Effect of nitrogen addition on the carbon metabolism of soil microorganisms in a Calamagrostis angustifolia wetland of the Sanjiang Plain, northeastern China

Xiaohong Weng1,2, Xin Sui1,2*, Yingnan Liu3†, Libin Yang2,3 and Rongtao Zhang3†

Abstract

Purpose: Soil microorganisms are important mediators of land ecosystem functions and stability. However, carbon sources in different amounts of nitrogen addition are known to affect the function of soil microbial communities. Thus, this study sought to evaluate the effects of nitrogen addition on the carbon utilization capacity of soil microorganisms in the Sanjiang Plain wetland, northeastern China.

Methods: Three nitrogen treatments (CK, 0 kg N ha⁻¹ a⁻¹; N40, 40 kg N ha⁻¹ a⁻¹; and N80 kg N ha⁻¹ a⁻¹) were evaluated in the Honghe National Nature Reserve of the Sanjiang Plain. The carbon metabolism capacity of soil microorganisms in the C. angustifolia wetland was investigated after five consecutive year's nitrogen addition treatment using the Bio-Eco technique.

Results: Different amounts of nitrogen addition conditions resulted in significant differences in pH, ammonium nitrogen (NH₄⁺), dissolved organic carbon (DOC), and soil microbial alpha diversity. The average well-color development (AWCD) in the Bio-Eco Plate assay increased gradually with incubation time, and different nitrogen levels significantly affected these AWCD values (P < 0.05), with the N40 treatment exhibiting the highest value. Furthermore, the N80 treatment had significantly lower Shannon and Pielou diversity indices (P < 0.05). N40 significantly promoted carbohydrate, amino acid, and ester utilization rates by soil microorganisms, whereas N80 significantly inhibited carbohydrate, amino acid, alcohol, amine, and organic acids utilization. Redundancy analysis (RDA) showed that the three treatments had remarkable differences in soil microbial community metabolism, and the cumulative variance contribution was 72.86%. In addition, RDA revealed that the N80 treatment was positively correlated with the TN, SMC, DON, and TOC but negatively correlated with DOC, NH₄⁺, pH, and NO₃⁻.

Conclusion: Long-term nitrogen addition leads to changes in soil microbial community structure and significantly alters the ability of soil microorganisms to utilize carbon sources in the Calamagrostis angustifolia wetland.

Keywords: Calamagrostis angustifolia wetland, Soil microorganism, Functional diversity, Bio-Eco Plate

Background

Soil nitrogen is one of the important nutrient elements in ecosystems and plays critical roles on ecosystem structure and function. In wetland ecosystems, nitrogen is considered to be one of the mainly limiting nutrient elements for primary productivity, but due to the
development of agricultural practice and long-term large amounts of nitrogen fertilizers utilization, the amounts of available nitrogen in the soil increase quickly and impact wetland ecosystem structure and function (Vitousek and Howarth 1991; Feng et al. 2015). Therefore, nitrogen-saturated inputs affect a number of ecological processes (Nakaji et al. 2001; Lu et al. 2015), such as soil acidification (Ouyang et al. 2005), affecting apoplastic decomposition (Song et al. 2011), and stimulated CO₂ emissions (Song et al. 2013; Tao et al. 2018), as well as reducing soil microbial diversity and affecting microbial ecological functions (Wang et al. 2018; Zhang et al. 2018), ultimately having an impact on wetland ecosystems.

Wetlands are important terrestrial ecosystems, covering 5 to 8% of the total area of the earth. Wetlands possess the characteristics of both terrestrial and aquatic ecosystems and are therefore highly biodiverse and productive, in addition to providing a wide range of ecosystem services (Wang et al. 2006a). Nitrogen addition affects the structure and function of wetland ecosystems by altering soil environmental conditions such as nitrogen content and soil organic matter, influencing soil microbial activity, as well as the composition and diversity of soil microbial communities (Li et al. 2006; Wang et al. 2006b). Soil microorganisms are major participants in soil nitrogen transformation, and have an important regulatory role in the soil nitrogen cycle (Zhang et al. 2009), and contribute significantly to the stability and function of wetlands. Since microbial response to environmental changes is very sensitive, so soil microbial structure and function is an important indicator for soil quality change (Yan et al. 2010).

Previous studies have shown that the continuous input of nitrogen affects soil microorganisms in different ecosystems. For example, Li et al. (2013) results showed that the soil microbial activity decreased significantly was changed when the ammonium sulfate concentration was higher than 528.5 mg/kg. In contrast, Wu et al. (2017) investigated the effect of nitrogen addition (NH₄NO₃) on soil microorganisms in coastal wetlands and found that soil microbial activity was increased under N addition (3 and 6 g N ha⁻¹ a⁻¹). The reason may be the different forms and amounts of N could affect the type of carbon source used by the soil microbiota. Fang et al. (2014) reported that nitrogen source inputs of ammonium and nitrate nitrogen significantly promoted microbial metabolic activity and utilization of carbon substrates. Long-term nitrogen input experiments conducted by Compton et al. (2004) at Harvard University showed that increased nitrogen decreased microbial biomass and reduced the utilization ability of microbial communities for carbon sources. It is seen that nitrogen input affects grassland ecosystems, forest ecosystems, and wetland ecosystems.

