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Nitrogen additions affect litter quality and soil biochemical properties in a peatland of Northeast China

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ABSTRACT

Nitrogen (N) is a limiting nutrient in many peatland ecosystems. Enhanced N deposition, a major component of global climate change, affects ecosystem carbon (C) balance and alters soil C storage by changing plant and soil properties. However, the effects of enhanced N deposition on peatland ecosystems are poorly understood. We conducted a two-year N additions field experiment in a peatland dominated by *Eriophorum vaginatum* in the Da Xing'an Mountains, Northeast China. Four levels of N treatments were applied: (1) CK (no N added), (2) N1 (6 g N m^{-2} yr^{-1}), (3) N2 (12 g N m^{-2} yr^{-1}), and (4) N3 (24 g N m^{-2} yr^{-1}). Plant and soil material was harvested at the end of the second growing season. N additions increased litter N and phosphorus (P) content, as well as β-glucosidase, invertase, and acid-phosphatase activity, but decreased litter C:N and C:P ratios. Litter carbon content remained unchanged. N additions increased available NH_4^+–N and NO_3^-–N as well as total Gram-positive (Gram+), Gram-negative (Gram−), and total bacterial phospholipid fatty acids (PLFA) in shallow soil (0–15 cm depth). An increase in these PLFAs was accompanied by a decrease in soil labile organic C (microbial biomass carbon and dissolved organic carbon), and appeared to accelerate decomposition and reduce the stability of the soil C pool. Invertase and urease activity in shallow soils and acid-phosphatase activity in deep soils (15–30 cm depth) was inhibited by N additions. Together, these findings suggest that an increase in N deposition in peatlands could accelerate litter decomposition and the loss of labile C, as well as alter microbial biomass and function.

1. Introduction

Enhanced atmospheric nitrogen (N) deposition is a major component of global climate change with potentially serious consequences for soil biogeochemistry and the nutrient balance of terrestrial ecosystems (BassiriRad, 2015; Gaudio et al., 2015). Peatland ecosystems represent an important store of carbon (C) and play a key role in the global C cycle. Nutrient-poor peatland ecosystems are particularly sensitive to N deposition (Payne, 2014). N deposition has been suggested as a potential threat to C sequestration of peatlands by altering C balances, accelerating peat decomposition, promoting C release, and weakening the role of peatlands as a C pool (Bragazza et al., 2006; Novak et al., 2015). Franzén (2006) observed that in nutrient-poor bog and fen systems, enhanced N deposition can change plant composition and soil properties; it may promoted microbiological activity; and may lead to accelerated decomposition of peat.

Increases in anthropogenic N deposition can lead to change of aboveground plant growth and physiology, as well as acidification and eutrophication of terrestrial ecosystems (Gao et al., 2014). Greater N availability leads to changes in nutrient uptake and photosynthetic efficiency by plants, ultimately controlling the quantity and biochemistry of litter input to the soil (Smemo et al., 2006). Many studies have shown that litter with higher N content and lower C:N ratios decomposes faster (Saiya-Cork et al., 2002; Wang et al., 2009; Mincheva et al., 2014; Tu et al., 2014). These changes to the rate of litter decomposition may have substantial impacts on soil C dynamics at local, regional and global scales. There is evidence that N deposition has altered soil respiration, soil organic C decomposition, enzymatic activity, and induced the loss of labile organic C (Du et al., 2014b). Moreover, as a part of N cycling, N deposition has affected N transformation processes (N

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mineralization and nitrification) and changed soil ammonium and nitrate content (Ochoa-Hueso et al., 2013; Gao et al., 2015). However, one of the major scientific uncertainties regarding increased N deposition is how it affects C and N cycling. In this paper we study how N additions influence nutrient cycling functions in soil microbial communities. We also study enzyme activities, which are important biochemical indicators of soil quality. The production of enzymes by microbes is closely related to the balance between the availability of and the demand for nutrients (Dong et al., 2015).

Enhanced N deposition can also alter the physiological potential of soil microbial communities. The microbial response may ultimately alter ecosystem C dynamics (Compton et al., 2004; Freedman and Zak 2014). Several studies have used phospholipid fatty acid (PLFA) analysis to study microbial community response to N deposition (Waldrop et al., 2004; Van Diepen et al., 2010). Given the important role of soil microbial communities in the cycling of organic C, an improved understanding of the microbial response to N deposition is needed. Soil enzymes play a critical role in organic matter decomposition. Enzyme activity can be used to assess nutrient dynamics in response to N deposition because it reflects the metabolic requirements of soil communities in relation to inorganic nutrient and substrate availability (Mineau et al., 2014).

Previous studies have found the activity of β-glucosidase, invertase, urease, and acid-phosphatase, which are involved in C, N, and P transformations respectively, responds rapidly and intensively to N deposition in different ecosystems (Wang et al., 2013; Zhou and Zhang, 2014). Furthermore, changes in soil enzyme activity are correlated with the degradation of soil organic matter (SOM) and plant litter (Keeler et al., 2009). Understanding the response of soil enzymes to N deposition is therefore important for determining the sensitivity of peatlands to atmospheric N pollution and for predicting the potential for a critical N threshold beyond which peatlands structure and function is irreversibly altered.

In the Da Xing'an Mountains, located in the Heilongjiang Province, Northeast China, approximately 12% of the land surface is covered by permafrost-affected peat bogs, with an average peat thickness of 0.5–1 m (Niu and Ma, 1995). N deposition in this area is reported to be approximately 13.33 kg ha^{-1} yr^{-1} (Lu and Tian, 2007). The primary objective of this study was to monitor the response of soil chemical and microbial properties of a peatland in the Da Xing'an Mountains to variable levels of N deposition. Soil microbial biomass carbon (MBC), dissolved organic carbon (DOC), and availiable N (i.e., NH_4^+–N, NO_3^-–N), and total nitrogen (TN) were measured as well as changes in PLFA concentrations and enzyme activity. To better understand the effect of N deposition on peatland C cycling in this ecosystem, we also examined the response of *Eriophorum vaginatum* litter (standing dead plant material) properties to N additions. The hypothesis was that enhanced N deposition in N-limited peatland ecosystems would increase plant N pools, and decrease the soil labile C pool (DOC and MBC) because of an increase in the microbial demand for C to assimilate excess N. We also expected the depression of soil microbial function (enzyme activity) with high levels of N deposition.

