *Carex pleistoguna*, *Blysmus sinocompressus*, *Carex nubigena*, *Deschamps caespitosa*, *Sanguisorba filiformis*, etc. The meadow supports *Potentilla anserina*, *Pedicularis longiflora* var. *tubiformis*, *Plantago asiatica*, *Artemisia japonica*, *Aster tataricus*, *Taraxacum mongolicum*, *Lancea tibetica*, etc. The cropland is used to grow buckwheat and highland barley.

### 2.2 Methods

For our research, typical natural wetland, meadow, and cropland located along topographical gradients from lowland to highland were selected. Three uniform plots of 10 m × 10 m were chosen from each site (natural wetland, meadow, and cropland) for sample collection, and the distance between plots was greater than 10 m.

#### 2.2.1 Soil Sampling

Soil sampling was conducted in May, July, September, and November of 2011. At least 5 soil cores (5 cm diameter) were randomly collected from the top 0–10 cm of each plot in natural wetland, grazing meadow, and cropland. Core samples from each plot were mixed thoroughly to form one composite sample, and three field samples were obtained for the respective land use type. Samples were kept in a cooler at 4 °C for laboratory analysis. Total organic carbon was measured with a total organic carbon analyzer. Total N content in soil was measured by the Kjeldahl method.  $\text{NH}_4^+$ -N was detected by Nessler's reagent colorimetry.  $\text{NO}_3^-$ -N was detected by the phenol disulfonic acid method. The soil water content at 10 cm depth was determined by an oven drying method.

#### 2.2.2 Soil N Mineralization

We used intact in situ soil cores, encased in PVC chambers, 5 cm in diameter and 25 cm deep, to quantify changes in net N mineralization in response to the land use change. Based on the method of DiStefano and Gholz (1986), we placed one resin bag on top of each core to deionize deposition from above and placed two resin bags on the bottom, with the upper one capturing ions leaching from the soil core and the lower one deionizing water entering the tube from below. In each resin bag, we combined approximately 3 g of a sulfonic acid-based cation resin (HCR-W2,  $\text{H}^+$  form) with 3 g of a trimethylbenzyl ammonium-based anion resin (21 K,  $\text{Cl}^-$  form) (Huizhu Resin Limited Company, Shanghai). We activated the resins by soaking them overnight in 5 M NaCl (Szillery et al. 2006). We placed one soil core in each of 54 points in 9 plots/fields from 16 May to 15 July 2011, from 16 July to 15 September 2011, and from 16 September to 15 November 2011 to quantify net N mineralization.

Following incubation in the field, we mixed the soil in each core and extracted a 15-g subsample in 75 mL of 2 M KCl for 1 h and then filtered the extracts through pre-leached filter paper and froze them. We analyzed the extracts colorimetrically for  $\text{NO}_3^-$  and  $\text{NH}_4^+$ . We discarded both outer resin bags and extracted the inner-bottom resin bag contents in 2 M KCl for 30 min. We estimated net N mineralization by subtracting the preincubation extractable  $\text{NH}_4^+$  and  $\text{NO}_3^-$  from the soil

from the postincubation extractable  $\text{NH}_4^+$  and  $\text{NO}_3^-$  from the soil core and inner-bottom resin (Hanselman et al. 2004).

### 2.2.3 Soil Denitrification

Denitrification was measured using the acetylene-block method (Tiedje et al. 1989). The soil cores were collected from each plot/field in natural wetland, grazing meadow, and cropland on 16 May, 16 July, 16 September, and 16 November 2011. All soil cores were collected between 9:00 am and 11:00 am. Six 5-cm diameter and 20-cm long PVC chambers were inserted 10 cm into the sediment/soil at each plot/field. Three groups of 6 soil cores (18 replicates) were kept in a cooler and transported to the laboratory and refrigerated at 4 °C. Acetylene gas was injected into the chambers until it occupied 10% (v/v) of the headspace volume. Then, the sampling cores were kept in the dark (to prevent the growth of algae) at 23 °C for 24 h. Headspace gas samples were then collected at 24 h after acetylene addition. Nitrous oxide produced by denitrification accumulated in the headspace and was analyzed using a gas chromatograph equipped with an electron capture detector (ECD, GC7900 System).

### 2.3 Statistical Analysis

The mean values of each land use site and its parameters were subjected to a one-way ANOVA using SPSS 18.0 to understand the impact of land-use types. Significant differences among the land-use types were determined using Tukey tests. Statistical significance was determined at  $P < 0.05$ . Relationships among mineralization, denitrification, and environmental variables were investigated using correlation analysis.

