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Article

Warming Amplified the Effect of Phosphorus Addition on N₂O Emissions in a Peatland

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Abstract: Natural montane peatlands generally are not a significant source of nitrous oxide (N₂O) due to environment limitation including phosphorus (P) scarcity and temperature lowness. Phosphorus enrichment and warming caused by global change are altering this limitation, likely to increase the source function of N₂O. However, the combined effects of P addition and warming on N₂O fluxes and biotic/abiotic factor in peatlands are still uncertain. To address this, we investigated the long-term (12 yrs) effects of P addition (5 and 10 kg ha⁻¹ yr⁻¹) and its interaction with warming on N₂O fluxes in a peatland. The results showed that although long-term P addition did not significantly affect the source/sink function of N₂O in the peatland, it stimulated enzyme activities, promoted peat decomposition. However, warming amplified the effect of P addition to increase N₂O emissions by stimulating enzyme activities and changing soil stoichiometry, even turned the peatland into a significant source of N₂O with an emission of approximate 100 g m⁻² during the growing season. Our study suggests that P enrichment under the current background of global warming will enhance the possibility of strong N₂O emissions in montane peatlands, which may increase the risk to further aggravate global warming.

Keywords: Enzyme activities; Changbai Mountains; Global change

Introduction

1. Introduction

Nitrous oxide (N₂O) is an important greenhouse gas with a warming potential 273 times that of carbon dioxide (CO₂) [1]. Montane peatlands are huge nitrogen (N) pools, but not a significant source of N₂O emissions due to phosphorus (P) limitation and low temperature [2–7]. However, the increase in P deposition and continuous warming caused by global changes will change the current status of peatlands, which may increase the possibility for peatlands to be a hot spot of N₂O emissions [8–10]. Nevertheless, the underlying mechanism about the response of N₂O emissions and biotic/abiotic factors in peatlands to the interaction of P addition and warming is rarely studied yet.

Phosphorus is limited in 43% of the global land soil [11,12], and it is also a key factor for controlling N₂O emissions in peatlands [13]. However, the continuous intensification of human activities and the availability of P increase, resulting in P may no longer being the limiting factor for ecological processes in peatlands [14–17]. Increase of P input will stimulate the activity of nitrifying and denitrifying bacteria, accelerate N turnover in soil, and promote soil N mineralization, which

may produce more N₂O [3,18–21]. Mori's meta-analysis found that the addition of P in a P limited ecosystem will meet the microbial demand for P, leading to an boom of microorganisms [22]. Li's study showed that P addition can stimulate soil enzyme activities, which will promote peat decomposition, accelerate cycling of C and N, thereby providing energy and substrates for nitrifying and denitrifying microorganisms, and promoting N₂O emissions in boreal peatlands [7,23].

In addition to directly affecting microorganisms and enzyme activities, P addition can also desorb available C from soil surface, such as dissolved organic carbon (DOC), providing substrates available for microorganisms, stimulating nitrification and denitrification and promoting N₂O emissions [24,25]. Phosphorus addition even increases microbial activity in P saturated soil, which may increase N₂O emissions [26]. This indicates that P addition does not only alleviate microbial P limitations, but also increases N₂O emissions through abiotic C transfer [27].

Global peatlands are gradually shrinking in area due to climate change and anthropic disturbance [28]. Under the current global warming, the sink function for the greenhouse gases CO₂ and N₂O in peatlands will be severely weakened [29]. Warming will stimulate soil enzyme and microbial activity, accelerate peat decomposition, change plant composition, and directly or indirectly promote N₂O emissions from peatlands [10,30,31]. Phosphate monoesters and orthophosphates were the dominant forms of P in wetlands [32]. Warming will promote the transformation of stable P into unstable P [33], accelerate soil P cycling, and break the P limitation in the surface soil, leading to a clear increase of P availability in wetlands [34,35].

Currently, we still know rather little on how N₂O emissions respond to the joint environmental changes, P addition and warming, in wetlands, especially peatland ecosystems. To address this, we conducted a field monitoring experiment in Hani peatland, and combined with laboratory experiments, to investigate the effect of P addition, as well as the interaction with warming on peatland net N₂O fluxes and the response of N₂O fluxes to changes in biological and abiotic factors. We hypothesized that: 1) P addition would alleviate P limitation, stimulate soil enzyme activities, accelerate peat decomposition, provide reaction substrates and energy sources for the biochemical process of producing N₂O, and lead to an increase in the net N₂O fluxes in the peatland, and the more P added the more N₂O emitted; and 2) warming would amplify the facilitative effect of P addition on N₂O emissions to turn the peatland into a significant source of N₂O.