2. Materials and methods

2.1. Site description and experimental design

The study site was located in the continuous permafrost peatlands in the Da Xing'an Mountains, China (Fig. 1). The soil is peat soil. The active layer ranges from 50 to 60 cm above the permafrost layer. Low temperature and continuous inundation considerably reduce the decomposition of litter and soil organic matter, thereby enhancing peat accumulation in this area. The dominant plant species are *Eriophorum vaginatum*, *Sphagnum* spp., *Calyculata Moench*, *Vaccinium uliginosum* L., and *Ledum palustre* L. The mean annual air temperature (1991–2010) is −3.9 °C with the monthly mean ranging from −31.9 °C in January to 19.8 °C in July, and the mean annual precipitation is 450 mm with 45% falling from July to August (Meng et al., 2014). During the growing season (from May to September) in 2012 and 2013, the air temperature averaged 12.1 and 13.0 °C, and precipitation averaged 347.1 and 505.2 mm (based our observed data), respectively.

During autumn 2011, the N addition experiment was established. Each experimental treatment was done in triplicate – there were 12 completely randomized experimental plots, 2-m × 2-m in size (Fig. 2). Boardwalks in the entire experimental area minimized disturbance to the plots. Plastic frames (PVC; 2-m × 2-m,

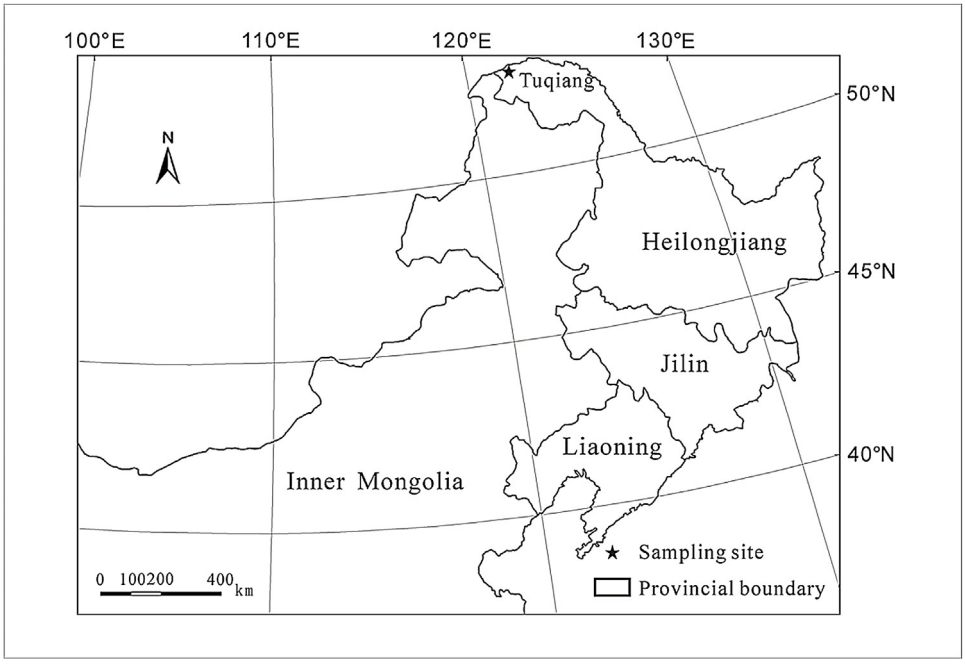

Fig. 1. Map of the sampling site on the Da Xing'an Mountains, Northeast China.

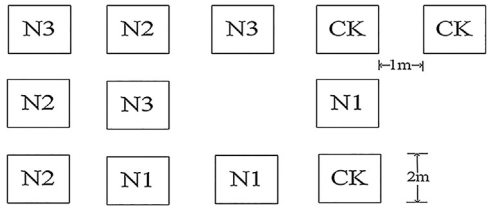

Fig. 2. The distribution of the nitrogen addition plots across the experimental field. CK, N1, N2 and N3 represent applications of 0, 6, 12, and 24 g N m^{-2} $year^{-1}$, respectively, applied monthly over the entire growing season.

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. Three concentrations of N additions and one control (CK) (i.e. no N addition) were used. To simulate natural exogenous N deposition, ammonium nitrate (NH_4NO_3) was applied monthly (from May to September) during the entire growing season in 2012 and 2013. In each year, NH_4NO_3 was divided into five equal doses and applied with an annual rate of 0 (CK), 6 (N1), 12 (N2), and 24 g N m^{-2} yr^{-1} (N3). For each N addition treatment, a concentrated solution of NH_4NO_3 with 1-L surface water was sprayed on each plot. At the same time, the CK treatment received 1 L surface water without NH_4NO_3.

E. vaginatum litter from individual plots was collected on 3 October 2013. Plant material was clipped above the soil surface, and then divided into two subsamples. Fresh samples were used to determine enzyme activity. The second subsample was oven-dried at 60 °C until constant weight. The dried samples were ground with a mini plant mill (FZ102, Tianjin City Taisite Instrument Co., Ltd. China), and then used to determine TC (total carbon), TN, and TP (total phosphorus). Soil samples were collected using a stainless steel soil core sampler (8-cm inner diameter) by taking four soil cores within each plot. Each core was separated into a shallow (0–15 cm) and deep (15–30 cm) layer. After removal of plant roots and debris, the samples were mixed thoroughly, and divided into two subsamples. One subsample for each depth was stored at 4 °C for determination of MBC, DOC, NH_4^+–N and NO_3^-–N content, PLFA content, as well as activity of soil enzymes (β-glucosidase, invertase, and urease). The second subsample was air-dried, ground using a mortar and pestle and passed through a 0.25-mm sieve prior to analysis for TC, TN and TP.

2.2. Sample analysis

TC of dried plant and soil material was determined by the dry combustion method using a Multi N/C 2100 analyzer (Analytik Jena, Germany). TN was measured by Kjeldahl digestion using a Kjeltec Auto Analyzer (Behr Labor Technik, Germany) (X.W. Wang et al., 2013). TP content was measured using the ammonium molybdate method after persulfate oxidation (Kuo 1996).

Soil MBC was measured using the fumigation–extraction method (Wu et al., 1990). Fumigated and non-fumigated fresh soils were extracted with 0.5 mol L^{-1} K_2SO_4 solution by shaking for 30 min. Organic C in the extracts was analyzed using a high-temperature combustion method (Multi N/C 2100 TOC analyzer, Analytik Jena, Germany). MBC was calculated using the following equation: MBC = E_C/0.45, where E_C was the difference in organic C between fumigated and non-fumigated samples.