Wetlands play an important role in ecological processes such as controlling greenhouse gas emissions, regulating climate, and maintaining ecosystem balance (Liu 2004). Many scholars have begun to focus on the effects of nitrogen addition on wetland soil microorganisms. Lu et al. (2021) found in the effect of nitrogen addition on soil microbial structure and function in coastal saline wetlands that high N treatment (200 kg N ha⁻¹ a⁻¹) with NH₄NO₃ as a nitrogen source increased nutrients in the soil but reduced soil microbial diversity. Wang et al. (2019) studied the effects of nitrogen addition on temperate marshland soils in northeastern China and found that nitrogen treatments (8 g N m⁻² a⁻¹) with NH₄NO₃ as a nitrogen source for nitrogen input reduced soil pH value and alteration in the microbial community.

The Sanjiang Plain is the largest freshwater marsh region in China. However, due to the extensive and intensive use of the region for agricultural production over the past 50 years, the natural freshwater marshes in this region often received increasing amounts of exogenous N inputs (Zhao 1999; Wang et al. 2010; Zhang et al. 2007). Studies have shown that nitrogen input promotes bacterial growth (Braganza et al. 2012), enhances methanogenic and anaerobic respiratory bacterial activity (Yavitt et al. 2012), accelerates soil denitrification processes (Francez et al. 2011), and inhibits aerobic methane oxidation (Lozanovska et al. 2016). The previous studies reported that the ratio of NH₄⁺-N/NO₃⁻-N has been reduced from 5 to 2 since the 1970s, so the nitrogen form has been changed (Hu et al. 2019). Our previous study also found that the bacterial and fungal diversities and compositions in the wetlands of the Sanjiang Plain were significantly changed by NH₄NO₃ addition (Sui et al. 2016). However, the function of soil microorganisms in the wetlands of the Sanjiang Plain by adding different nitrogen forms is still unknown. Therefore, we aim to (i) evaluate the variation of soil physicochemistry properties in different amounts NO₃⁻ addition and (ii) clarify the changes of soil microbial carbon metabolism and function diversity in different amounts NO₃⁻ addition.

The Bio-Eco Plate method is a technique that allows researchers to identify the different types of carbon sources utilized by soil microorganisms (Garland 1997; Preston et al. 2002). This technique has been widely used in recent years as it provides a simple and rapid means of assessing the function of soil microbial communities (Konopka et al. 1998; Garland and Mills 1991). In this study, the carbon utilization capacity of soil microorganisms was investigated using the Bio-Eco Plate technique based on long-term field simulations of NH₄ addition in the Honghe National Nature Reserve in the Sanjiang Plain. Our findings thus provide important insights into the mechanisms by which wetland ecosystems adapt
Results

Effect of different nitrogen levels on soil physicochemical characteristics

Table 1 summarizes the average values of main soil physicochemical properties under different nitrogen application levels. Different nitrogen levels had a significant effect on soil pH, $\text{NH}_4^+$ content, and DOC ($P < 0.05$). The contents of SMC, DON, and TN showed an increasing trend with the increase of nitrogen addition concentration, as order CK < N40 < N80; the contents of soil pH and NO$_3^-$ showed a decreasing trend with the increase of nitrogen addition concentration, as order CK > N40 > N80; the $\text{NH}_4^+$, DOC, and TOC contents of each treatment showed a decreasing and then increasing phenomenon with the increase of nitrogen addition concentration.

Effects of simulated nitrogen addition on soil microbial carbon source metabolic activity

As illustrated in Fig. 1, the average well color development (AWCD) of the soil microbial community under different nitrogen addition concentrations increased with culture time. Specifically, this value increased rapidly at

---

Table 1  Physicochemical properties of wetland soil with different nitrogen addition conditions

| Treatment | SMC (mg/kg) | pH | NO$_3^-$ (mg/kg) | NH$_4^+$ (mg/kg) | DOC (mg/kg) | DON (mg/kg) | TN (%) | TOC (%) |
|-----------|-------------|----|------------------|-----------------|-------------|-------------|--------|---------|
| CK        | 0.43 ± 0.06a| 4.4 ± 0.18a| 23.8 ± 6.34a     | 21.2 ± 3.45a    | 3897.9 ± 625.37a | 299.7 ± 130.71a | 0.57 ± 0.23a | 5.34 ± 2.23a |
| N40       | 0.48 ± 0.03a| 4.3 ± 0.0ab| 23.4 ± 0.89a     | 4.3 ± 0.30b     | 836.0 ± 135.58b | 374.2 ± 17.77a  | 0.77 ± 0.16a | 4.62 ± 0.80a  |
| N80       | 0.50 ± 0.04a| 4.1 ± 0.12b| 21.7 ± 1.41a     | 9.4 ± 2.28b     | 1575.6 ± 325.29b| 444.2 ± 134.93a | 0.94 ± 0.29a | 5.31 ± 1.44a  |