2. Materials and Methods

2.1. Study site

Hani peatland (126°31'E, 42°13'N), located in the Changbai Mountains in Northeast China, with an altitude of 900 m and an average peat depth of 4 m. The mean annual temperature is 3°C and the mean annual precipitation is 800 mm. The vegetation in the peatland is dominated by peat mosses (such as *Sphagnum magellanicum* Brig., *S. imbricatum* Hornsch. ex Russow etc.) and vascular plants (such as the shrub *Betula ovalifolia* Rupr. and the graminoid *Carex lasiocarpa* Ehrh.).

2.2. Experimental design

The field measurements were conducted at a 12-yr global change simulation experiment platform of Hani peatland during the 2019 growing season (May to September). We selected 20 plots (0.8 m×0.8 m), including 5 treatments {CK (control), P1 (5 kg P ha⁻¹ yr⁻¹), P2 (10 kg P ha⁻¹ yr⁻¹), P1W (P1+Warming), and P2W (P2+Warming)}, each with 4 replicates. We used NaH₂PO₄·2H₂O as the P fertilizer, dissolved it in 300 mL water, and evenly sprayed in plots once a month during the growing season. The same amount of distilled water was sprayed in CK plots. The warming was passively achieved by the open top chamber (OTC), which increased the air temperature about 0.6°C during the growing season. For more information, see Bu, et al. [36], Yi, Lu and Bu [30] and Lu, Wu, Yi, Xu, Wang, Sundberg and Bu [6].

2.3. Collection and analysis of N₂O

The N₂O fluxes were measured by the static chamber-gas chromatography method. Gas samples (60 mL) were collected by a syringe from the static chamber at 10-minute intervals over a 30-minute period (0, 10, 20 and 30 minutes) and then measured by utilizing electron capture detector (ECD) of gas chromatography (GC system, Agilent 7980B, Santa Clara, USA) to calculate the concentration of N₂O, and determined the N₂O flux by establishing a regression equation for gas concentration and sampling time (more details in Yi, Lu and Bu [30]).

2.4. Environmental factors

Environmental factors of each plot were measured while monitoring N₂O fluxes, including soil temperature at 5 cm and 20 cm below the moss surface, air temperature at 20 cm above the moss surface, soil humidity (SM), water table depth (WTD), and pH of peat water *in situ*. The plant covers, including *Sphagnum*, vascular plants, and then total plant cover, were estimated by visual inspection at the end of July 2019 [37]. More details can be seen in Yi, Lu and Bu [30].

2.5. Water and soil sampling

In August 2019, peat samples were collected from plots and analyzed for their physicochemical indicators, including total nitrogen (TN), total carbon (TC), total phosphorus (TP), and DOC [30].

2.6. Determination of soil enzyme activities

β-D-glucosidase (BDG), N-acetyl-β-glucosaminidase (NAG) and phosphatase (PHO) are three types of hydrolytic enzymes highly related to the decomposition of organic carbon in peatlands [38–40]. The former three enzymes can hydrolyze cellulose into glucose, decompose chitin and catalyze phosphate monoesters to increase C, N and P availability for microorganism and plants, respectively. Phenol oxidase (POX) can partially oxidize phenolic substances into simple organic compounds, and the activity of POX is crucial for the accumulation of organic matter in peatlands [41,42]. Microporous plate fluorescence method was used to determine the activities of the above three hydrolases and one oxidase in peat soil [6,43].

2.7. Statistical analysis

The data were checked by normality test before analysis, and logarithmic transformation was done when needed. The effects of P addition, and interaction of P addition and warming on mean N₂O fluxes during the growing season were analyzed by repeated measures analysis of variance (ANOVA). We used two-way ANOVAs to analyze the effects of P addition and the interaction of P addition and warming on N₂O fluxes and biotic/abiotic factors. One-way ANOVA was used to analyze the influences of different treatments on biotic/abiotic factors. Tukey's HSD test was used to assess the differences among the treatments when ANOVAs have significance ($P < 0.05$). Pearson correlation was used to assess the relationships between N₂O fluxes and biotic/abiotic factors, as well as for the correlation between biotic and abiotic factors. We used principal component analysis (PCA) to assess the effects of different treatments on fluxes and biotic/abiotic factors. Structural equation modeling (SEM) was used to establish the relationships between all biotic/abiotic factors. All statistical analyses were performed in R 4.1.4 (Development Core Team, 2019).

3. Results

3.1. N₂O fluxes

Both low and high levels of P addition alone did not have a significant impact on the N₂O source and sink function of Hani peatland (Figure 1, Table 2). The plots with low level of P addition tends to emit N₂O ($38 \pm 24 \text{ g m}^{-2}$, $t=1.548$, $P=0.219$), while those with high level of P addition tends to uptake N₂O ($-39 \pm 49 \text{ g m}^{-2}$, $t=0.763$, $P=0.484$). P addition with warming had a marginal effect on the

cumulative N₂O flux during the growing season ($P=0.121$, Table 2), but had a significant impact on the mean N₂O flux ($P=0.010$, Table 2). Low level of P addition with warming did not have a significant impact on the source/sink function of N₂O in the peatland, while high levels of P addition under warming significantly promoted N₂O emissions ($t=3.361$, $P=0.041$) (Figure 1), with a flux of 101 ± 30 g m⁻².