Soil DOC was assayed following the procedures presented by Ghani et al. (2003). Fresh soil samples were extracted with 30 mL of distilled water by shaking for 30 min. Next, 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 dissolved 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 the difference between total dissolved C and dissolved inorganic C.

Soil mineral N (NH_4^+–N + NO_3^-–N) was extracted with a 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 UV spectrophotometry at 220 and 275 nm. Measurement at two wavelengths allows for correction for interference by dissolved organic matter. Soil pH in air-dried soils was determined by using a 1:5 soil-water ratio (Wood and Lawrence, 2008). Measurements were taken with a PHS-3C pH meter with composite electrode (REX Instrument Factory, Shanghai, China).

Soil microbial community was characterized using PLFA analysis. PLFA was extracted from the soil using the procedure outlined by Bossio and Scow (1998). Fatty acid methyl esters were separated, quantified, and identified using capillary gas chromatography (GC). Qualitative and quantitative fatty acid analyses were performed using an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) and the MIDI Sherlock Microbial Identification System (MIDI Inc., Newark, DE, USA). Fatty acids were quantified by calibration against standard solutions of FAME 19:0 (Matreya Inc., State College, PA, USA) (Chen et al., 2012). Fatty acids used as biomarkers for specific groups of soil organisms are listed in Table 1.

β-Glucosidase activity was assayed using the method of Tabatabai (1994) and expressed as μg *p*NP g^{-1} h^{-1}. Invertase and urease activity was assayed following the methods of Guan (1986). Invertase activity was expressed as mg g^{-1} 24 h^{-1}. Urease activity was expressed as mg NH_4^+–N g^{-1} 24 h^{-1}. Acid phosphatase activity was assayed with 5 mL *p*-nitrophenyl phosphate (*p*NPP) substrate (Zhao and Jiang, 1986), expressed as mg *p*NP g^{-1} 12 h^{-1}.

Table 1
Phospholipid fatty acids used in the analysis of microbial community composition.

Microbial group	Phospholipid fatty acid signatures
Gram+ bacteria	i14:0, i15:0, i16:0, i17:0, a15:0, a17:0, 18:1ω7c (Cao et al., 2010; Liu et al., 2013)
Gram− bacteria	14:1ω5c, 15:1ω6c, 16:1ω7c, cy17:0, 18:1ω5c (Cao et al., 2010; Switzer et al., 2012)
Bacteria in general	14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 16:1ω5c, i17:1ω5c (Groffman and Fisk, 2011; Li et al., 2012; Switzer et al., 2012; Jin et al., 2014)
Fungi	18:1ω9c, 20:1ω9c, 18:2ω6,9 (Ponder et al., 2009; Kaiser et al., 2010; Zhao et al., 2014)
Actinomycetes	10Me17:0 (Zhao et al., 2014)

2.3. Statistical analyses

Statistical analyses were conducted with SPSS 11.5 package. Means (n = 3) and standard errors (SE) were calculated. One-way analysis of variance (ANOVA) was used to determine the differences among different N treatments. Post hoc mean comparisons were conducted using the Duncan test. All data were normally distributed and met the assumptions of ANOVA (data not shown). Significance for all statistical analyses was accepted at the $\alpha = 0.05$ level.

3. Results

3.1. C, N, P concentrations, and enzyme activity in E. vaginatum litter

N additions affected the quality of *E. vaginatum* litter, by changing the N and P content, and by affecting enzyme activity. N and P concentrations, but not C in *E. vaginatum* litter increased significantly with the N additions compared to the control (CK) treatment (Table 2). This resulted in a significant decrease in lit-

Table 2
Content of TC (Total carbon); TN (Total nitrogen); TP (Total phosphorus); C:N (TC:TN); N:P (TN:TP); C:P (TC:TP) of *Eriophorum vaginatum* litter after two years of N additions.

	TC (mg g^{-1})	TN (mg g^{-1})	TP (mg g^{-1})	C:N	N:P	C:P
CK	450(±17) a	5.46(±0.31) b	0.44(±0.06) c	79.5(±4.1) a	13.0(±1.8) a	1030(±141) a
N1	479(±21) a	9.01(±1.21) a	0.70(±0.07) b	48.2(±4.2) b	13.0(±0.5) a	625(±75) b
N2	497(±6) a	9.02(±21.95) a	0.71(±0.12) b	48.4(±4.8) b	12.8(±1.7) a	603(±28) b
N3	478(±10) a	12.20(±2.32) a	0.94(±0.07) a	36.2(±4.0) b	13.3(±2.2) a	465(±47) c

CK, N1, N2, and N3 represent application of 0, 6, 12, and 24 g N m^{-2} yr^{-1} respectively applied monthly over the entire growing season. Values in parentheses are standard errors of the means for each treatment (n = 3). Means with different lowercase letters in the same column are significantly different at $P < 0.05$.

ter C:N ($P < 0.01$) and C:P ratios ($P < 0.05$). N additions significantly enhanced litter β-glucosidase and invertase activity ($P < 0.001$, Figs. 3a and 2b). In addition, N additions tended to increase acid phosphatase activity of the litter, but only with the N3 treatment was the difference significant ($P < 0.05$, Fig. 3c).

3.2. Soil MBC and DOC concentrations

Soil MBC and DOC concentrations varied with soil depth and N addition. Soil MBC concentrations under all treatments varied from 862 μg g^{-1} to 2210 μg g^{-1} in the shallows soil and from 381 μg g^{-1} to 1360 μg g^{-1} in the deep soil. Consistent with our hypothesis, soil MBC decreased as N additions increased. Differences were significant for the N2 ($P < 0.01$) and N3 ($P < 0.01$) treatments in the shallow soil and the N3 treatment in the deep soil ($P < 0.01$) (Fig. 4a). Soil DOC concentrations ranged from 357 μg g^{-1} to 558 μg g^{-1} in the shallow soil (upper 15 cm depth) and from 333 μg g^{-1} to 453 μg g^{-1} in the deep soil (15–30 cm depth). All N addition treatments significantly decreased DOC concentrations in the shallow soil ($P < 0.05$), but only the N2 treatment significantly reduced DOC concentrations in the deep soil ($P < 0.05$, Fig. 4b).