Each treatment was performed in triplicate. The data are expressed as the mean ± standard deviation; lowercase letters indicate significant differences ($P < 0.05$)

CK control, N40 40 kg N ha$^{-1}$ a$^{-1}$, N80 80 kg N ha$^{-1}$ a$^{-1}$, SMC soil moisture contents, DOC dissolved organic carbon, DON dissolved organic nitrogen, TN total nitrogen, TOC total organic carbon

---

Fig. 1  Average well color development (AWCD) of the soil microbial community under different nitrogen addition concentrations as a function of incubation time
0–96 h, indicating a high microbial metabolic activity at this culture stage. These increases decelerated after 120 h and stabilized thereafter. Therefore, the AWCD values that were incubated for 120 h were selected for subsequent analysis. The AWCD (i.e., and indicator of carbon utilization) of the microbial communities under different nitrogen addition conditions exhibited the following order: N40 > CK > N80. Interestingly, the N40 treatment exhibited the highest carbon metabolic capacity.

**Changes in soil microbial functional diversity**

To further determine the effect of different nitrogen concentrations on soil microbial carbon source utilization, different diversity indices were used to evaluate the AWCD value at 120 h of culture. As indicated in Table 2, there was no significant difference between the soil microbial functional diversity of the N40 and CK treatments, but there was a significant difference between N80 and CK (P < 0.05).

**Utilization of different carbon sources by the soil microbial community**

Figure 2 illustrates the effects of different nitrogen addition levels on the utilization rates of different types of carbon sources by soil microorganisms. A total of 31 carbon sources were evaluated with the Bio-Eco Plate assay, which in turn were divided into six categories: carbohydrates (7), amino acids (6), alcohols (3), esters (4), amines (3), and organic acids (8). As shown in Fig. 2, N40 significantly promoted carbohydrate, amino acid, and ester utilization rates by soil microorganisms (P < 0.05), whereas N80 inhibited carbohydrate, amino acid, alcohol, amine, and organic acids utilization (P < 0.05).

As shown in Fig. 3, the metabolic fingerprint of the N40-treated soil was constituted by eight kinds of carbon sources with AWCD > 2.0, including α-D-lactose, β-methyl-D-glucoside, D-cellobiose, L-serine, D-malic acid, and α-D-glucose-1-phosphate, and β-methyl-D-glucoside in N80 were significantly lower than in CK and N40; in group II, the AWCD values of D-galacturonic acid, N-acetyl-D-glucosamine, D-mannitol, D-cellobiose, α-D-lactose, and β-methyl-D-glucoside in N80 were significantly lower than in CK and N40; in group III, putrescine, L-Serine, D-malic acid, and α-D-glucose-1-phosphate were significantly higher in CK and N40 than in N80; and in group IV, the AWCD values of D-glucosaminic acid in N80 were significantly lower than in CK and N40. These results demonstrated that different nitrogen concentrations had significantly different effects on the activity and carbon source utilization preference of the soil microbiota.

**Factors influencing soil microbial carbon source utilization patterns under different nitrogen addition treatments**

Table 3 summarizes the correlation coefficients of the main components of the 31 carbon sources. As shown in Table 3, a total of 21 carbon sources constitute the first principal component (PCA1), including five carbohydrates, four amino acids, two esters, three alcohols, three amines, and three organic acids. Among them, D-cellobiose was the carbon source most related to PCA1 with a 0.989 load value, followed by β-methyl-D-glucoside (0.987) and α-D-glucose-1-phosphate (0.979). Therefore, D-cellobiose, β-methyl-D-glucoside, and α-D-glucose-1-phosphate had a major effect on PCA1. Additionally, seven kinds of carbon sources constituted the second principal component (PC2), including two carbohydrates, two amino acids, one ester, and two organic acids. Among them, D-xylose is the most relevant carbon source for PC2 (loading value of −0.841), followed by L-phenylalanine (0.805) and glycogen (0.785). Collectively, nitrogen addition makes the carbon source metabolic activity of soil microorganisms most correlated...
with D-cellobiose and D-xylose in the *Calamagrostis angustifolia* wetland of the Sanjiang Plain.

The microorganisms in the soil samples containing varying nitrogen levels were cultured for 120 h. The results of the redundancy analysis (RDA) showed that the variance contributions of RDA1 and RDA2 were 55.36% and 17.5%, respectively, and the cumulative variance contribution was 72.86%. As illustrated in Fig. 5, Soil microbial Biolog-substrate utilization patterns were separated with the alteration of nitrogen addition. Among them, the total N80 treatment clustered farthest from the total CK and N40 treatments, whereas the total CK and N40 treatments clustered closer to each other. Therefore, we concluded that the N80 treatment significantly changes the carbon source utilization capacity of soil microorganisms. In addition, the N80 treatment was positively

**Fig. 2** Utilization of different carbon sources by soil microbial communities under different nitrogen addition concentrations. 1 Carbohydrate, 2 amino acids, 3 alcohols, 4 esters, 5 amine, 6 acids; lowercase letters indicate significant differences (*P* < 0.05). CK, control; N40, 40 kg N ha$^{-1}$ a$^{-1}$; N80, 80 kg N ha$^{-1}$ a$^{-1}$
correlated with the TN, SMC, DON, and TOC but negatively correlated with DOC, \( \text{NH}_4^+ \), pH, and \( \text{NO}_3^- \).

