Weakening of the 'enzymatic latch' mechanism following long-term fertilization in a minerotrophic peatland

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ABSTRACT

Despite the global importance of understanding the effects of nitrogen (N) and phosphorus (P) deposition on carbon (C) cycling in northern peatlands, the control of N and P interactions through the 'enzymatic latch' mechanism has been largely overlooked. A long-term fertilization experiment in a moderate-rich fen in northeast China was conducted to evaluate the underlying mechanisms of how continuous N and P addition regulate key extracellular enzymes and their interactions, and the subsequent influences on organic C storage in peatlands. The results demonstrated that the growth of *Sphagnum* moss and vascular plants were both reduced by N addition but enhanced by P addition. Phenolic concentrations were higher in P addition treatments, as were phenol oxidase activities. In general, N addition played a critical role in regulating the stoichiometry of β-D-glucosidase, N-acetyl-β-glucosaminidase and phosphatase, while P addition was more important in regulating their activities. The direct and indirect effects due to fertilization and shifts in vegetational composition, respectively, weakened the 'enzymatic latch' mechanism that controls the decomposition of organic matter after long-term fertilization. Our results indicate that P likely plays a more important role than N in controlling microbial extracellular enzymatic activities and organic matter decomposition in northern minerotrophic peatlands. Consequently, the interactions between N and P is likely of primary significance in regulating the biogeochemical cycling of peatlands.

1. Introduction

Northern peatlands are important carbon (C) sinks that store one-third of the global soil C (500 ± 100 Gt), but only comprise approximately 3% of the total land surface (Gorham, 1991; Yu et al., 2010). Moreover, more C is stored in *Sphagnum* moss and litter than is fixed by all terrestrial global plants every year (Clymo and Hayward, 1982; Page and Baird, 2016). In China, peatlands are primarily located in the northeast and southwest, comprise an area of ~10,441 km², and store ~1.39 Gt organic carbon (Ma, 2013). The harsh conditions associated with northern peatlands (i.e., low nutrients, cold temperatures, and acidic and waterlogged soils) greatly limit microbial activity and

decrease organic matter decomposition rates (Moore and Basiliko, 2006; Rydin and Jeglum, 2013).

Nitrogen (N) and phosphorus (P) are critical nutrients in peatlands and have profound effects on plant production, litter decomposition, and biogeochemical cycles in these environments (Limpens et al., 2006; Walbridge and Navaratnnam, 2006; Rydin and Jeglum, 2013). N and P are also important nutrients for microorganisms and are involved in many microbial biochemical processes (Sinsabaugh and Moorhead, 1994). Atmospheric reactive N deposition has increased by 59% from 12.64 kg N ha^{-1} yr^{-1} in the 1960s to 20.07 kg N ha^{-1} yr^{-1} in the past decade, due to increased human activities and massive resource consumption (Lü and Tian, 2014). The northern peatlands also encounter

high rates of N deposition, which affect plant productivity, organic matter decomposition, and weaken their C sequestration capacities (Bragazza et al., 2012; Larmola et al., 2013; Wang et al., 2015b). The natural and anthropogenic emission of P increased globally from 2.6 to 4.0 Tg P yr^{-1} between 1850 and 2013 (Wang et al., 2017). P addition may relieve P limitation, while stimulating biological N fixation (Benner and Vitousek, 2007), although it may not alleviate physiological stresses on *Sphagnum* mosses due to excessive N addition (Fritz et al., 2012). It is well known that interactions between N and P play key roles in regulating ecological processes and functions in a wide range of ecosystems (e.g., Elser et al., 2000; Zechmeister-Boltenstern et al., 2015). However, few studies have investigated the effects of long-term N and P inputs on the ecological processes and functions in peatlands (Bubier et al., 2011; Song et al., 2011; Larmola et al., 2013; Keuskamp et al., 2015). Such studies would provide a better understanding of the response and feedback mechanisms of N and P inputs into northern peatlands. In addition, changes in plant biomass and vegetation structure due to long-term nutrient input should be an important consideration underlying ecological function in peatlands (Bobbink et al., 2010).

Soil enzymes are the principal media by which soil microorganisms participate in soil biogeochemical processes including organic matter decomposition and nutrient cycling, and they are sensitive to soil nutrient characteristics (Kuijper et al., 2012). The 'enzymatic latch' is a well-known mechanism positing that the low phenol oxidase (POX) activity within peatlands leads to the build-up of phenolics ([PHEN]), which then inhibits the activity of hydrolytic enzymes, and thereby facilitates the accumulation of organic matter (Freeman et al., 2001, 2004, 2012). Phenolics inhibit decomposition by binding to the reactive sites of hydrolytic enzymes (Brouns et al., 2014). Importantly, POX partially oxidize phenolics into simple organic compounds (Duran et al., 2002; Freeman et al., 2004). Due to their importance in peatland nutrient cycling, POX activity and the concentration of soluble phenolics within peatlands have been investigated in recent years in the context of warming climates (Jassey et al., 2012) and long-term fertilization (Pinsonneault et al., 2016a). However, the effect of long-term fertilization on POX, and especially the interaction of N and P, is still unclear. For example, Bragazza et al. (2006) observed that POX activity increased nearly three-fold when the external N input was increased from 0.2 to 2 g N m^{-2} yr^{-1}. However, Pinsonneault et al. (2016a) suggested that POX activity could be significantly reduced by long-term N additions. Nevertheless, most studies have not accounted for the role of P in regulating POX activity.