3.3. Soil NH_4^+–N and NO_3^-–N concentrations

Soil NH_4^+–N and (to a lesser extent) NO_3^-–N concentrations varied with soil depth and N addition. Soil NH_4^+–N concentrations ranged from 22.7 μg g^{-1} to 84.9 μg g^{-1} in the shallow soil and from 6.5 μg g^{-1} to 46.5 μg g^{-1} in the deep soil. For the N2 and N3 treatments, NH_4^+-N concentrations increased by 2.8 and 3.7 times in shallow soil, and by 2.5 and 7.1 times in deep soil compared to the control treatment, respectively (Fig. 5a). Soil NO_3^-–N concentrations ranged from 16.5 μg g^{-1} to 26.1 μg g^{-1} in the shallow soil and from 8.3 μg g^{-1} to 14.0 μg g^{-1} in the deep soil. NO_3^-–N concentrations increased in the shallow soil with an increase in N additions, but were not affected in the deep soil (Fig. 5b).

3.4. Soil C, N, and P concentrations and soil pH

Soil TC and pH did not change because of N additions. The response of soil TN and TP depended on soil depth and N addition concentration. Soil TC ranged from 357.6 mg g^{-1} to 386.9 mg g^{-1} in the shallow soil and from 304.3 mg g^{-1} to 358.7 mg g^{-1} in the deep soil. N additions did not significantly change soil TC ($P < 0.05$, Fig. 6a). TN and TP increased significantly in the shallow soil for the N3 treatment, by 14.3% and 20.4% respectively, compared to the control treatment. Deep soil TN concentrations did not change, and deep soil TP concentrations decreased significantly with all N additions ($P < 0.05$, Fig. 6b and c). Soil pH ranged from 5.2 to 5.4 in the shallow soil and from 5.2 to 5.3 in the deep soil. Two years of N additions did not have a significant effect on soil pH ($P > 0.05$, Fig. 6d).

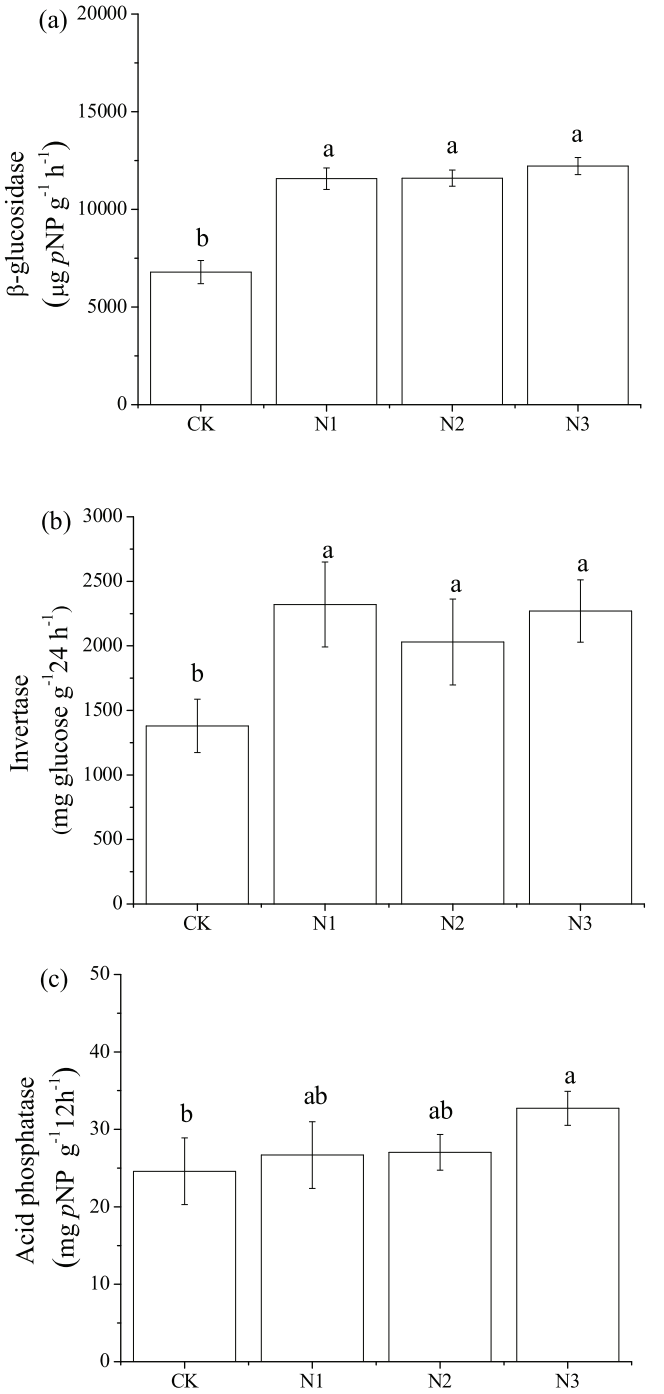

Fig. 3. Effects of two years of N addition on (a) β-glucosidase, (b) invertase, and (c) acid phosphatase activitiy in *Eriophorum vaginatum* litter. CK, N1, N2 and N3 represent applications of 0, 6, 12, and 24 g N m^{-2} year^{-1}, respectively, applied monthly over the entire growing season. Values are the means ± SE of each treatment. Bars within each subgraph followed by the same letter are not significantly different at $P < 0.05$.

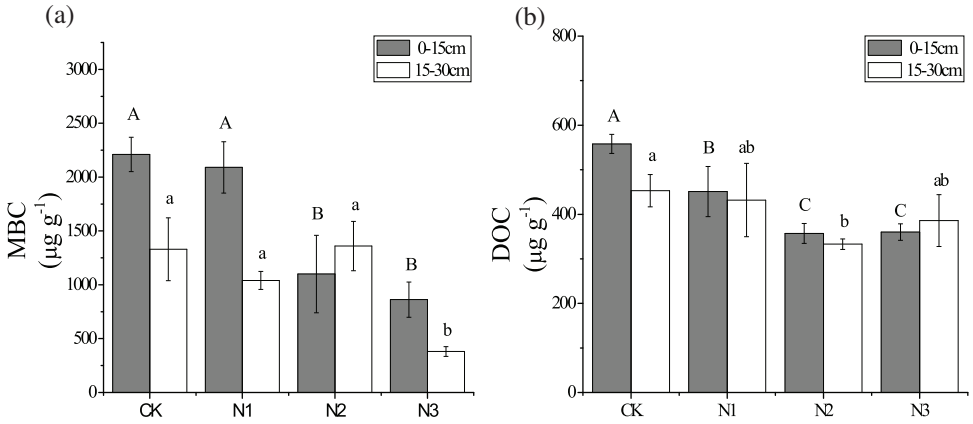

Fig. 4. Changes in soil (a) MBC (microbial biomass carbon), and (b) DOC (dissolved organic carbon) concentrations in the 0–15 cm and 15–30 cm layers after two years of N addition. CK, N1, N2, and N3 represent applications of 0, 6, 12, and 24 g N m^{-2} $year^{-1}$, respectively, applied monthly over the entire growing season. Values are the means ± SE of each treatment (n = 3). Bars with different upper- and lower-case letters are significantly different ($P < 0.05$) in the 0–15 cm and 15–30 cm layers soil, respectively.

