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Short-term effects of nitrogen addition and vegetation removal on soil chemical and biological properties in a freshwater marsh in Sanjiang Plain, Northeast China

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ABSTRACT

Nitrogen (N) is commonly a limited nutrient in wetland ecosystems. Nitrogen addition affects the ecosystem carbon (C) balance and alters soil C storage through soil chemical and biological changes. In the present study, the effects of N addition and vegetation removal after one growing season on soil properties were examined in a *Calamagrostis angustifolia* freshwater marsh in Northeast China. Specifically, available N (NH_4^+–N and NO_3^-–N), microbial biomass C (MBC), and dissolved organic C (DOC) concentrations in the soil were analyzed. In addition, activities of soil enzyme (β-glucosidase, invertase, and urease) were investigated. The results showed that N addition resulted in significant increase of NH_4^+–N and NO_3^-–N in both topsoil and subsoil, and vegetation removal enhanced these effects. Soil MBC concentrations increased after N addition and vegetation removal but DOC concentrations did not change significantly. As to soil enzyme activities, N addition stimulated the β-glucosidase activity in the topsoil and invertase and urease activities in both soil layers. Vegetation removal enhanced the effect of N addition on β-glucosidase and invertase activities but inhibited the effect on urease activity. These results suggested that N addition affects soil biochemical process indirectly through marshland vegetation.

1. Introduction

Nitrogen (N) is commonly a limited nutrient in wetland ecosystems, and it directly affects the productivity of the wetland (Mitsch and Gosselink, 2000). In recent decades, reclamation of natural wetlands has been one of the major land use changes in Northeast China. Sanjiang Plain, the largest freshwater marshland in China, has experienced intensive and extensive cultivation over the past 50 years (Zhao, 1999). An increasing number of marshes have been drained for conversion into agricultural lands, and the un-drained ones have received a large amount of exogenous N input from the adjacent agricultural land because of fertilization. Nitrogen addition can affect the ecosystem carbon (C) balance and alter the C storage and N cycling rate in wetland soils through chemical and biological changes (Min et al., 2011). Studies have shown that exogenous N significantly increased the CH_4 emission rate and N_2O fluxes (Zhang et al., 2007a,b), and accelerated litter decomposition, thus causing substantial soil C losses in the freshwater marshes in the Sanjiang Plain (Song et al., 2011). However, the mechanisms responsible for the observed responses are poorly understood.

Labile organic C (LOC) fractions, with short turnover terms, such as microbial biomass C (MBC) and dissolved organic C (DOC), are valuable indicators of soil quality (Xu et al., 2010; Yang et al., 2012). Previous studies show that the effect of N addition on soil LOC varies with time. Soil MBC increased initially (Wang et al., 2010; Zhang and Zak, 1998) but decreased after long-term N addition (Chen et al., 2012). Park et al. (2002) suggested that N addition reduces the cumulative DOC release, whereas Pregitzer et al. (2004) found that chronic N addition increases the production and leaching of DOC. Except for its chemical property, biological indicators of soil quality have also been increasingly used because of their sensitivity to land use changes (Mijangos et al., 2006). Soil enzymes are mediators and catalysts of biochemical processes that are important in soil functions (An et al., 2008). Thus, enzyme activities have great potential in providing a unique integrative biological assessment of soils (Alkorta et al., 2003). Previous literatures found that N addition alters soil β-glucosidase, invertase, and urease activities in grassland, forest, and cropland ecosystems (Ajwa et al., 1999; Dalmonech et al., 2010; Iyyemperumal and Shi, 2008; Saiya-Cork et al., 2002). However, more studies are still required to better understand the effect of N addition on these soil chemical and biological parameters as well as the nutrient dynamics in wetland ecosystems.