β-D-glucosidase (BDG), N-acetyl-β-glucosaminidase (NAG), and phosphatase (PHO) directly constrain the decomposition of organic C, and changes in their activities are reliable indicators of organic C decomposition in peatlands (Freeman et al., 1995; Shackle et al., 2000; Kuijper et al., 2012). BDG is an enzyme used to acquire C via the hydrolysis of cellulose to glucose, which is a labile C resource that supports microbial metabolisms (Kuijper et al., 2012; Dunn et al., 2014). Increases in external nutrient inputs result in the release of greater BDG to meet C demands (Sinsbaugh and Moorhead, 1994; Dunn et al., 2014). POX activity may be influenced by fertilization and can also affect the activity of BDG (Freeman et al., 2004). Evolutionary-economic principles (Allison et al., 2010) assert that microorganisms produce enzymes due to the balance between resource supply and demand. Consequently, NAG, the primary enzyme for N acquisition via chitin decomposition (Kang et al., 2005), may exhibit increased activity due to the addition of P. Similarly, N additions can also lead to increases in the activity of PHO, which releases phosphate by

catalyzing the breakdown of phosphate monoesters (Dunn et al., 2014). Therefore, the stoichiometric trade-off between N and P (i.e., N and P interactions) may be the primary factor controlling the above-mentioned biochemical processes in peatlands.

In this study, the effects of N and P addition on the 'enzymatic latch' mechanism were evaluated in a 10-year N and P fertilization experiment in the Hani peatland in the Changbai Mountains (Bu et al., 2011). To the best of our knowledge, no investigation has evaluated the effects of N and P interactions on the regulation of soil enzymes involved in organic C cycling via the 'enzymatic latch' mechanism. We hypothesized that (1) sufficient nutrient supply would enhance POX activity, therefore decreasing [PHEN]; (2) the demand for P by microorganisms would increase in the long-term N fertilized plots due to the possible shift towards P limitation; (3) microorganisms would release more NAG to enhance the acquisition of N and stoichiometrically balance N and P as P deficiency subsided after P application; (4) hydrolase activities would increase owing to lower [PHEN], thereby accelerating soil organic matter decomposition.

2. Materials and methods

2.1. Study sites

The study was conducted at the Hani Peatland (42°13′5″N, 126°31′05″E) in the Changbai Mountains in northeast China, which comprises an area of ~16.78 km². This area exhibits a continental monsoon climate within a cold temperate region, with a mean annual precipitation of 757–930 mm and a mean annual air temperature of 2.5–3.6 °C (Bu et al., 2011). The peat in the area averages ~4 m in thickness with a maximum depth of 9.6 m (Qiao, 1993). Hani Peatland is dominated by shrubs, including *Betula fruticose* Pall. Var. *ruprechtiana* Trautv., *Vaccinium uliginosum* L., and *Rhododendron tomentosum* Harmaja, in addition to herbaceous plants, including *Eriophorum polystachion* L., *Carex lasiocarpa* Ehrh., and *Smilacina japonica* A. Gray. The underlying moss layer is dominated by *Sphagnum magellanicum* Bird. and *Sphagnum palustre* L. (Bu et al., 2017). Atmospheric N deposition in northeast China is about 1.4 g N m^{-2} yr^{-1}, which is close to the national average of 1.3 g N m^{-2} yr^{-1} (Lü and Tian, 2007), but significantly higher than in other global areas at a similar latitude (<0.6 g N m^{-2} yr^{-1}) (Bobbink et al., 2010).

2.2. Experimental design and field sampling

A long-term N and P fertilization experiment was initiated in August 2007. Plots of *Sphagnum* hummocks (0.8 m × 0.8 m) were established in the open areas dominated by *Sphagnum palustre*. The levels of fertilizer application were estimated based on the specific characteristics of the Hani Peatland, as described in previous studies (Güsewell et al., 2003; Gunnarsson and Rydin, 2000) (Table 1). A factorial design was used with three levels of N addition (0, 5, and 10 g N m^{-2} yr^{-1}; i.e., 0N, 5N, 10N) and three levels of P addition (0, 0.5, and 1 g P m^{-2} yr^{-1}; i.e., 0P, 0.5P, 1P). There were four replicates for each treatment, totaling 36 experimental plots. N was applied as ammonium nitrate (NH_4NO_3) and P as sodium dihydrogen phosphate ($NaH_2PO_4·2H_2O$) in 300 mL of distilled water. Fertilizers were added once a month during the growing season with three additions in 2007 and five additions from May to September thereafter. The control plots were supplied with only 300 mL of distilled water.

Samples were collected 10 days after the latest fertilization when no precipitation had occurred over the period in order to avoid

Table 1
Design of the nitrogen (N) and phosphorus (P) fertilization experiment.

Treatment	0N0P	5N0P	10N0P	0N0.5P	5N0.5P	10N0.5P	0N1P	5N1P	10N1P
N (g m^{-2} yr^{-1})	0	5	10	0	5	10	0	5	10
P (g m^{-2} yr^{-1})	0	0	0	0.5	0.5	0.5	1	1	1

confounding effects from the increase of nutrients due to nutrient leaching caused by heavy rains. To minimize destructive sampling, peat samples were collected in small patches over the entire plot to form a composite sample for each plot (~20 g). Living mosses were removed from samples, and the remainder of the sample was placed in a sterile plastic bag and transported back to the laboratory on ice. In the laboratory, clean scissors were used to cut samples and remove coarse roots and twigs. Peats were then homogenized, subsampled, and stored at 4 °C prior to analyses within seven days.