Table 4 presents the relationships between different soil microbial functional diversity indices and soil physicochemical properties in the Sanjiang Plain. The AWCD value was highly positively correlated with pH \( (R^2 = 0.69, P < 0.01) \). Furthermore, the Shannon-Wiener index (H), Simpson index (D), and Pielou index were highly correlated with pH, with \( R^2 \) values reaching 0.68, 0.70, and 0.74, respectively.

**Discussion**

Different soil conditions can strongly affect soil microbial composition and diversity, and therefore, shifts in the functions of the soil microbiota can be used as relevant ecological indicators. Long-term nitrogen input ultimately affects the structure and function of wetland ecosystems, and therefore, assessing the impact of long-term nitrogen addition on soil microbial function is critical to gain a comprehensive insight into wetland ecosystem dynamics. In this study, the AWCD value of the soil microbial community increased with culture time, and the different experimental treatments exhibited the following order: \( N40 > CK > N80 \) (Fig. 1). Different times of N addition and different nitrogen forms had significant differences in soil microbial carbon metabolism. For example, Yuan et al. (2012) found that the AWCD of N treatments (\( \text{CO(NH}_2\text{)}_2 \)) in a Chinese fir plantation after seven consecutive year N addition showed nitrogen treatment (60 kg N ha\(^{-1}\)
a⁻¹) significantly increased the AWCD, but nitrogen treatment (120 kg N ha⁻¹ a⁻¹) significantly decreased the AWCD value. However, Yu et al. (2013) found that treatment with applied NH₄NO₃ (150 kg N ha⁻¹ a⁻¹) promoted AWCD in shrub, but nitrogen treatment (50 kg N ha⁻¹ a⁻¹) inhibited AWCD in shrub after 1 month of N addition. Sui et al. (2016) conducted simulating nitrogen addition (NH₄NO₃), and four consecutive years on the functional diversity of soil microorganisms in the *Calamagrostis angustifolia* wetland of the Sanjiang Plain showed that after AWCD, values increased with increasing nitrogen concentration, showing HN (8 g N ha⁻¹ a⁻¹) > LN (4 g N ha⁻¹ a⁻¹) > CK. The differences in the results of these studies may be related to differences in the form and the concentration of N addition. In soils with short-term nitrogen addition, nitrogen application helps to alleviate nitrogen limitation and improve soil available nitrogen content, thereby promoting the functional activity of soil microbial carbon metabolism (Liu et al. 2010). However, long-term high nitrogen addition can result soil acidification and reduced organic carbon content, affect the effectiveness of heterotrophic microbial communities on substrate utilization, and reduce soil microbial productivity (Deforest 2004).

Our findings indicated that higher nitrogen addition substantially decreased soil microbial activity, which also coincided with higher soil moisture. In soils with low nitrogen content, nitrogen application helps to alleviate nitrogen limitation and improve soil

![Fig. 4 Heat map and hierarchical cluster analysis based on the average well color development (AWCD) at 120 h of soil microbial communities under different nitrogen addition treatments. The samples are grouped based on their similarity to each other. The clustering results are arranged horizontally. Higher AWCD values are indicated in dark green, whereas lower AWCD values are indicated in yellow. CK, control; N40, 40 kg N ha⁻¹ a⁻¹; N80, 80 kg N ha⁻¹ a⁻¹](image-url)
available nitrogen content, thereby promoting the functional activity of soil microbial carbon metabolism (Liu et al. 2010). Therefore, our study is consistent with the results of Sui et al. (2016) and Wu et al. (2017) that low concentration of nitrogen addition promotes the functional activity of carbon metabolism in soil microorganisms. However, long-term high concentration of nitrogen addition can affect the effectiveness of heterotrophic microbial communities on substrate utilization and reduce soil microbial productivity (Deforest 2004; Compton et al. 2004) conducted a long-term nitrogen input experiment in Harvard Forest, and the study showed that high nitrogen addition will lead to the decrease of soil microbial carbon biomass, which reduces the utilization rate of soil microorganisms for substrates. In addition, excessive nitrogen additions can lead to soil acidification (Table 1), and lower soil pH leads to changes in microbial biomass and microbial communities (Li et al. 2019). This may be the reason that the high concentration of nitrogen addition decreased the soil microbial AWCD value. However, Frey et al. (2004) carried out nitrogen addition experiments in Harvard Forest, and their studies have shown that the utilization of substrates by soil microorganisms in broad-leaved forests and mixed forests is not significantly related to nitrogen increase. So, the changes of soil microbial carbon metabolism under different nitrogen addition conditions may be related to ecosystem types, nitrogen application time, study period, nitrogen application amount and nitrogen application form, etc. Therefore, the effect mechanism of nitrogen addition on soil microbial metabolic activity still needs to be further studied.