## 3 Results

### 3.1 Soil Physicochemical Properties

The water content in the natural wetland soils was significantly higher than that in the grazing meadow and cropland soils ( $P < 0.05$ ). The bulk density was significantly lower in the natural wetland soils than that in the grazing meadow and cropland soils ( $P < 0.05$ ). Organic carbon, total N, and  $\text{NH}_4^+$ -N were significantly higher in the natural wetland soils than those in the grazing meadow and cropland soils ( $P < 0.05$ ).  $\text{NO}_3^-$ -N and inorganic N in the natural wetland and grazing meadow soils showed significant change with the growing season but were comparable in the cropland soils (Table 1). This observation may have resulted from fertilization of the croplands. In addition,  $\text{NH}_4^+$ -N accounted for 51–91% of inorganic N in the natural wetland soils. In contrast,  $\text{NO}_3^-$ -N was the main form of the inorganic N in May and

July, but  $\text{NH}_4^+$ -N was the main form of the inorganic N in September and November in the grazing meadow and cropland soils.

### 3.2 Net N Mineralization and Nitrification

The net N mineralization rate was higher in the natural wetland soils than that in the grazing meadow soils, whether in spring ( $P > 0.05$ ) and summer ( $P > 0.05$ ) or autumn ( $P < 0.05$ ) (Fig. 1a). The net N mineralization rate of the cropland soils was higher than that of the natural wetland soils in spring ( $P > 0.05$ ) and summer ( $P > 0.05$ ) but was lower in autumn ( $P > 0.05$ ). The net nitrification rate was higher in the natural wetland soils than that in the grazing meadow soils in spring ( $P < 0.05$ ) and summer ( $P > 0.05$ ) but lower in autumn ( $P > 0.05$ ). The net nitrification rate was higher in the natural wetland soils than that in the cropland soils in spring ( $P < 0.05$ ) and autumn ( $P > 0.05$ ) but lower in summer ( $P > 0.05$ ) (Fig. 1b). The ammonification rate was lower in the natural wetland soils than that in the grazing meadow and cropland soils in spring ( $P > 0.05$ ) and summer ( $P < 0.05$ ) but higher in autumn ( $P < 0.05$ ) (Fig. 1c). Of the processes we measured, net nitrification was the major process of net N mineralization in the grazing meadow and cropland soils, and net ammonification was the major process of net N mineralization in the natural wetland soils.

### 3.3 Denitrification

We observed a consistent seasonal pattern in denitrification rates over the sampling period, with higher rates in May and July and lower rates in September and November (Fig. 2). Specifically, the denitrification rate in the cropland soils decreased from May to November. Along with the seasonal patterns, we found a spatial gradient of denitrification rates. Cropland soils had relatively higher denitrification rates than those of natural wetland and grazing meadow soils in May, September, and November ( $P > 0.05$ ). In contrast, natural wetland and grazing meadow soils had relatively higher denitrification rates than those of cropland soils in July ( $P > 0.05$ ) (Fig. 2). The denitrification rates of the natural wetland soils were higher than those of the grazing meadow soils.

## 4 Discussion

The pools of total nitrogen and organic carbon in the upper 10 cm of the natural wetland soils were significantly higher than those of the grazing meadow and cropland soils, suggesting that the conversion of natural wetland greatly reduced nitrogen pools and carbon stocks, as noted in previous reports (Yang et al. 2008; Uri et al. 2008; Tripathi and Singh 2009; Yang et al. 2010), confirming that there is a strict significant

**Table 1** Selected physicochemical properties of 0–10 cm soil depth samples in natural wetland (NW), grazing meadow (GM), and cropland (CL). The means (with SD in parentheses) with a different letter in the same row are significantly different ( $n = 3$ ,  $P < 0.05$ )