2.3. Physicochemical properties

The peat subsamples were oven dried at 65 °C to a constant weight, and their moisture content was then measured. Dried peat was ground with a ball mill (MM400, RETSCH, Germany) for further chemical analyses. Total nitrogen (TN) and total carbon (TC) were determined by dry combustion on an elemental analyzer (vario MACRO cube, elementar, Germany). Total organic carbon (TOC) was measured on a TOC analyzer (TOC-L, SHIMADZU, Japan). To determine total phosphorus (TP), samples were digested in concentrated sulphuric acid and hydrogen peroxide with selenium and lithium sulphate as catalysts (Parkinson and Allen, 1975). TP was then determined colorimetrically using the ammonium molybdate-ascorbic acid method (Murphy and Riley, 1962) on a continuous flow analyzer (San + +, Skalar Analytical B.V., Breda, The Netherlands). The data of *Sphagnum* increment and vascular plant importance values were used as described in Bu et al. (2011) and Liu et al. (2015).

2.4. Enzymatic activities and phenolic abundances

The measurement of enzymatic activities and phenolics concentrations were adopted from Dunn et al. (2014) and repeated in triplicate. POX activity was measured using the quantitative colorimetric method with L-3,4-dihydroxy phenylalanine (L-DOPA) on a full-wavelength microplate reader (MULTISKAN GO, THERMO FISHER, Finland) and measurement at 475 nm. Absorbances were converted into activities (μmol diqc g^{-1} min^{-1}; where diqc refers to 2,3-dihydroindole-5,6-quinone-2-carboxylate) using the formula: ((substrate value – blank value) $\times$ 10 $\times$ 1000 $\times$ 2)/εb/dry weight of 1 g fresh peat/reaction time, where ε is the molar absorption coefficient for phenol oxidase and b is the path length.

[PHEN] was measured on a full-wavelength microplate reader (MULTISKAN GO, THERMO FISHER, Finland) using an improved Folim-Ciocalteu method modified from Box (1983). Specifically, phenol was used to prepare the standard over a range of 0–45 mg/L. For [PHEN] measurements, 1 g of peat was placed in a centrifuge tube with a 0.45 μm filter insert (Costar spin-X, Cole-Parmer) and centrifuged at 10,000 RPM for 5 min. The mixture (250 μL filtrate + 12.5 μL Folin's reagent + 37.5 μL Na_2CO_3 solution) was allowed to incubate in a 96 well clear microplate for 1.5 h. Subsequently, the absorbance was

measured at 750 nm and then converted into a concentration value.

Methods for measuring BDG, NAG, and PHO activities were adopted from Dunn et al. (2014), using microplate-based fluorescence analysis. A 4-methylumbelliferone (MUF) sodium salt was used as the standard. For measurements of each of the enzymes, 1 g of fresh peat was placed in a stomacher bag with 7 mL of substrate labeled with 4-MUF. The stomacher bags were then incubated at field temperatures for 45 min for PHO or 60 min for the other enzymes. The fluorescence of the supernatant was measured at an excitation wavelength of 330 nm and an emission wavelength of 450 nm on a multi-function microplate reader (M200PRO, TECAN, Switzerland).

2.5. Statistical analyses

Data normality and the homogeneity of variance were tested with Shapiro-Wilk tests and Levene's tests, respectively. General linear models with two-way ANOVAs were used to examine the fertilization effect on soil physicochemical properties and enzymatic activities using N, P, and their interaction as fixed factors in the SPSS 23.0 software package. A post-hoc multiple comparison was conducted using Tukey's test at the significance level of $P < 0.05$. If the interaction between N and P was not significant, different levels of N and P were grouped to only assess the main effect. The relationships between soil physicochemical properties and/or enzymatic activities were examined by ordinary least squares (OLS) and standard major axis (SMA) regressions using the *lmodel2* package (Legendre, 2018) for R 3.5.1 (R Core Team, 2018).

3. Results

3.1. Nutrient and plant community characterization

The effects of N and P interactions on peat physicochemical properties were generally significant ($P < 0.05$). For example, under the same level of P application, increasing N addition increased TN ($P < 0.001$) and TP concentrations ($P = 0.034$; Table 2). Under the same level of N, increasing P additions increased TP. In contrast, P increased TN only in the 10N treatment group. TC and TOC concentrations did not change significantly, while N and P interacted to affect soil TOC ($P = 0.047$). TOC decreased with increasing N additions in N-only treatments. At the highest level of N application (i.e., 10N), P additions increased TOC concentrations by 10.5%.

N and P did not exhibit interactive effects with regards to nutrient stoichiometry. N additions significantly decreased TC:TN and TOC:TN ratios ($P < 0.001$; Fig. 1a, c). Further, P additions significantly decreased TC:TP and TOC:TP ratios ($P < 0.001$; Fig. 1b, d). Ratios of TC:TN, TOC:TN, TC:TP, and TOC:TP ranged from 25.6 to 43.4, 24.3–39.8, 414.9–813.6 and 381.6–756.9, respectively. Similarly, N and P did not exhibit significant interactive effects towards vegetation characteristics (Fig. 2). However, the growth of *Sphagnum* mosses and

Table 2
Concentrations of total carbon (TC), total organic carbon (TOC), total nitrogen (TN), and total phosphorus (TP) (mean ± SE, $n = 4$) in peat under different treatments.