The Shannon diversity index, Simpson index, and Pielou index are composite indicators of the richness and evenness of microbial species (He et al. 2013a). Our findings indicated that different levels of nitrogen addition significantly changed the alpha functional of the soil microbial community (Table 2). Specifically, the Shannon-Wiener, Simpson, and Pielou indices of the soil microorganisms were significantly higher in the CK and N40 treatments compared to N80. These findings were consistent with those of Sui et al. (2016), whose study demonstrated that the Shannon and Simpson indices increased with the low concentration of nitrogen addition (4 g N ha$^{-1}$ a$^{-1}$) and decreased with the high concentration of nitrogen addition (8 g N ha$^{-1}$ a$^{-1}$) in wetland soils, and the indices differed significantly between treatments. This may be because moderate nitrogen application favors the growth of soil microorganisms, whereas high nitrogen content promotes the proliferation of some microbial populations while suppressing others, resulting in a decrease in microbial community diversity indices.

The heat map shows that different nitrogen concentrations had different effects on the carbon source utilization patterns of the microbes in the soil samples. Combining Fig. 2 and Fig. 3, it can be seen that N40 promoted the utilization of all carbon sources in carbohydrates, amino acids, and esters by soil microorganisms. N80 inhibited the utilization of all carbon sources in carbohydrates, amino acids, alcohols, amines, and organic acids, except for 4-hydroxy benzoic acid and γ-hydroxybutyric acid in organic acids. In addition, the most relevant D-cellobiose and D-xylose for PC1 and PC2 are both derived from carbohydrates, which exactly correspond to the results

### Table 3 Correlation coefficients of major components in 31 kinds of carbon sources

| Category | Biolog EcoPlate well | Carbon source types | PC1     | PC2     |
|----------|-----------------------|---------------------|---------|---------|
| Carbohydrate | B2 | D-xylose | 0.383  | −0.841 |
|           | H1 | α-D-lactose | 0.969  | −0.205 |
|           | A2 | β-methyl-D-glucoside | 0.987  | −0.117 |
|           | G2 | α-D-glucose-1-phosphate | 0.979  | −0.092 |
|           | E1 | α-cyclodextrin | 0.434  | 0.719  |
|           | F1 | Glycogen    | 0.785  |         |
|           | G1 | D-cellobiose | 0.989  | −0.119 |
| Amino acids | A4 | L-arginine | 0.463  | 0.607  |
|           | B4 | L-asparagine | 0.809  | 0.018  |
|           | C4 | L-phenylalanine | 0.469  | 0.805  |
|           | D4 | L-serine    | 0.96   | 0.09   |
|           | E4 | L-threonine | 0.746  | −0.119 |
|           | F4 | Glycyl-L-glutamic acid | 0.917  | 0.343  |
| Esters    | B1 | Pyruvic acid methyl ester | 0.699  | 0.29   |
|           | C1 | Tween 40    | −0.226 | 0.262  |
|           | D1 | Tween 80    | 0.723  | −0.213 |
|           | A3 | D-galactonic acid-y-lactone | −0.072 | 0.088  |
| Alcohols  | C2 | i-erythritol | 0.575  | −0.178 |
|           | D2 | D-mannitol | 0.968  | −0.113 |
|           | H2 | D, L-α-glycerol phosphate | 0.852  | 0.04   |
| Amine     | G4 | Phenylethyl-amine | 0.408  | −0.825 |
|           | H4 | Putrescine  | 0.963  | −0.103 |
|           | E2 | N-acetyl-D-glucosamine | 0.976  | 0.036  |
| Acids     | B3 | D-galacturonic acid | 0.956  | −0.063 |
|           | F2 | D-glucosaminic acid | 0.926  | −0.073 |
|           | C3 | 2-hydroxy benzoic acid | 0.381  | 0.697  |
|           | D3 | 4-hydroxy benzoic acid | −0.0396 | 0.089  |
|           | E3 | γ-hydroxybutyric acid | −0.169 | −0.261 |
|           | F3 | Itaconic acid | 0.392  | −0.772 |
|           | G3 | α-ketobutyric acid | 0.334  | 0.116  |
|           | H3 | D-malic acid | 0.966  | −0.181 |

PC1 principal component 1, PC2 principal component 2
in Fig. 2 and Fig. 3. Frey et al. (2004) also evaluated the effect of simulated nitrogen settlement on the soil microbial community of a hardwood forest and found that the carboxylic acids and carbohydrates were significantly higher in the low nitrogen plots, whereas the citric acid and malonic acid were significantly higher in the high nitrogen plots. Chakraborty et al. (2011) reported that the application of nitrogen fertilizer reduced the ability of soil microbial groups to decompose organic acids and amines. Zhu et al. (2014) found that anaerobic bacteria increased the use of amino acids and decreased the use of organic acids and carbohydrates after nitrogen application. Furthermore, gram-negative bacteria increased the use of carboxylic acids and decreased the use of amino acids and polymeric carbon sources. In contrast, yeast increased the use of carboxylic acids and polymeric carbon sources. These findings demonstrate that the differences in the carbon source utilization patterns of soil microorganisms may be an adaptation to soil environmental changes.