Item		Water content (%)	Bulk density ( $\text{g cm}^{-3}$ )	Organic carbon ( $\text{g kg}^{-1}$ )	Total N ( $\text{g kg}^{-1}$ )	$\text{NH}_4^+\text{-N}$ ( $\text{mg kg}^{-1}$ )	$\text{NO}_3^-\text{-N}$ ( $\text{mg kg}^{-1}$ )
May	NW	77.13 $\pm$ 3.11a	0.18 $\pm$ 0.05c	231.08 $\pm$ 21.48a	133.26 $\pm$ 9.96a	75.60 $\pm$ 9.79a	13.99 $\pm$ 3.51c
	GM	12.73 $\pm$ 0.93b	1.27 $\pm$ 0.15a	30.22 $\pm$ 12.39b	31.91 $\pm$ 9.04b	28.76 $\pm$ 4.08b	55.33 $\pm$ 8.98a
	CL	15.65 $\pm$ 2.87b	1.06 $\pm$ 0.08b	24.93 $\pm$ 1.82b	22.90 $\pm$ 0.83b	19.80 $\pm$ 4.89b	37.55 $\pm$ 1.63b
July	NW	66.88 $\pm$ 2.76a	0.33 $\pm$ 0.02b	252.51 $\pm$ 7.45a	129.02 $\pm$ 8.87a	61.11 $\pm$ 8.12a	58.15 $\pm$ 2.83a
	GM	10.87 $\pm$ 0.76b	1.38 $\pm$ 0.03a	32.39 $\pm$ 2.89b	26.25 $\pm$ 2.57b	30.41 $\pm$ 3.16b	67.59 $\pm$ 10.05a
	CL	11.65 $\pm$ 1.63b	1.36 $\pm$ 0.03a	40.37 $\pm$ 0.48b	16.02 $\pm$ 4.94b	24.79 $\pm$ 4.75b	44.28 $\pm$ 5.47b
September	NW	85.47 $\pm$ 3.69a	0.15 $\pm$ 0.04b	225.89 $\pm$ 26.98a	138.87 $\pm$ 30.99a	67.39 $\pm$ 9.59a	6.92 $\pm$ 0.79b
	GM	27.15 $\pm$ 0.85b	1.13 $\pm$ 0.06a	37.06 $\pm$ 12.62b	29.36 $\pm$ 12.89b	33.22 $\pm$ 14.97b	5.85 $\pm$ 0.36b
	CL	27.05 $\pm$ 2.38b	1.08 $\pm$ 0.03a	46.53 $\pm$ 4.62b	30.60 $\pm$ 1.37b	29.65 $\pm$ 4.68b	28.73 $\pm$ 6.98a
November	NW	75.88 $\pm$ 27.28a	0.24 $\pm$ 0.02b	249.87 $\pm$ 5.52a	138.71 $\pm$ 15.57a	75.77 $\pm$ 8.19a	9.68 $\pm$ 0.14b
	GM	12.68 $\pm$ 1.53b	1.47 $\pm$ 0.49a	33.98 $\pm$ 10.45c	26.83 $\pm$ 5.22b	13.78 $\pm$ 1.84b	2.56 $\pm$ 0.12b
	CL	14.55 $\pm$ 4.45b	1.15 $\pm$ 0.16a	51.40 $\pm$ 4.02b	30.56 $\pm$ 2.54b	17.17 $\pm$ 3.11b	14.87 $\pm$ 8.09a

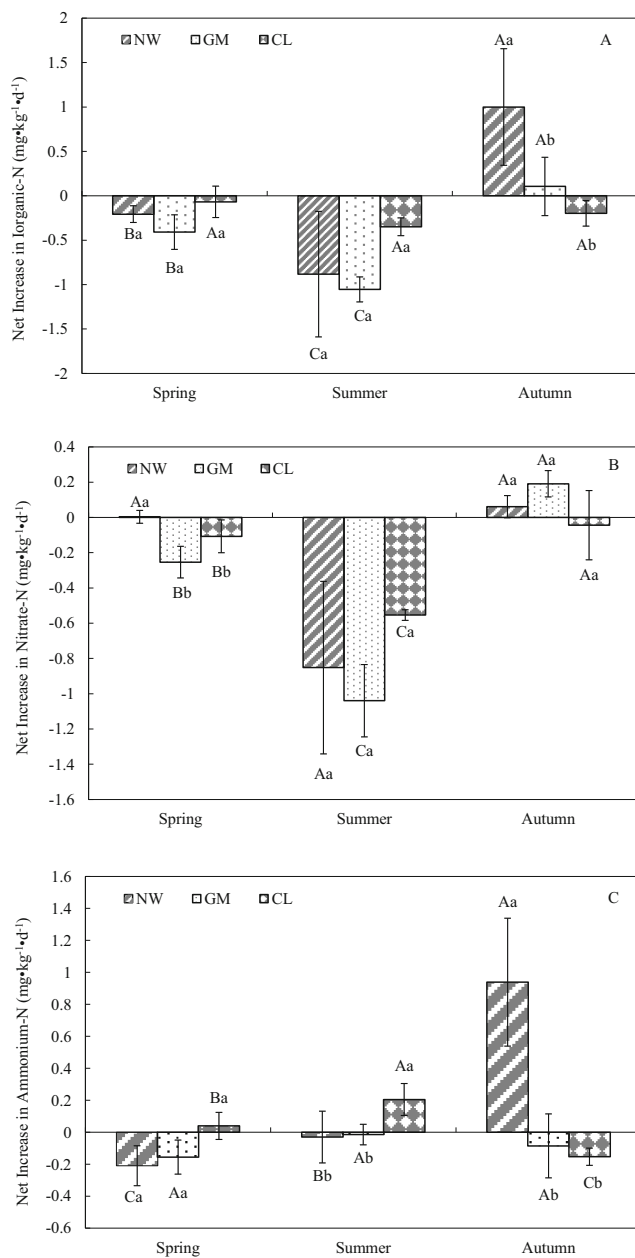
correlation between total nitrogen and soil organic carbon (Uri et al. 2008; Liu et al. 2018).