Treatment	TC (mg/g dry peat)	TOC (mg/g dry peat)	TN (mg/g dry peat)	TP (mg/g dry peat)
0N0P	411.0 ± 3.8	381.0 ± 3.6[Aa]	9.8 ± 0.7[Ab]	0.5 ± 0.1[Aa]
5N0P	423.7 ± 4.7	394.1 ± 2.0[Aab]	12.8 ± 0.8[Ab]	0.5 ± 0.0[Aa]
10N0P	387.2 ± 4.3	361.7 ± 3.2[Bb]	16.6 ± 0.8 [Ba]	0.6 ± 0.0[Aa]
0N0.5P	412.8 ± 9.9	376.2 ± 10.4[Aa]	10.0 ± 1.1[Aa]	0.8 ± 0.1[Ba]
5N0.5P	425.0 ± 5.3	391.5 ± 2.3[Aa]	11.8 ± 0.8[Aa]	0.7 ± 0.1[Ba]
10N0.5P	393.6 ± 14.9	379.7 ± 11.2[ABa]	12.9 ± 1.5[Ca]	0.8 ± 0.1[Ba]
0N1P	421.2 ± 10.2	387.1 ± 9.0[Aa]	10.0 ± 1.1[Ac]	0.9 ± 0.0[Ba]
5N1P	414.9 ± 7.0	381.0 ± 5.4[Aa]	14.3 ± 0.9[Ab]	1.1 ± 0.0[Cab]
10N1P	431.3 ± 8.1	399.1 ± 6.8[Aa]	20.5 ± 0.8[Aa]	1.2 ± 0.0[Cb]

Different lowercase letters represent significant differences among N treatments with the same P addition level. Different uppercase letters represent significant differences among P treatments with the same N addition level.
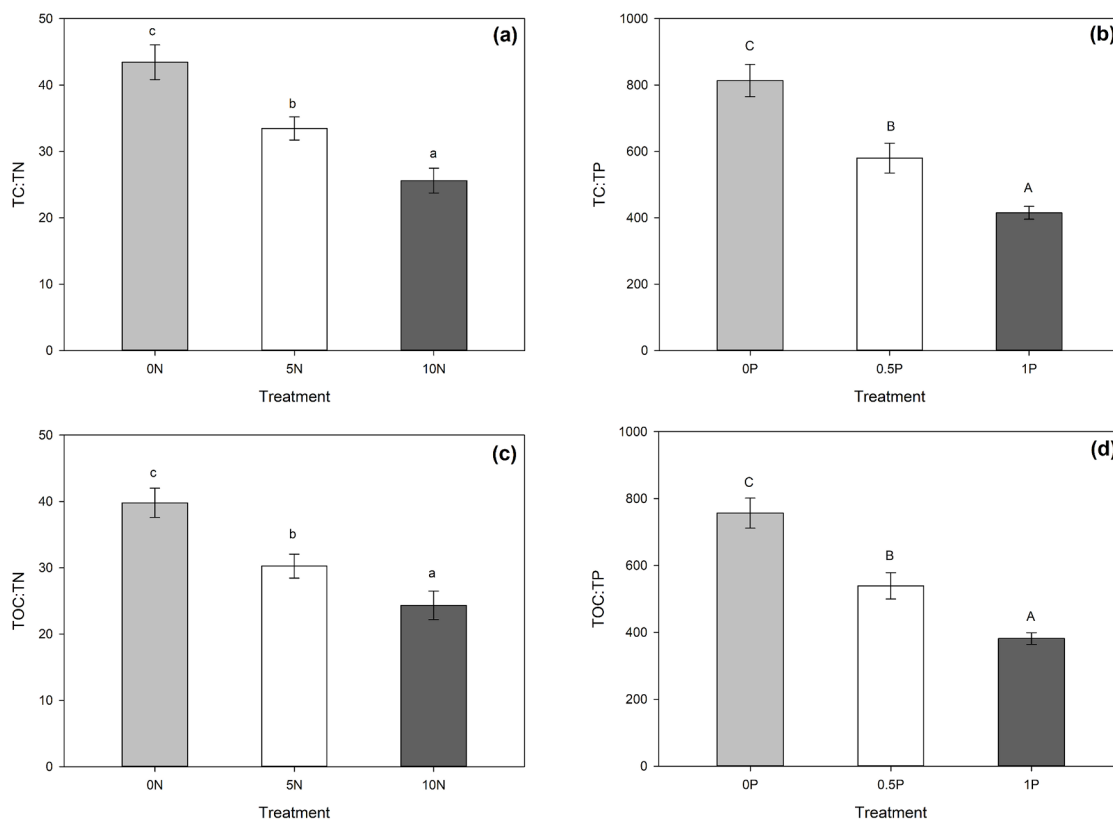
Fig. 1. Stoichiometric ratios of total carbon (TC) to total nitrogen (TN) (a) and total phosphorus (TP) (b), in addition to total organic carbon (TOC) to TN (c) and TP (d) under different treatments. Error bars indicate standard errors. Different lowercase letters represent significant differences among N treatments at the same P addition level. Different uppercase letters represent significant differences among P treatments at the same N addition level ($n = 4$). Treatment abbreviations are described in Table 1.

the importance value of shrubs decreased in the 10N treatments (Fig. 2a). P addition increased plant growth, with the exception of the importance value of evergreen shrubs in the 1P treatments (Fig. 2b).

3.2. Effects of fertilization on microbial enzymatic activity

Long-term fertilization significantly affected hydrolytic and oxidative enzyme activities in addition to [PHEN] in peat (Fig. 3). The effect of N addition on POX activity depended on P addition levels. Specifically, N increased POX activity under N-only treatments, but decreased POX activity with moderate (i.e., 0.5P) or high levels (i.e., 1P) of P addition (Fig. 3a). P addition increased POX activity, which was highest when TP concentrations were 0.90 mg/g in peat (Fig. 4a). Two-way

ANOVAs indicated that changes in [PHEN] concentrations were marginally significant in response to N additions ($P = 0.054$) without significant N and P interaction ($P = 0.423$). [PHEN] significantly increased at high levels of P addition (Fig. 3b) and exhibited a positive linear relationship with peat TP concentrations ($P < 0.05$; Fig. 4b).