The presence of vegetation is important because it significantly affects soil biological and biochemical properties (Lucas-Borja et al., 2010; Roldan et al., 1994). Vegetation provides a source of C and other nutrients for the soil decomposer communities in the form of litter and root exudates (Porazinska et al., 2003; Rodríguez-Loinaz et al., 2008). Greater N availability leads to changes in nutrient uptake and photosynthetic efficiency by plants, ultimately controls the quantity

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and biochemistry of litter inputs to the soil and alters substrates that can be leached or microbially decomposed (Smemo et al., 2006). Sanaullah et al. (2011) reported that MBC is significantly higher in vegetated soils than in the unvegetated control, and the difference is probably caused by rhizodeposition. Vegetation can also influence soil enzyme activities by excreting exogenous enzymes and affect microbial species composition and diversity by releasing exudates and oxygen into the rhizosphere, which in turn indirectly affect enzyme activities (Singh and Kumar, 2008; Zhang et al., 2010). Previous studies showed that invertase (Kandeler et al., 2002), urease (Garcia et al., 2005; Zhang et al., 2011), and β-glucosidase (Garcia et al., 2005) activities in planted soil decreased, compared with that in unplanted soil. These changes in MBC and enzyme activities can provide an early indication for slower, less easily detectable soil organic matter changes following vegetation removal.

This study focuses on the changes in soil N (i.e., NH_4^+–N, NO_3^-–N, and total N) concentrations, LOC fractions (i.e., MBC and DOC), and enzyme (i.e., β-glucosidase, invertase, and urease) activities following one growing season of N addition and vegetation removal in a *Calamagrostis angustifolia* freshwater marsh in Northeast China. The aim of this study is to evaluate the influence of N addition on soil chemical and biological properties and how these properties are affected by vegetation in marshland.

2. Materials and methods

2.1. Study area

The study was conducted at the Sanjiang Marsh Wetland Experimental Station (47°35′N, 133°31′E), Chinese Academy of Sciences, which is located in the Sanjiang Plain, Heilongjiang Province, Northeast China. The area features an annual average temperature of 2.5 °C with growing season duration of 125 days. The annual precipitation in this area is 550 mm to 600 mm, of which more than 60% falls from June to August. The typical type of seasonal waterlogged *C. angustifolia* (90% area coverage; few other plants included) marsh was selected as the field experiment. The soil type in the study site is meadow marsh soil and the soil properties are as below: the concentration of total organic C (TOC) and total N (TN) is 23.60 and 2.18 mg g^{-1}, respectively. Soil bulk density is 0.88 g cm^{-3}, and pH (H_2O) is 5.58.

2.2. Experimental design

The experimental setup was a factorial design with four different treatment combinations and three replicates: control plots with vegetation, no N added (CK), plots with vegetation and N addition (N), unvegetated plots (Unvegetated), and unvegetated with N addition plots (Unvegetated + N). Nitrogen was applied as NH_4NO_3 at 4.8 g N m^{-2} every month (from May to September) in the N addition plot during the whole growing season in 2011, totaling to 24 g N m^{-2}. This addition level was used to study the response of wetland ecosystem to highly N saturated condition that may occur in the future. In the vegetation removed plot, the above-ground vegetation was repeatedly removed using scissors to avoid disturbing the soil surface. Board walks, providing access to the whole experimental area, were installed to minimize further impacts to the plots. Plastic frames (PVC; 1.5 m × 1.5 m and 0.8 m deep) were installed to prevent horizontal movement and lateral loss of the added N. Each plot was separated by a 1 m buffer zone.

2.3. Sample collection and analysis

Soil samples of 0–15 cm and 15–30 cm layers were collected respectively by taking four soil cores from each plot on 3 October 2011 (about 30 days after the last N addition in September). Soil samples were sieved through 2-mm screen to remove vegetation roots and other debris. Subsequently, the samples were mixed thoroughly and then divided into two subsamples. Half of each sample was kept at 4 °C for NH_4^+–N, NO_3^-–N, MBC, and DOC content determination, and the other half was freeze-dried and used to determine soil enzymes (i.e., β-glucosidase, invertase, and urease) activities, TOC and TN contents.