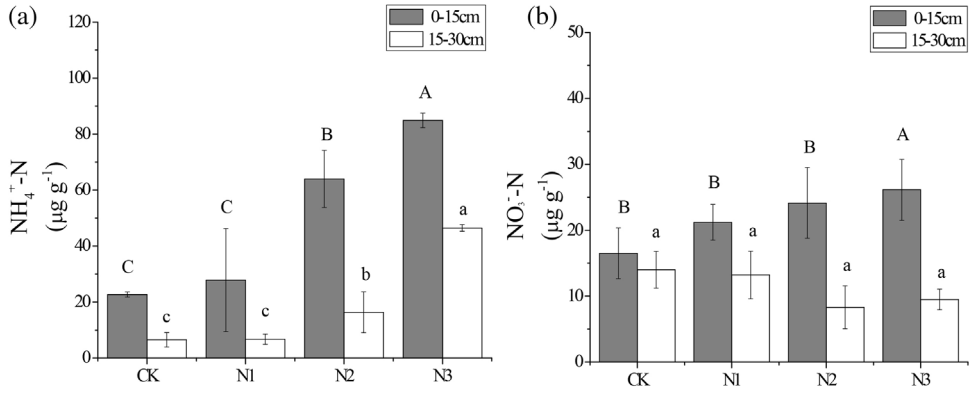

Fig. 5. Changes in soil (a) NH_4^+–N, and (b) NO_3^-–N concentrations in the 0–15 cm and 15–30 cm layers after two years of N addition. CK, N1, N2, and N3 represent applications of 0, 6, 12, and 24 g N m^{-2} $year^{-1}$, respectively, applied monthly over the entire growing season. Values are the means ± SE of each treatment (n = 3). Bars with different upper- and lower-case letters are significantly different ($P < 0.05$) in the 0–15 cm and 15–30 cm layers soil, respectively.

3.5. Soil microbial community composition and biomass

N additions tended to increase total and individual PLFA. However, there were no significant differences in PLFA in the deep soil among the treatments (Fig. 7). Significant increases in total PLFA and Gram-negative (Gram−) PLFA of soil samples were observed at all the N addition treatments in shallow soil ($P < 0.05$, Fig. 7a–d). Shallow soil Gram-positive (Gram+) PLFA and bacterial PLFA were significantly increased in the N3 treatment ($P < 0.05$). Fungi PLFA in the shallow soil ranged from 3.83 nmol g^{-1} to 6.52 nmol g^{-1} and was significantly higher in the N1 treatment compared with CK, with an increase of 70.2% (Fig. 7e). Soil actinomycete PLFA ranged from 0.44 nmol g^{-1} to 0.76 nmol g^{-1} in the shallow soil and from 0.40 nmol g^{-1} to 0.73 nmol g^{-1} in the deep soil, and did not significantly change with N additions ($P > 0.05$, Fig. 7f). Finally, N additions did not significantly affect fungi:bacteria PLFA ratios and Gram+:Gram− bacteria PLFA ratios ($P > 0.05$, Fig. 7g and h).

3.6. Soil enzymatic activity

Responses of soil enzyme activity to N additions varied with different enzymes and with soil depth. Two years of N additions significantly reduced soil invertase and urease activity in the shallow soil ($P < 0.05$), but not in the deep soil ($P > 0.05$, Fig. 8a and c). Different from our original hypotheses, soil β-glucosidase activity was not affected by N additions in either soil layer ($P > 0.05$, Fig. 8b). Soil acid phosphatase activity in the deep soil significantly decreased by 47% and 43% in the N2 and N3 treatments respectively, compared with the control treatment (Fig. 8d).

4. Discussion

4.1. Litter quality

N and P are indispensable major elements for plant physiological functions and are important components of organic matter. Litter C:N has been recognized as an index for the effects of litter quality on rates of organic matter decomposition. Litter C:P affects gross P mineralization and gross phosphate immobilization in decomposing litter (Mooshammer et al., 2012). Results from our study show that N additions enhanced plant N and P uptake as indicated by the significant increase of *E. vaginatum* litter N and P content; C:N and C:P ratios decreased because C was not affected by N additions. The mean C:N ratio decreased with increasing N additions in *Sphagnum* litter in a previous study by Bragazza et al. (2006), and indicated that enhanced N addition had the potential to profoundly affect litter chemistry through a higher accumulation of N. This was in line with our initial hypothesis. *E. vaginatum* has the ability to take up nitrate in peatland ecosystems (McKane et al., 2002). An increase in plant tissue N content with N additions also was found in a temperate steppe by Wang et al. (2014). P is essential to a plant's growth, the increase in P content is probably because of the self-adaptation to absorb more P to alleviate the negative N effect from enhanced N assimilation (Du et al., 2014a). Similarly, earlier studies