In this study, KNO₃ was the main nitrogen source in this experiment; therefore, the NO₃⁻/NH₄⁺ increased in the soil, so this may reduce the soil microbial activity and used the ability of some carbon substrate. Soil carbon
concentration also affected significantly on soil microbial function and diversity. Fang et al. (2014) reported that high nitrogen concentration significantly increases DOC content in soil by changing the metabolic activity of microorganisms and the way of utilizing carbon substrate, and nitrogen has no effect on it. This reason may be the soil organic carbon is an important nutrient on soil microbial function, and carbon metabolism activity and the variation of soil DOC concentration would directly affect the soil microbial composition and diversity and therefore change the soil microbial function (He et al. 2013b). The soil carbon metabolism types of N80 treatment were positively correlated with the TN, SMC, DON, and TOC but significantly negatively correlated with DOC and NH$_4^+$, pH, and NO$_3^-$ (Fig. 5). The increasing N addition concentration also significantly reduced soil pH and NH$_4^+$ content (Table 1). The reason may be adding nitrogen will lead to reduce the soil pH and affect the soil carbon metabolism ability. Liu et al. (2010) found that long-term high nitrogen addition would lead to soil acidification, affecting the soil microbial metabolism change and activity decline. Sui et al. (2021) reported that soil pH and total nitrogen had a significant effect on soil bacterial community structure. Fierer and Jackson (2006) demonstrated that soil pH significantly affected the composition of the bacterial community. Furthermore, Diao et al. (2019) found that soil pH was the main factor affecting microbial carbon source utilization under different nitrogen levels. Additionally, our redundancy analyses showed that there was a distinct separation between the different nitrogen treatments tested herein, while the effect of the N80 treatment was significantly greater than that of the N40. It is possible that a nonlinear threshold is crossed somewhere between the N40 and N80 treatments, which in turn allows the N80 treatment to have a greater effect on the composition, diversity, and carbon metabolism patterns of the microbial community than the N40 treatment. With the development of agriculture and industry in the future, it can be predicted that the amount of nitrogen input in the Sanjiang Plain will continue to increase, which will affect the structure and function of soil microorganisms. Carbon is the most important component in nature that can be utilized by soil microorganisms, and it is also the most consumed and utilized nutrient substrate. A large amount of nitrogen input will increase the amount of litter, change the quantity and quality of soil organic matter (Grandy et al. 2009; Liu et al. 2010), and also directly change the quality of soil microbial decomposition substrates (C:N:P ratio), quantity, and soil environment. Therefore, nitrogen addition affects soil nutrient availability, causing changes in microbial utilization of carbon sources and ultimately altering the structure and function of microbial communities (Chakraborty et al. 2011).

In summary, the simulated nitrogen addition treatments had a significant effect on the soil microbial communities of Sanjiang Plain, demonstrating that increases in nitrogen addition rates will invariably change the physicochemical properties of the wetland environment. Nevertheless, the Biolog analysis method can only reflect changes in microbial functions based on carbon source utilization patterns and thus cannot fully illustrate the functional diversity of soil microorganisms. Therefore, this approach must be combined with high-throughput sequencing technology, molecular biology, and phospholipid fatty acid analysis to better characterize the variations of microbial function diversity.

**Conclusions**

The carbon source utilization capacity of the soil microorganisms in the Calamagrostis angustifolia wetland of the Sanjiang Plain changed significantly under different nitrogen addition conditions. Particularly, N80 treatment significantly reduced the Shannon, Simpson, and Pielou diversity indices of soil microorganisms and significantly changes the carbon source utilization capacity of soil microorganisms. However, nitrogen addition affects the aforementioned parameters, thus affecting the carbon source utilization capacity of soil microorganisms. Therefore, once nitrogen addition rates exceed a certain threshold in the future, the stability of the temperate wetland ecosystem will be inevitably compromised if no prevention strategies are implemented.

**Methods**

**Study area**

The experimental site was located in the Honghe National Nature Reserve of Sanjiang Plain (47° 42′ 18″–47° 52′ 07″ N, 133° 34′ 38″–133° 46′ 29″ E) (Fig. 6). This region has a total area of 2.18 × 10$^4$ ha, accounting for more than half of the total area of the reserve. The reserve exhibits a temperate humid/semi humid monsoon climate with long winters, severe cold and snow, and short spring and autumn seasons. The average annual temperature (MAT) of this region is 1.9 °C, the average annual evaporation is 1166 mm, and the average annual precipitation (MAP) is 585 mm, with precipitation mostly concentrated between July and September (Qu et al. 2015). The study site exhibits primarily bleached stagnant soil and fibrous organic soil, its main vegetation types are meadows and swamps, and the dominant plants are Calamagrostis angustifolia, Glyceria spiculose, Carex lasiocarpa, and Carex pseudocuraica.