Differences among inorganic N forms were particularly pronounced among the three land use types. Natural wetland soils were characterized by low  $\text{NO}_3^-\text{-N}$ , with the  $\text{NH}_4^+\text{-N}$  to  $\text{NO}_3^-\text{-N}$  ratio ranging from 1.1 to 9.7 across the growing season. In contrast,  $\text{NO}_3^-\text{-N}$  was the dominant form of inorganic N in grazing meadow and cropland soils, with the  $\text{NH}_4^+\text{-N}$  to  $\text{NO}_3^-\text{-N}$  ratio less than 0.5 in May and July.  $\text{NH}_4^+\text{-N}$  was the dominant form of inorganic N in grazing meadow and cropland soils, with the  $\text{NH}_4^+\text{-N}$  to  $\text{NO}_3^-\text{-N}$  ratio ranging from 1.0 to 5.7 in September and November. Differences in inorganic N forms may be due to different patterns and activities of microbial biomass (Burger and Jackson 2003; Liu et al. 2019), which controls N mineralization, nitrification, denitrification, and the uptake of inorganic N by microbes or plants (Uri et al. 2008). A consistently higher level of  $\text{NH}_4^+\text{-N}$  in natural wetland soils than that in grazing meadow and cropland soils indicates that nitrogen was transformed mainly in the form of ammonium. In contrast, a higher level of  $\text{NO}_3^-\text{-N}$  in grazing meadow and cropland soils than that in natural wetland soils in May and July indicates that nitrate was accumulating. Although the causes for the difference in the main form of inorganic N between spring-summer and autumn in grazing meadow and cropland soils remain to be determined, the difference might be related to qualitative differences in ammonia-oxidizing bacteria and ammonia-oxygen archaea communities in the substrates (Zhong et al. 2014).

The pattern of higher rates of net N mineralization and nitrification observed in natural wetland soils than in grazing meadow soils (Fig. 1) is similar to the results of past studies in tropical montane and lowland regions, where conversion from forest to pasture markedly reduced net N mineralization rates (Tripathi and Singh 2009; Yang et al. 2010). The pattern of a

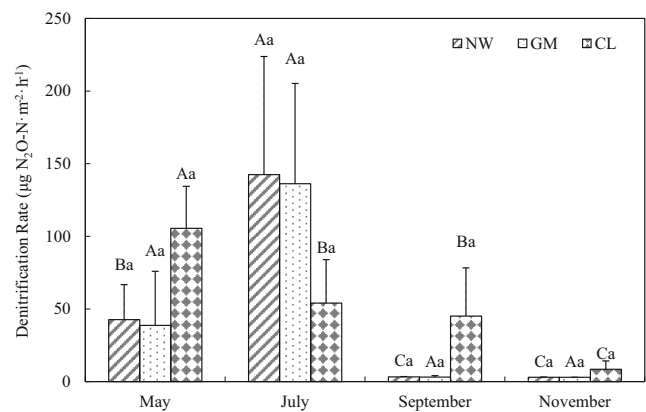
higher net N mineralization rate in cropland soils than that in natural wetland soils in spring and summer (Fig. 1) is similar to the results of studies in the Los Haitises region (Templer et al. 2005), where the net mineralization was significantly greater in agricultural soils. We attribute these differences to the amount of water content ( $R^2 = 0.44$ ,  $P = 0.24$ ), organic carbon ( $R^2 = 0.21$ ,  $P = 0.59$ ),  $\text{NO}_3^-\text{-N}$  concentration ( $R^2 = -0.77$ ,  $P = 0.02$ ), and inorganic N ( $R^2 = -0.56$ ,  $P = 0.12$ ). Large declines in organic carbon and water content and increases in  $\text{NO}_3^-\text{-N}$  concentration and inorganic N follow the conversion of natural wetland to grazing meadow and reduced soil mineralization. It is evident that human disturbance speeds up organic matter decomposition and loss, especially in cropland soils, along with the removal of N and other nutrients by annual crop harvesting. Larger losses of organic matter and soil microbial community in autumn reduced soil mineralization in cropland soils (Fig. 1).