Nitrogen addition significantly increased BDG activity under N-only treatments ($P < 0.05$), whereas the increases in BDG activity were diminished when P was added (Fig. 3c). Moderate (i.e., 5N) or high levels (i.e., 10N) of N addition resulted in decreasing BDG activity due to P addition. Similarly, N addition significantly increased NAG activity under N-only treatments, but could decrease NAG activity under moderate or high levels of P addition (Fig. 3d). However, P addition generally resulted in decreased NAG activity. A strong positive
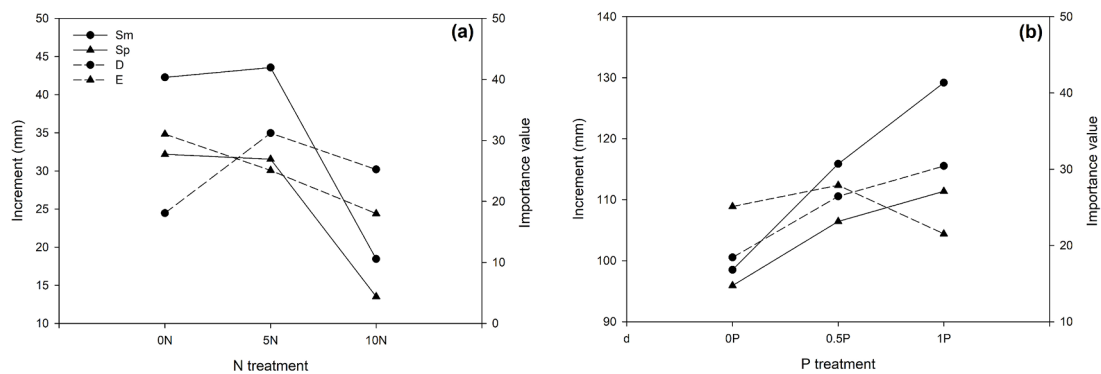