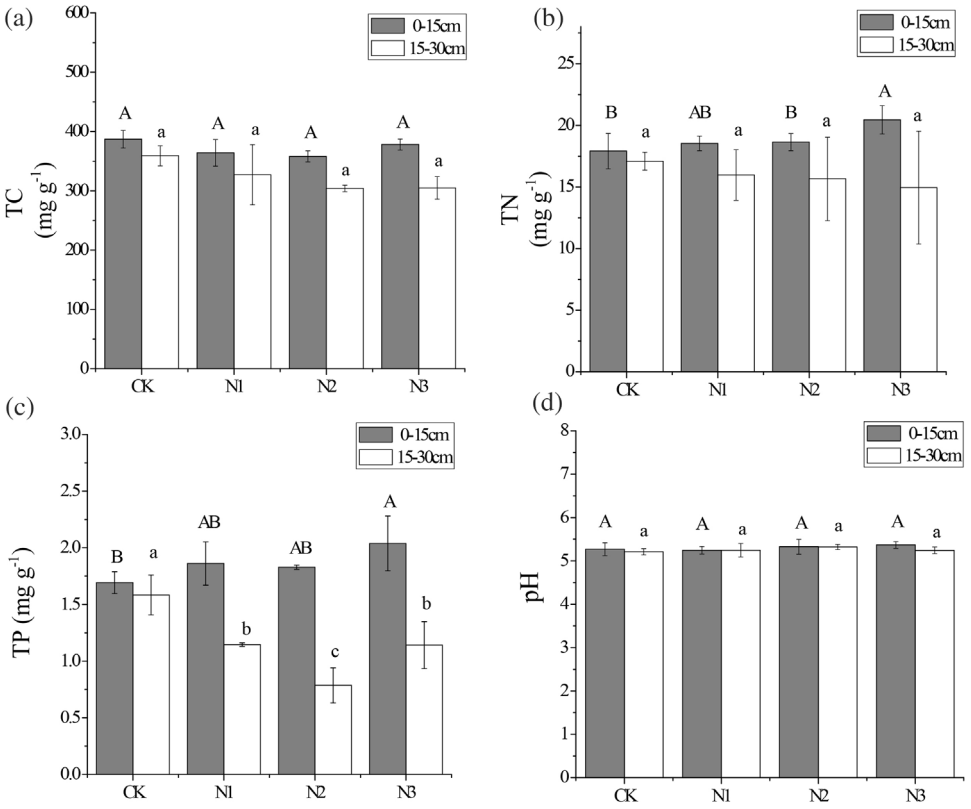

Fig. 6. Changes in soil (a) TC (total carbon), (b) TN (total nitrogen), (c) TP (total phosphorus) and (d) pH in the 0–15 cm and 15–30 cm layers after two years of N addition. CK, N1, N2, and N3 represent applications of 0, 6, 12, and 24 g N m^{-2} $year^{-1}$, respectively, applied monthly over the entire growing season. Values are the means ± SE of each treatment (n = 3) plots. Bars with different upper- and lower-case letters are significantly different ($P < 0.05$) in the 0–15 cm and 15–30 cm layers soil, respectively.

also observed that N additions lead to higher plant P and lower C:P at the species level (Han et al., 2014). Mooshammer et al. (2012) found that the decomposition rate was higher for litter with high internal N content and lower C:N; in addition, rates of P mineralization were negatively correlated with litter C:P. Thus, decreased C:N and C:P ratios with N additions should stimulate litter decomposition and accelerate C and nutrient cycling in peatlands.

Nutrient mineralization from plant litter occurs via the enzymatic activities of microbial communities that become established on litter surfaces (Kourtev et al., 2002). Enzyme analysis can be used as an index for litter decomposition rates in wetlands (Kang and Freeman, 2009). Two years of N additions enhanced β-glucosidase, invertase, and acid phosphorase activity in *E. vaginatum* litter. N additions have also been shown to enhance activity of β-glucosidase in *Acer saccharum* litter (Saiya-Cork et al., 2002), acid phosphatase activity in *E. vaginatum* litter (Johnson et al., 2010), and invertase activity in *Populus tremula* litter (Chigineva et al., 2011). Both β-glucosidase and invertase have a function in litter decomposition. Invertase catalyzes the hydrolysis of sucrose to glucose and fructose. Beta-glucosidase is involved in the enzymatic degradation of cellulose, the main component of plant polysaccharides. Acid phosphatase is responsible for the P release from the litter. All three of these enzyme activities are closely linked to litter mass loss (Zhang et al., 2009; Waring, 2013). Decomposition of litter is a crucial ecosystem process that regulates the cycling of C and P between plants and soils (Waring, 2013). Greater microbial respiration rates and P release rates are associated with an increased rate of mass loss during decomposition (Zhang et al., 2014; Bargali et al., 2015). Therefore, increases in β-glucosidase, invertase, and acid phosphatase activity under N additions may promote litter decomposition and increase the rate of C and P release.

4.2. Soil labile C

In this study, soil TC did not change in response to N additions. However, high levels of N additions decreased soil MBC and DOC concentrations, indicating that soil labile organic C, an energy source for microbial growth, responded to N additions. Such changes are most likely because N additions stimulated microbial growth and changed C-use efficiency because of the exhaustion of labile C substrates. Li et al. (2013) reported that N additions decreased soil MBC concentrations. In contrast, shorter-term N fertilization has been shown to increase MBC concentrations (Zhang and Zak, 1998). These contradictory results might be because of differences in the initial status of the microbial communities, soil pH, organic matter, and soil nutrient content (Li et al., 2013).

In our study, N additions enhanced soil DOC loss by 19.2%–36.0% in the shallow soil and by 4.6%–26.5% in the deep soil. Fang et al. (2014) suggest that the decrease of DOC concentration in soil was a consequence of changing microbial decomposition and humification processes. DOC concentrations in porewater also tended to decrease when ammonium was supplied. The result was that C released by plants was rapidly respired by root associated or soil heterotrophic microbes before it was able to contribute to the DOC pool (Currey et al., 2011). Soil DOC represents the main source of substrate and energy for microbial metabolism, and microbial metabolites also constitute a significant proportion of DOC (Magill and Aber, 2000; Tian et al., 2013). The same response by both MBC and DOC in our study suggests labile substrate availability is regulated by microbial growth as affected by N additions. The effects of increased N on MBC and DOC have important consequences on the ecosystem and at larger scales.