Denitrification requires anoxic conditions, which can occur in water-saturated soils or in aggregates of unsaturated soils. Generally, soil moisture and available organic carbon have been identified as major controlling factors for denitrification in wetlands (Hill and Cardaci 2004; Shrestha et al. 2012). The denitrification rate was much higher in natural wetland soils than that in grazing meadow soils in May and July (Fig. 2). We attribute this difference to the soil moisture and the amount of organic carbon and total N as the water content, the amount of organic carbon, and total N varied strictly in the same order as denitrification rates. It is evident that larger declines of soil moisture and organic matter resulting from the conversion of natural wetland to grazing meadow reduced soil denitrification rate. There is a negative correlation of  $\text{NO}_3^-\text{-N}$  concentration with water content ( $R^2 = -0.44$ ,  $P = 0.16$ ), organic carbon ( $R^2 = -0.30$ ,  $P = 0.35$ ), and total N ( $R^2 = -0.33$ ,  $P = 0.29$ ) and a positive relationship with denitrification rates ( $R^2 = 0.67$ ,  $P = 0.02$ ). These data suggest that soil moisture, organic



**Fig. 1** Net increases in inorganic N ( $\text{NH}_4^+\text{-N}$  plus  $\text{NO}_3^-\text{-N}$ ),  $\text{NH}_4^+\text{-N}$ , and  $\text{NO}_3^-\text{-N}$  over a 60-day incubation in spring (from 16 May to 15 July), summer (from 16 July to 15 September), and autumn (from 16 September to 15 November) in soils of natural wetland (NW), grazing meadow (GM), and cropland (CL). Samples were taken from 0 to 10 cm soil depth. Lowercase letters indicate significant differences among land uses on the same period, while capital letters represent significant differences among sampling times for the same land use. Vertical bars are standard errors of the mean ( $n = 6$ )

carbon, and total N play important roles in regulating nitrogen availability, while the  $\text{NO}_3^-\text{-N}$  availability affects denitrification strongly. In contrast, the denitrification rate was much higher in cropland soils with lower soil water content, whereas the  $\text{NO}_3^-\text{-N}$  concentration was higher with annual fertilization in cropland (Table 1). The general importance of  $\text{NO}_3^-\text{-N}$



**Fig. 2** Denitrification rate measured in natural wetland (NW), grazing meadow (GM), and cropland (CL) soils. Samples were taken from 0 to 10 cm soil depth. Lowercase letters indicate significant differences among land uses for the same period, while capital letters represent significant differences among sampling times for the same land use. Vertical bars are standard errors of the mean ( $n = 6$ )

availability for denitrification is indicated by the high statistical significance for  $\text{NO}_3^-\text{-N}$  as the explaining factor for denitrification rate in the cropland soils ( $R^2 = 0.44$ ,  $P = 0.24$ ).

## 5 Conclusions

We found a significant variation in soil C and N pools, N availability, and N transformation processes among natural wetland, grazing meadow, and cropland soils. The conversions of natural wetland to grazing meadow or cropland appear to result in significant declines in water content, organic C, total N, and inorganic N concentration while increasing soil bulk density. The conversions of natural wetland to grazing meadow also result in a substantial decrease in net N mineralization, net nitrification, and denitrification. The conversions of natural wetland to cropland increase N mineralization in spring and summer and denitrification rates in May, September, and November. Natural wetland soils have higher N availability and transformation rates than those of grazing meadow soils. Cropland soils have lower N availability and higher transformation rates. The derived ecosystems, particularly grazing meadow and cropland soils, have substantially different soil characteristics, fertility, and C and N cycling process.

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