**Plot setting and sample collection**

To investigate the nitrogen addition concentration on soil microbial function in Sanjiang Plain, sample plots were established in May 2016, and nine plots (5 m × 30 m) were randomly established in the *Calamagrostis angustifolia* wetland experiment station, which were treated with three nitrogen concentration levels. The nitrogen addition treatments included control (CK), 0 kg N ha\(^{-1}\) a\(^{-1}\); N40, 40 kg N ha\(^{-1}\) a\(^{-1}\); and N80, 80 kg N ha\(^{-1}\) a\(^{-1}\) treatments. Each treatment was performed in triplicate and randomly assigned. Nitrogen addition concentrations were set based on the amount of local agricultural fertilizer utilization. Applying large doses of N in the short term can effectively mimic long-term small-dose N concentration inputs (Dise and Stevens 2005). Nitrogen additions greater than the exogenous N input to the wetland ecosystem were used to study the response of the ecosystem to a possible future high N saturation state, and different N additions were set to observe the effects of N input over the next 50 years. The nitrogen source KNO\(_3\) was dissolved in water and sprayed uniformly with a sprinkler during the growing season which is mainly associated with agricultural fertilization (Sun et al. 2007), in May each year. The control plots were sprayed with an equal amount of water.

Sampling was conducted in October 2020 within each sample plot in the *Calamagrostis angustifolia* wetland. Five points (the center point of the diagonal line and the sample point on the diagonal line at an equal distance from the center sample point) were selected using the diagonal 5-point sampling method in each of the three treatment sample plots, and the soil was collected from the surface soil layer (0–20 cm) with a 4-cm diameter soil auger. After removing plant debris and other impurities in the collected soil samples, the samples were pooled, stored in a 4 °C cooler, and transported to the laboratory. One part of the sample was used for the Bio-Eco Plate experiments, whereas the other part was naturally dried and used for soil physicochemical analyses.

**Determination of soil physicochemical factors**

Soil pH was determined via the leaching and potentiometric method with a ratio of 2.5:1; soil moisture was determined via the drying method and determined gravimetrically by oven drying at 105 °C for 24 h; soil total nitrogen (TN) was determined with an elemental analyzer; NO\(_3^-\)-N was determined via the phenol disulfonic acid colorimetric method; NH\(_4^+\)-N was determined via the potassium chloride leaching-indophenol blue colorimetric method; dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) were determined via the K\(_2\)SO\(_4\) leaching method with a soil-liquid ratio of 1:5, extracted for 30 min, centrifuged and filtered, and then measured by TOC analyzer (Jones and Willett 2005); total organic carbon (TOC) was determined with a TOC analyzer; and NH\(_4^+\)-N and NO\(_3^-\)-N were determined by the Elemental Analyzer (Flash EA 1112 N, Thermo Fisher, Waltham, MA, USA) (Murphy et al. 2000).
Determination of the functional diversity of the soil microbial community

The Bio-Eco Plate incubation method was used to determine the ability of soil microbial communities to utilize 31 different kinds of carbon sources (Qu et al. 2015). The Bio-Eco Plate has 96 microtiter wells, 1 replicate for every 32 wells, and 3 replicates in total. The first well was used as a control without carbon source, while the other wells contain different carbon sources and tetrarazolium salt dyes. The microorganisms use the carbon source to respire and cause the dye tetrarazolium in the microtiter wells to change color by redox reaction (Xi and Hu 2003). A portion of the soil sample was activated in a thermost at 25 °C for 24 h. Afterward, 10 g of fresh soil was weighed into a 200 mL triangular flask, to which 90 mL of 0.85% sterile NaCl solution was added. The mixture was then shaken at room temperature for 30 min at 200 r/min. The plates were continuously incubated at 25 °C for 168 h. During the incubation period, the absorbance was measured at 590 nm at 24 h intervals, and the absorbance values were recorded (Feng et al. 2021).

Statistical analysis

AWCD was calculated as follows (Velasco et al. 2009; Jin et al. 2014; Liao et al. 2013):

\[ AWCD = \frac{\sum (C_i - R)}{31} \]  

where \( C_i \) is the 590 nm absorbance value of the well containing a carbon source, \( R \) is the absorbance value of the control well, and 31 is the number of holes in the ECO plate. If \( C_i - R \leq 0 \), the value was recorded as 0.

The functional diversity of the soil microbial communities was calculated using 120-h cultivation data. The soil diversity indices were calculated as follows (Pielou 1975; Whittaker 1972):

Shannon-Wiener diversity index: \( H = P_i \ln P_i \)  

Simpson diversity index: \( D = 1 - \sum (P_i)^2 \)  

Pielou diversity index: \( J = \frac{H}{\ln S} \)

where \( P_i \) is the ratio of the \( i \)th relative absorbance value to the sum of the relative absorbance values of all samples.