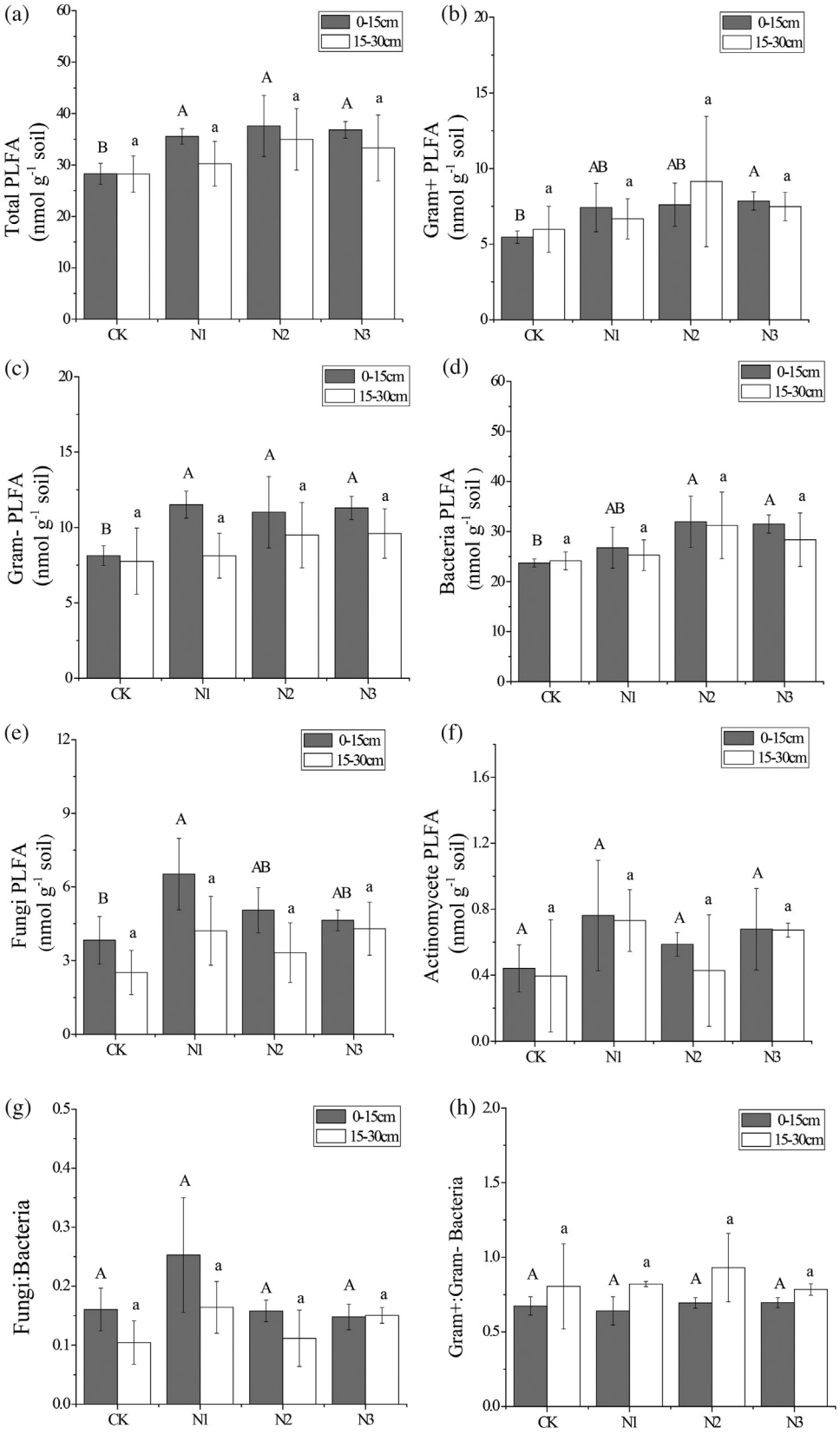

Fig. 7. Changes in soil phospholipid fatty acids (PLFA) (a) total, (b) Gram+, (c) Gram−, (d) bacteria, (e) fungi, (f) actinomycetes, (g) fungi:bacteria ratio, and (h) Gram+:Gram− bacteria ratio at 0–15 cm and 15–30 cm depths after two years of N addition. CK, N1, N2, and N3 represent applications of 0, 6, 12, and 24 g N m^{-2} $year^{-1}$, respectively, applied monthly over the entire growing season. Values are the means ± SE of each treatment (n = 3). Bars with different upper- and lower-case letters are significantly different ($P < 0.05$) in the 0–15 cm and 15–30 cm layers soil, respectively.

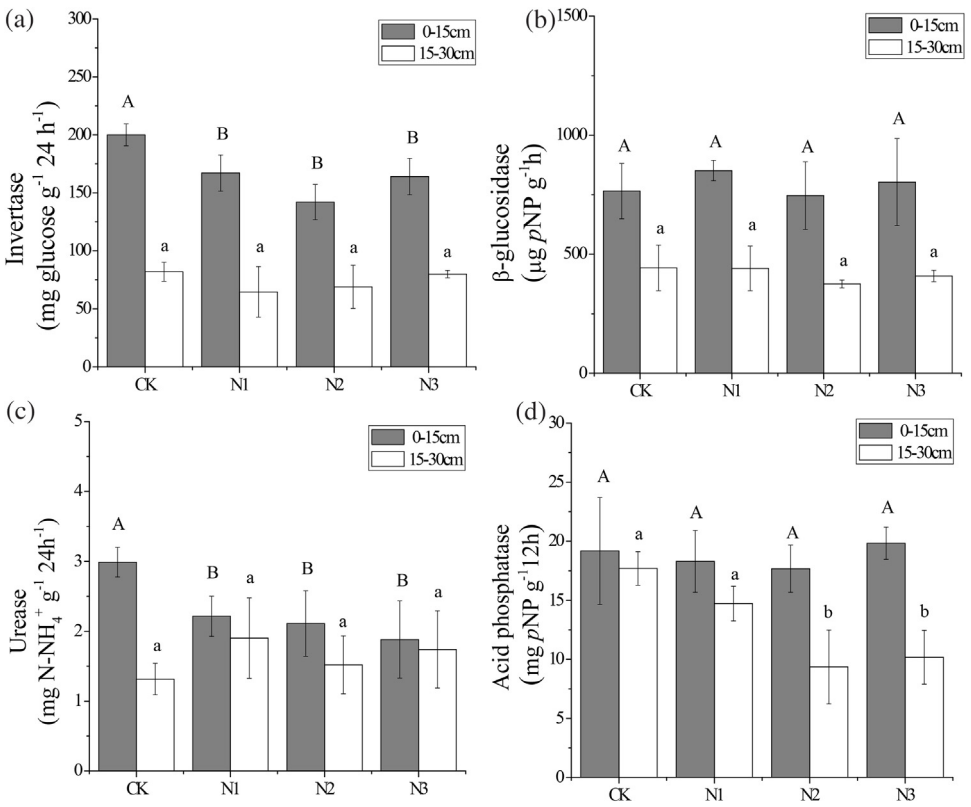

Fig. 8. Changes in soil (a) β-glucosidase, (b) invertase, (c) urease and (d) acid phosphatase activities in the 0–15 cm and 15–30 cm layers after two years of N addition. CK, N1, N2, and N3 represent applications of 0, 6, 12, and 24 g N m^{-2} $year^{-1}$, respectively, applied monthly over the entire growing season. Values are the means ± SE of each treatment (n = 3). Bars with different upper- and lower-case letters are significantly different ($P < 0.05$) in the 0–15 cm and 15–30 cm layers soil, respectively.