Fig. 2. Effects of nitrogen (N) (a) and phosphorus (P) (b) addition on the increment of *Sphagnum magellanicum* (Sm) and *S. palustre* (Sp), and the importance of deciduous (D) and evergreen shrubs (E). The importance value indicates the relative importance of plant species in the community where importance value = relative coverage + relative density + relative frequency. Data are derived from Bu et al. (2011) and Liu et al. (2015). Treatment abbreviations are described in Table 1.
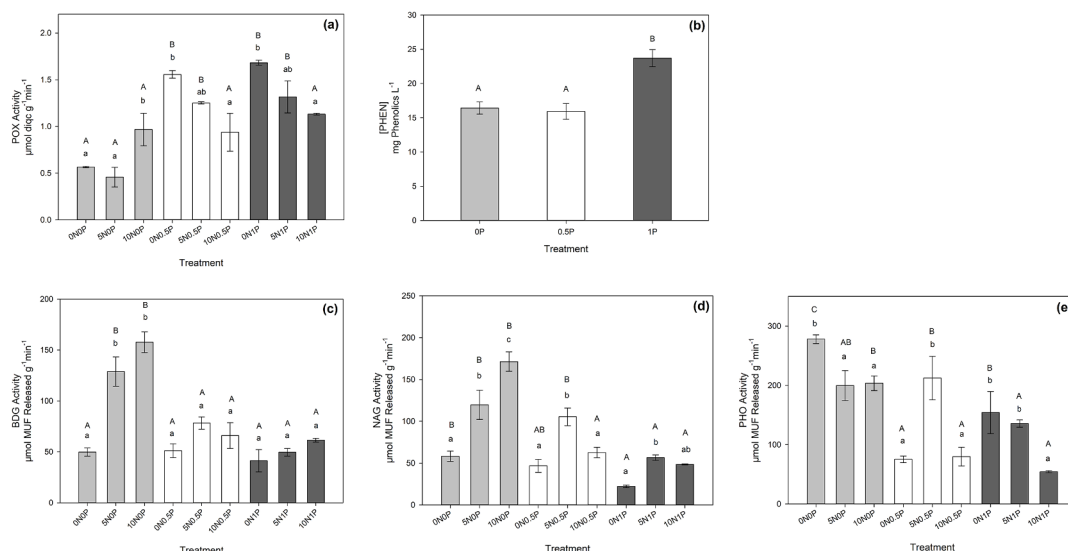
Fig. 3. Activities of phenol oxidase (POX; a) and concentration of phenolics ([PHEN]; b), in addition to the activities of β-D-glucosidase (BDG; c), N-acetyl-β-glucosaminidase (NAG; d), and phosphatase (PHO; e) under different nitrogen (N) and phosphorus (P) fertilization treatments. Error bars indicate standard errors. Different lowercase letters represent significant differences among N treatments at the same P addition levels. Different uppercase letters represent significant differences among P treatments at the same N addition levels ($n = 4$). Treatment abbreviations are described in Table 1.
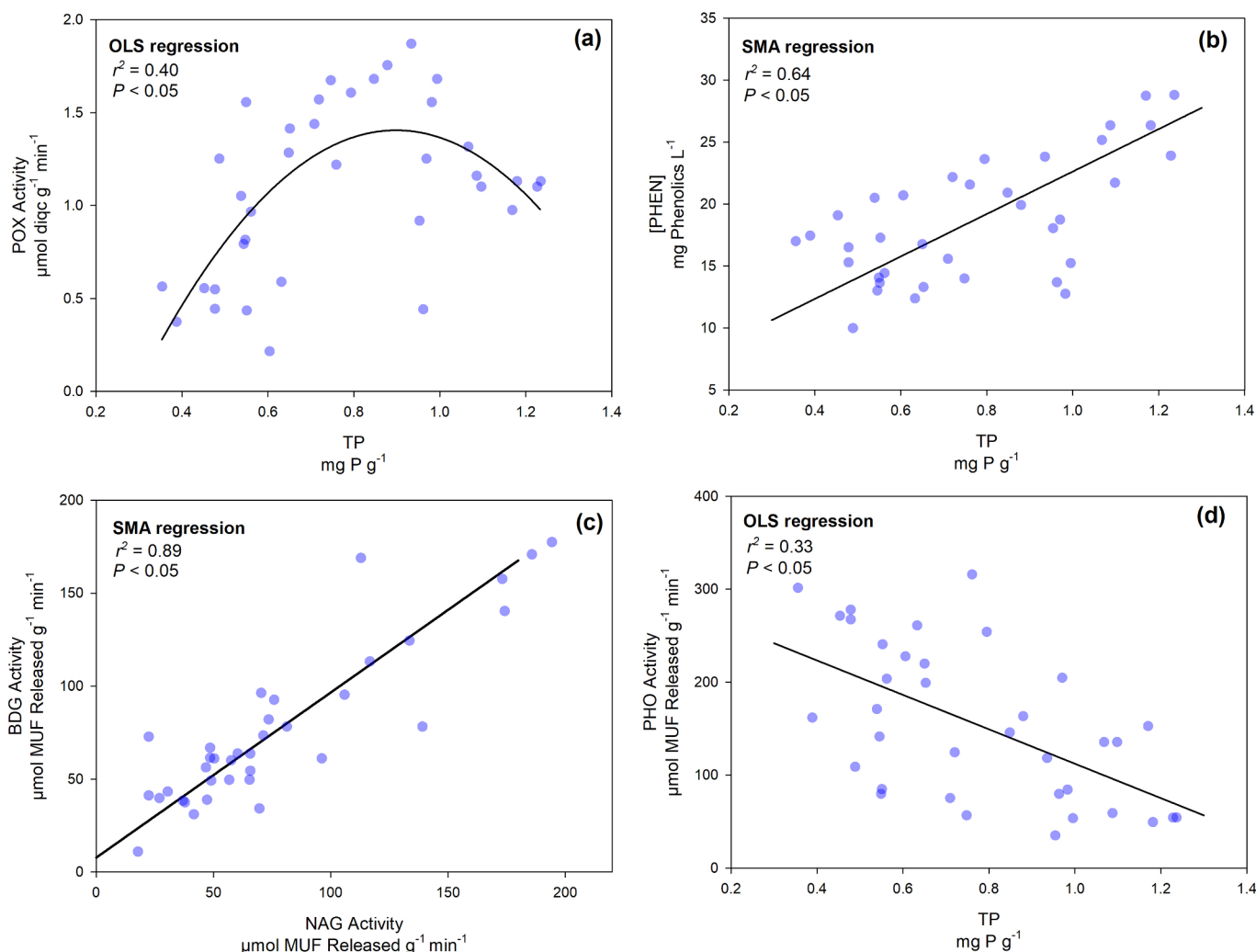

Fig. 4. Regressions between nutrient concentrations and enzymatic activities ($n = 36$). **(a)** $y = -3.79x^2 + 6.82x - 1.66$. **(b)** $y = 17.13x + 5.5$. **(c)** $y = 0.89x + 7.79$. **(d)** $y = -185.15x + 297.41$.
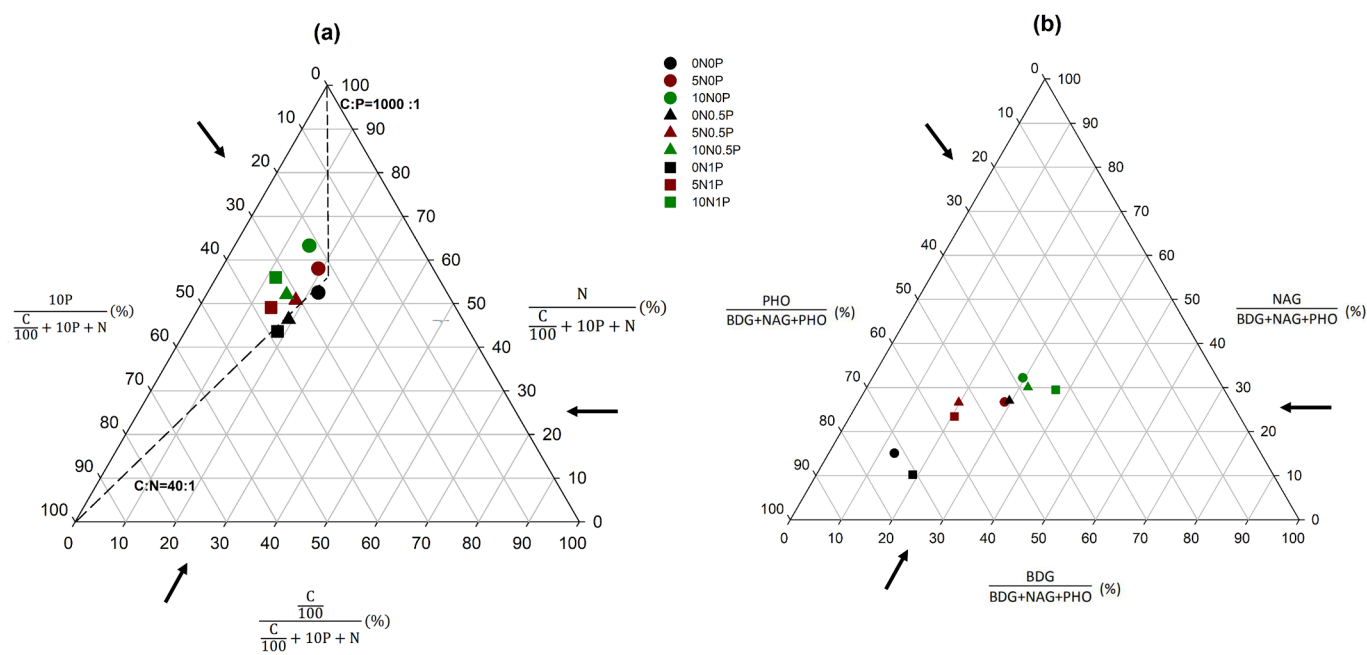
Fig. 5. Ternary diagrams showing carbon (C), nitrogen (N), and phosphorus (P) concentrations (a), and β-D-glucosidase (BDG), N-acetyl-β-glucosamini-dase (NAG) and phosphatase (PHO) activities (b) in peat samples. Dashed lines indicate the critical ratios of C:N (40) and C:P (1,000) as indicated by Wang et al. (2015b). Treatment abbreviations are described in Table 1.