All data were analyzed before processing using Excel 2010. One-way ANOVA was performed using the SPSS 25.0 software with the test level set at 0.05. Scatter plots with trend lines were generated using Excel 2010, and histograms were generated using SigmaPlot 10.0. Diversity index analysis, heat map, and redundancy analysis (RDA) were performed with R (Vegan package).

Abbreviations

CK: Control; N40: 40 kg N ha\(^{-1}\) a\(^{-1}\); N80: 80 kg N ha\(^{-1}\) a\(^{-1}\); AWCD: The average well-color development; RDA: Redundancy analysis; SMC: Soil moisture contents; DOC: Dissolved organic carbon; DON: Dissolved organic nitrogen; TN: Total nitrogen; TOC: Total organic carbon.

Acknowledgements

We are grateful to the Scientific Paper Editing Co. Ltd. for language editing.

Authors’ contributions

XW and XS performed the experiments, analyzed the data, and wrote this manuscript. YL designed this experiment and revised the manuscript. LY helped to analyze the data, and RZ helped to do the experiment and took the soil samples. The author(s) read and approved the final manuscript.

Funding

The work was funded by the Heilongjiang Provincial Academy of Sciences Special Plan (YZ202003), special projects for the central government to guide the development of local science and technology (ZY20B15), Natural Sciences Foundation of Heilongjiang Province (LH2020C088), and Outstanding Youth Foundation of Heilongjiang University (JCL202006), supported by the Application Technology Research and Development Plan Project of Heilongjiang Province (GA19C006-6), and supported by the Key Laboratory of Forest Plant Ecology, Ministry of Education (K2020A02).

Availability of data and materials

The original data is recorded in an Excel file named “Data record sheet” and has been attached to this article.

Declarations

Ethics approval and consent to participate

The study did not violate ethics, and all participants agreed to publish the paper.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

1 Engineering Research Center of Agricultural Microbiology Technology, Ministry of Education, Heilongjiang University, Harbin 150500, China. 2 Heilongjiang Provincial Key Laboratory of Ecological Restoration and Resource Utilization for Cold Region, School of Life Sciences, Heilongjiang University, Harbin 150080, China. 3 Institute of Natural Resources and Ecology, Heilongjiang Academy of Sciences, Harbin 150040, China.

Received: 29 October 2021  Accepted: 15 April 2022

Published online: 07 May 2022

References

Braganza L, Buttler A, Habermacher J, Brancaleoni L, Gerdol R, Fritze H, Hanajk P, Laiho R, Johnson D (2012) High nitrogen deposition alters the decomposition of bog plant litter and reduces carbon accumulation. Glob Chang Biol 18(3):1163–1172

Chakraborty A, Chakrabarti K, Chakraborty A, Ghosh S (2011) Effect of long-term fertilizers and manure application on microbial biomass and microbial activity of a tropical agricultural soil. Biol Fertil Soils 47(2):227–233

Compton J, E, Watruda L, Porteous L, A, DeGroot S (2004) Response of soil microbial biomass and community composition to chronic nitrogen additions at Harvard forest. For Ecol Manage 196(1):143 -158.

DeForest J (2004) Atmospheric nitrate deposition and the microbial degradation of cellulose and vanillin in a northern hardwood forest. Soil Biol Biochem 36(6):965–971
Yan H, Wu XY, Huang J, He ZM (2010) Microbial indicator of soil quality evaluation and its studying methods. J Shanxi Agricul Sci 38(10):78–81
Yavitt JB, Yashiro E, Cadillo-Quiroz H, Zinder SH (2012) Methanogen diversity and community composition in peatlands of the central to northern Appalachian Mountain region, North America. Biogeochemistry 109:117–131
Yu PY, Zhu F, Wang ZY, Yan WD, Su SF, Li TP (2013) Effects of nitrogen addition on metabolic function of microbial community in red soil of Cinnamomum camphora forest. J Cent South Univ For Technol 33(3):70–74
Yuan YH, Fan HB, Li HX, Liu WF, Shen FF, Guo HB (2012) Effects of simulated nitrogen deposition on soil microorganism in a Chinese fir plantation. Sci Silvae Sin 48(9):8–14
Zhang J, Lin XG, Yi R (2009) Advances in functional gene diversity of microorganism in relation to soil nitrogen cycling. Chin J Eco-Agric 17(5):1029–1034
Zhang L, Song C, Wang D, Wang Y (2007) Effects of exogenous nitrogen on freshwater marsh plant growth and N₂O fluxes in Sanjiang Plain, north-east China. Atmos Environ 41(5):1080–1090
Zhang TA, Chen HYH, Ruan HH (2018) Global negative effects of nitrogen deposition on soil microbes. ISME J 12:1817–1825
Zhao KY (1999) Mires in China. Science Press, Beijing
Zhu F, Li TP, Yu PY, Su SF, Hong XY, Chen T (2014) Carbon source utilization of soil microbial communities in response to nitrogen addition in the Cinnamomum camphora plantation. Sci Silvae Sin 50(8):82–89

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.