Soil inorganic N (NH_4^+–N + NO_3^-–N) was extracted using 2 mol L^{-1} KCl solution. After extraction, NH_4^+–N was analyzed using the indophenol blue spectrophotometric method, and NO_3^-–N was analyzed using the ultraviolet spectrophotometry at 220 nm and 275 nm (Lu, 2000). The measurement of the two wavelengths enables the correction for the interference due to the dissolved organic matter by calculating the difference between both absorbance readings. The TN amount was measured by the Kjeldahl method using the Kjeltec Auto Analyzer (Behr Labor Technik, Germany).

Soil MBC was measured using the chloroform fumigation extraction method (Wu et al., 1990). Soils with or without fumigated chloroform were extracted using 0.5 mol L^{-1} K_2SO_4 by shaking for 30 min and then filtered. Organic C in the extracts was analyzed using high-temperature combustion (Multi N/C 2100 TOC analyzer, Analytik Jena, Germany). The MBC was calculated from the difference between the K_2SO_4-extracted C of the chloroform-treated and -untreated soils, and calibrated using the extraction efficiency factor of 0.45. Soil DOC was assayed following the procedures presented by Ghani et al. (2003). Soil samples were extracted using 30 mL of distilled water by shaking for 30 min. The samples were centrifuged for 20 min at 3500 rpm. All supernatants were filtered through a 0.45 μm filter into separate vials for C analysis. Total organic C and inorganic C in the water were measured using a Multi N/C 2100 analyzer (Analytik Jena, Germany). Soil DOC was calculated by determining difference between total dissolved C and dissolved inorganic C. TOC was determined by the dry combustion method using a Multi N/C 2100 analyzer (Analytik Jena, Germany).

Soil β-glucosidase activity was determined using the method of Tabatabai (1994). 0.5 g soil was put into a 50 mL flask and mixed with 0.25 mL of toluene, 4 mL of modified universal buffer (MUB) (pH 6.0), and 1 mL of 0.5 mol L^{-1} *p*-nitrophenyl-β-D-glucoside (*p*NPG) solution, and then incubated for 1 h at 37 °C. The reaction was terminated by adding 1 mL of 0.5 mol L^{-1} $CaC1_2$ and 4 mL of 0.1 mol L^{-1} pH 12 tris(hydroxymethyl)aminomethane buffer. The soil suspension was allowed to develop a yellow color. *p*-nitrophenol (*p*NP), the product of the reaction, was determined spectrophotometrically at 410 nm. Soil β-glucosidase activity was expressed as μg *p*NP g^{-1} h^{-1}. Invertase and urease activities were measured following the methods of Guan (1986). Invertase activity was determined by incubating 1.0 g soil with 15 mL of 8% sucrose solution and 5 mL of phosphate buffer (pH 5.5) at 37 °C for 24 h and by measuring the reducing sugars as glucose through colorimetry at 578 nm using 3,5-dinitrosalicylic acid. The results were expressed as mg glucose g^{-1} 24 h^{-1}. Urease activity was determined using a colorimetric technique based on the determination of the NH_4^+–N released when 1.0 g soils were incubated with 10 mL of 10% urea solution and 20 mL of citrate solution (pH 6.7) at 37 °C for 24 h. The released ammonium was determined through the indophenol blue method, and the results were expressed as mg NH_4^+–N g^{-1} 24 h^{-1}. All determinations of enzymatic activities were performed in triplicate. Controls were included for each sample to which substrate was added after incubation.

2.4. Statistical analyses

Statistical analyses were conducted using the SPSS 16.0 package. Means (n = 3) and standard errors (SE) were calculated. Two-way analysis of variance (two-way ANOVA) procedures were performed on all experimental variables to detect the interactions between N addition and vegetation removal treatment using the General Linear Models (GLM) procedure. One-way analysis of variance (one-way ANOVA) was used to determine significant differences between two soil layers. Significance for all statistical analysis was accepted at $\alpha = 0.05$ level.