4.3. Soil available N and TN

Ehanced N deposition has become increasingly recognized as an important factor that alters N cycling in terrestrial ecosystems, especially for soil N transformations (mineralization, nitrification, gaseous emissions, and leaching) (Moldan and Wright, 2011; Gao et al., 2014). N additions increased the amount of available N. NH_4^+–N increased in both soil layers but NO_3^-–N only increased with the addition of 24 g N m^{-2} y^{-1}, and only in the shallow soil, possible due to the more aerobic nature of shallow soil environment. Many studies have shown that exogenous N input will stimulate microbe-mediated N immobilization and transformation (Fang et al., 2004; Gao et al., 2013), enhance net mineralization (Vestgarden et al., 2003), and nitrification (Moldan and Wright, 2011). Our result showed that soil TN content in N3 addition plots was significantly higher than those in controls. Similarly, Zhang et al. (2013) also found accumulation effect of N additions on soil TN. Given that N is the primary growth-limiting nutrient in many peatland ecosystems, increasing pools of available N and TN likely will stimulate vegetation growth.

4.4. Soil PLFA

Diverse soil microbial communities contribute to the decomposition of organic matter and the breakdown of organic molecules (Li et al., 2014). Bacteria and fungi are the main constituents of soil microbial biomass and play important roles in C and nutrient cycling (Bååth and Anderson, 2003; Deacon et al., 2006). Two years of N additions led to significant increases of bacterial PLFA in the shallow soil. However, only the N1 treatment significantly increased fungi PLFA in the shallow soil, suggesting that bacteria were more sensitive than fungi to N additions because bacteria have higher nutrient requirements and metabolic activities (Alster et al., 2013). Lee et al. (2015) also reported that bacteria groups were more easily affected by N additions, while fungi groups were resistant to them. Gram+ and Gram− bacteria use older C and fresh plant material, respectively, as substrates (Börjesson et al., 2012). We observed a significant increase of total PLFA, bacterial Gram+, and Gram− PLFA in the shallow soil, implying that SOM decomposition will be stimulated by N deposition. Similar effects were also found by Liu et al. (2015).

This study found contrasting responses of total PLFA and MBC to N additions. Such differences may be because PLFA and MBC analytical methods measure different components of the microbial community (Bardgett et al., 1999). Soil MBC is determined from the flush of C that is rendered water-soluble by fumigation with chloroform, whereas PLFA measures the amount of phospholipids in intact microbial membranes, and so measures active microbial biomass (Calderón et al., 2001). The two-year field experiment only reflected the short-term responses of soil microorganisms to N additions in peatland soils. Whether the microbial soil communities respond differently to long term N additions also needs to be investigated.

4.5. Soil enzyme activity

N deposition can alter the chemistry of organic matter and affect decomposition rates by changing the expression of key microbial enzymes (Grandy et al., 2008). In our study, the reduction of invertase activity was accompanied by a decrease of soil MBC in the topsoil, indicating that this enzyme is closely related to soil microbial biomass. Invertase plays a critical role in releasing low molecular weight sugars that are important as energy sources for microorganisms (Zhou et al., 2012). Beta-glucosidase activity

in soil was not altered by the N additions, which suggests that other factors, for example ecosystem productivity and SOM content influence activity of this enzyme. Other studies have found variable effects of N additions on soil β-glucosidase activity. For example, Ochoa-Hueso et al. (2013) observed that N addition was negatively related to soil β-glucosidase activity by decreasing the biomass of the main producers of β-glucosidase in soil (Zheng et al., 2015). However, Saiya-Cork et al. (2002) and Kim and Kang (2011) found that β-glucosidase was elevated with N additions. The mechanism underlying this response may be that N additions alleviate N limitation (Kim and Kang, 2011).

Urease catalyzes the hydrolysis of urea to carbon dioxide and ammonia, and plays an important role in N cycling. N additions may have negatively affected urease activity in shallow soil because microbial production of urease in soils can be repressed through end product inhibition or through byproducts formed from the microbial assimilation of excessive N (Saha et al., 2008). Ajwa et al. (1999) also found that N fertilization suppresses soil urease activity. In contrast, Saiya-Cork et al. (2002) and Hu et al. (2010) reported that N additions did not suppress soil urease activity because soil N availability was not increased enough to reduce the production of N-degrading enzymes by soil microbes.

Acid phosphatase, which hydrolyzes phosphate from phospholipids and phosphosaccharides, can be influenced by N additions (Saiya-Cork et al., 2002; Zhou et al., 2012). In this study, high levels of N additions decreased soil acid phosphatase activity in the deep soil, indicating that N deposition may inhibit soil P mineralization. Similar to our findings, Kang and Lee (2005) reported that N additions could reduce acid phosphatase activity by altering microbial allocation to enzyme production or through shifts in the abundance of soil microbes that produce specific enzymes (Weand et al., 2010). Also, decreased soil P content under N additions because of plant uptake may lead to a reduction of acid phosphatase activity in the deep soil. In contrast, Li et al. (2014) found that acid phosphatase activity increased with N additions. The effects of N amendments on phosphatase activity may vary depending on the rate of N applied, the form in which N is applied, and the type of ecosystem receiving the N (Hopkins et al., 2008; Zhou et al., 2012).

5. Conclusions

In conclusion, we found that two years of N additions to a peatland in the Da Xing'an Mountains in northeast China increased plant litter N and P content, and invertase and β-glucosidase activity, and decreased litter C:N and C:P ratios significantly. These changes in litter quality should result in faster decomposition rates and stimulate nutrient release from litter to the soil. The highest N addition treatment increased NH_4^+–N, NO_3^-–N, and TN in shallow soil (0–15 cm depth), and also increased topsoil total PLFA, Gram+ PLFA, Gram− PLFA, and bacterial PLFA. In line with our initial hypothesis, our study found significant negative effects of high levels of N additions on MBC and DOC pools, urease and invertase activity in the shallow soil and acid phosphatase activity in the deep soil (15–30 cm). Therefore, increased N deposition can modify key processes associated with carbon and nutrient cycling in peatlands. In addition our findings indicate that urease and invertase activity would be useful tools for assessing soil labile C stocks. Longer-term experimental studies are critical to better understand the response of peatlands to increased chronic atmospheric N deposition.

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