correlation between BDG and NAG activity was observed via regression analysis ($r^2 = 0.89$, $P < 0.05$; Fig. 4c). N additions resulted in decreased PHO activity, except under moderate P addition treatment, whereas P addition decreased PHO activity ($P < 0.05$; Fig. 3e). Lastly, PHO activity was negatively correlated with TP concentrations (Fig. 4d).

The responses of the stoichiometric ratios between C, N, and P to fertilization with N and P were stronger than those of their corresponding nutrient acquiring enzymes (Fig. 5). Specifically, peat C:N:P stoichiometric ratios varied within a relatively small range in response to long-term N and P input. (Fig. 5 a). The distinct ratios of hydrolase activities were mainly driven by different levels of N addition, with the PHO% decreasing sharply from 72% to 34% as N addition levels increased from 0 to 10 g m^{-2} yr^{-1} (Fig. 5b).

4. Discussion

The effects of long-term fertilization on the enzymatic activities within the Hani peatland are complex. Long-term N and P inputs can directly affect 'enzymatic latch' mechanisms and plant growth, and the latter can subsequently indirectly affect enzymatic interactions, thereby regulating organic matter decomposition. To the best of our knowledge, the present study is the first to assess how the interaction of long-term N and P addition affects the interactions of key oxidase and hydrolase activities in northern minerotrophic peatlands.

4.1. Phenol oxidase and phenolics

Changes in plant growth, community composition, and the lignin content of litters are the primary controls underlying the differences in POX activity and [PHEN] among fertilization experiments (Knorr et al., 2005; Jassey et al., 2012). POX activity is very sensitive to changes in lignin content, where higher lignin content of peat generally leads to higher POX activities (Boot et al., 2016). In our study, POX activity increased by 63.1% after the addition of 10 g m^{-2} yr^{-1} of N. These results are consistent with those of Guo et al. (2010), wherein POX activity increased by about 60% after six months of N application (0.6 g N m^{-2} month^{-1}). The growth of vascular plants in our study was

subsequently enhanced when P was added alone or with N (Fig. 2b). The lignin content of vascular plants is much higher than that of *Sphagnum* mosses, and thus the greater input of vascular plant litter due to enhanced growth may account for the reduction in POX activity (Hall et al., 2014; Wang et al., 2015a).

In partial support of our first hypothesis, increased nutrient supply increased POX activity. However, [PHEN] exhibited an opposite response that is probably due to the proliferation of vascular plants after long-term fertilization. Specifically, *Sphagnum* mosses typically comprise a polyphenol network that contains little lignin (van Breemen, 1995) and their growth decreased with N addition but increased with P addition. Increasing cover of vascular plants also results in numerous water-soluble phenolic compounds after P addition (Wang et al., 2015a). Accordingly, [PHEN] increased dramatically when P was added alone or with N, which may result in the preferential recruitment of polyphenol decomposers within peat (Fog, 2010). After the long-term application of high levels of P (i.e., 1 gP m^{-2} yr^{-1}), the enhanced growth of *Sphagnum* mosses and vascular plants have likely resulted in the large amount of phenolic compounds in peat that far exceed the oxidation capacity of microorganisms, thereby leading to the significant increases of [PHEN].

4.2. Responses of hydrolase activities to fertilization

Nitrogen additions have been shown to weaken the carbon sequestration capacity of peatlands (Bragazza et al., 2006, 2012; Limpens et al., 2011; Larmola et al., 2013). Specifically, N additions decrease peat C:N ratios and consequently enhance organic matter mineralization (Fellman et al., 2008; Pinsonneault et al., 2016b). Meanwhile, N is an indispensable nutrient that microorganisms require to produce various biomolecules including enzymes. Accordingly, the synthesis of BDG and NAG was promoted after N was added by itself in our experiments, which is consistent with the results of Dong et al. (2015). However, PHO activity declined after N addition, contrasting with our second hypothesis. Because plant growth was inhibited by N addition (Fig. 2a), diminished P acquisition may explain the decline in PHO activity in the N-only treatments. Moreover, P may play a more important role than N in nutrient cycling and organic matter decomposition in peatlands (e.g., Wang et al., 2015b). P addition attenuated

the activities of BDG, NAG, and PHO, in contradiction to our third hypothesis. The 'enzymatic latch' principle hypothesizes that increased [PHEN] upon high levels of P addition would inhibit hydrolase activities. However, when P was added, the effects of N on hydrolase activities became complex and dependent on the level of P addition. These results indicate that P addition can regulate the effects of N supplementation on peatland nutrient cycling.

Soil microorganisms are expected to follow evolutionary-economic principles when synthesizing extracellular enzymes (Allison et al., 2010). For example, increased P input leads to less demand for P scavenging to support microbial metabolism, thereby leading to the reduction of PHO activity (Güsewell and Freeman, 2005; Knorr et al., 2005; Currey et al., 2009; Burns et al., 2013). However, the applicability of these principles is not always straightforward due to complex biotic and abiotic controls, and especially considering their interactions (Currey et al., 2009). For example, NAG activity did not decrease following relief from N limitation (Fig. 3d). Nevertheless, a strong positive correlation was observed between BDG and NAG activity, supporting the hypothesis that the mineralization of C and N is closely coupled (Olander and Vitousek, 2000). Therefore, when evaluating the dynamics of hydrolases, multiple related enzymes should be considered simultaneously (Limpens et al., 2004; Carfrae et al., 2007).

4.3. Nutrient and enzymatic stoichiometry

Nitrogen deposition has long been recognized to cause the gradual replacement of *Sphagnum* mosses by vascular plants (Limpens et al., 2011; Bragazza et al., 2012; Larmola et al., 2013). In contrast, our results suggest that high N application rates inhibit the growth of vascular plants (Fig. 2a). P application significantly promoted the growth of vascular plants, suggesting that P limitation is critical for plant growth at Hani. N application led to the reduction of *Sphagnum* moss biomass, which could lead to P retention in peat soils due to the inhibition of its adsorption and assimilation by plants.

Our results indicate that variation in N affects the stoichiometry of microbial nutrient acquiring enzymes in peat, while variation in P may play a greater role in regulating enzymatic activities (Figs. 3–5). A much smaller C, N, and P acquisition ratio (BDG:NAG:PHO = 1:1:6) was observed here than the global average (1:1:1) (Sinsabaugh et al., 2008), indicating a much stronger P-acquiring potential in Hani peatland. The activities of hydrolases that were significantly affected by long-term fertilization are probably sub-optimal for C:N:P stoichiometry in the Hani peatland. This could be due to the presence of a diversity of microbial N acquiring pathways and the resultant strong correlation between carbon and nitrogen acquisition (Sinsabaugh et al., 2008). The observed inconsistency between C:N:P stoichiometry and the related hydrolases in response to N and P additions may suggest that the 'enzymatic latch' mechanism is weakened in the northern peatlands of China.

4.4. Weakening of the 'enzymatic latch' mechanism

Overall, our results suggest that the applicability of the 'enzymatic latch' mechanism in explaining the influence of external N and P inputs on soil organic carbon dynamics in peatlands is complex and may be limited in northern peatlands. Long-term fertilization conditions may lead to an attenuated influence of the 'enzymatic latch' mechanism, due to the effects arising from shifts in vegetation composition (Bobbink et al., 2010) that are closely coupled to N and P interactions. The subsequent effect of altered litter inputs on peat chemistry due to shifts in vegetational composition may mask the direct effects of N and P inputs on soil enzyme activities. Accordingly, a straightforward negative relationship between [PHEN] and hydrolase activities, as hypothesized by the 'enzymatic latch' mechanism, was not observed (hypothesis 4).

In summary, the effects can be attributed to a number of mechanisms (see graphical abstract). Specifically, long-term nutrient input promotes plant growth and a shift in vegetation composition favoring vascular plants at the expense of *Sphagnum* mosses, thereby resulting in lignin-rich litter that increases POX activity. Concomitantly, the decline in soil C:N ratios after N additions increases the activities of several key enzymes, but weakens the carbon sequestration capacity of the peatlands. Furthermore, the 'enzymatic latch' mechanism becomes less relevant to biogeochemical cycling in these systems and also becomes more complex. A larger abundance of phenolics is directly added to these soils due to the shift in vegetation composition, while long-term P addition regulates the effect of N acquisition and also potentially plays a more important role than N in controlling peatland soil C dynamics. We note that the chemical environment and stoichiometry of peat soils can be directly altered by nutrient additions as well as indirectly affected by shifts in vegetation composition due to long-term fertilization. However, the respective contributions of the direct and indirect mechanisms towards regulating biogeochemical cycling remains unclear. Thus, short-term fertilization experiments are warranted in the future to disentangle the direct and indirect effects of fertilization.

5. Conclusions

In this study, we quantified the nutrient concentrations and enzymatic activities in a minerotrophic peatland in northeast China after 10 years of N and P fertilization. N and P interacted closely to affect the interactions of enzymatic activities, and the stoichiometry of peat. However, P was more important than N in regulating enzymatic activities and their interactions. The effects of N addition on enzymatic activities differed, sometimes drastically, under different P addition treatments. This could lead to significant acceleration of organic matter decomposition in the absence of P addition, indicating that P addition regulates the effects of N additions in peatland biogeochemistry. Our results suggest that long-term fertilization leads to a complex influence of the 'enzymatic latch' mechanism on soil activities that is weakened due to shifts in vegetation composition, nutrient interactions, and likely other competing mechanisms.

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