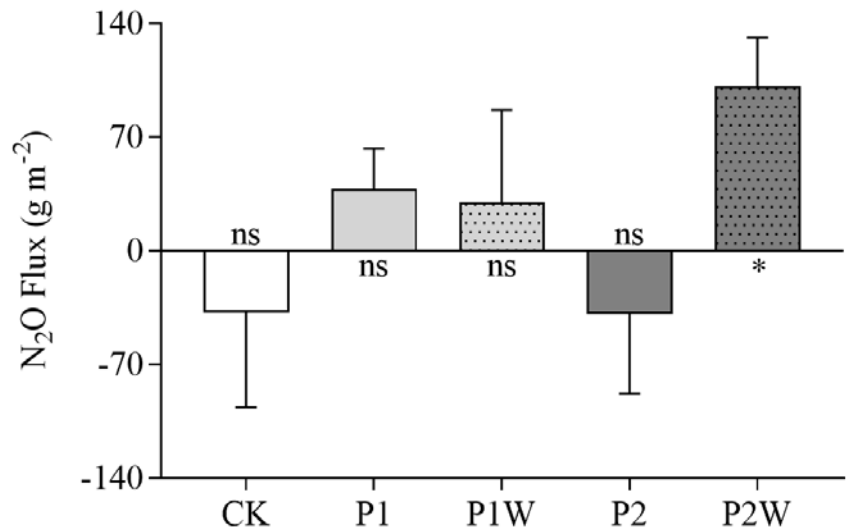


Figure 1. Cumulative N₂O fluxes (mean ± SEM, $n = 4$) in Hani peatland in the growing season of 2019. CK, control; P1, P addition with 5 kg P ha⁻¹ yr⁻¹; P2, P addition with 10 kg P ha⁻¹ yr⁻¹; P1W, P1 + warming; P2W, P2 + warming. Asterisks denote N₂O flux significantly different from zero. * $P < 0.05$; ns, no significant difference.

Table 1. Direct and warming interaction-mediating effect of P addition on environmental factors in Hani peatland (Two-way ANOVA).

Parameter	Warming		P addition		Warming × P addition	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
T _{soil} , 5 cm	1.748	0.206	0.325	0.727	0.178	0.679
T _{soil} , 20 cm	5.396	0.034*	0.3558	0.704	2.645	0.124
WTD	4.515	0.050*	8.386	0.003***	5.671	0.031**
DOC	0.052	0.822	26.020	0.000**	1.834	0.196
pH	0.140	0.713	2.219	0.143	1.266	0.278
TN	6.763	0.020**	1.621	0.230	0.394	0.539
TC	1.899	0.188	1.707	0.367	3.248	0.091*
TP	19.812	0.000***	17.754	0.000***	1.759	0.205
N:P	11.052	0.005***	18.979	0.000***	0.356	0.560
C:N	5.396	0.034**	2.256	0.139	0.056	0.816

Note: WTD, water table depth; DOC, dissolved organic carbon; TN, total nitrogen; TC, total carbon; TP, total phosphorus. Asterisk represents a significant difference, *** $P < 0.01$, ** $P < 0.05$, * $P < 0.1$.

Table 2. Effect of warming and P addition on dynamic (repeated measurement two-way ANOVA) and cumulative N₂O fluxes (two-way ANOVA) in the growth season of 2019 in Hani peatland.

Treatment	Dynamic fluxes			Cumulative fluxes		
	<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>
Warming	1	0.181	0.674	1	3.456	0.082*
P addition	2	0.003	0.997	2	0.453	0.718
Warming × P addition	1	7.658	0.010**	1	2.603	0.121

Note: ** $P \leq 0.01$.

3.2. Abiotic factors

WTD was significantly reduced after P addition (Table 1, Figure 2a), but in P1W plots it was significantly higher than that in other treatment plots ($P < 0.007$) (Figure 2a). Phosphorus addition alone significantly increased DOC concentration (P1: $P < 0.08$, P2: $P < 0.001$) (Figure 2b), and DOC concentration in P2 plots was the highest among the five treatments ($P < 0.09$), approximately as twice as that of CK. DOC in P1W treatment was not significantly different from that in CK, while it in P2W plots was significantly higher than that in CK ($P < 0.05$, Figure 2b). There was no significant difference of TN concentration among different treatments, but TN in CK plots was the lowest and in P2W plots was the highest (Figure 2d). Phosphorus addition singly or jointly with warming significantly increased TP concentration in peat water (Table 1, Figure 2e). In addition, there was no significant difference in TC concentration and pH among different treatments (Figure 2c, h). There was no significant difference in N:P ratio and C:N ratio between different treatments but these two indices showed a decreasing trend with combined P addition and warming (Figure 2f and g).

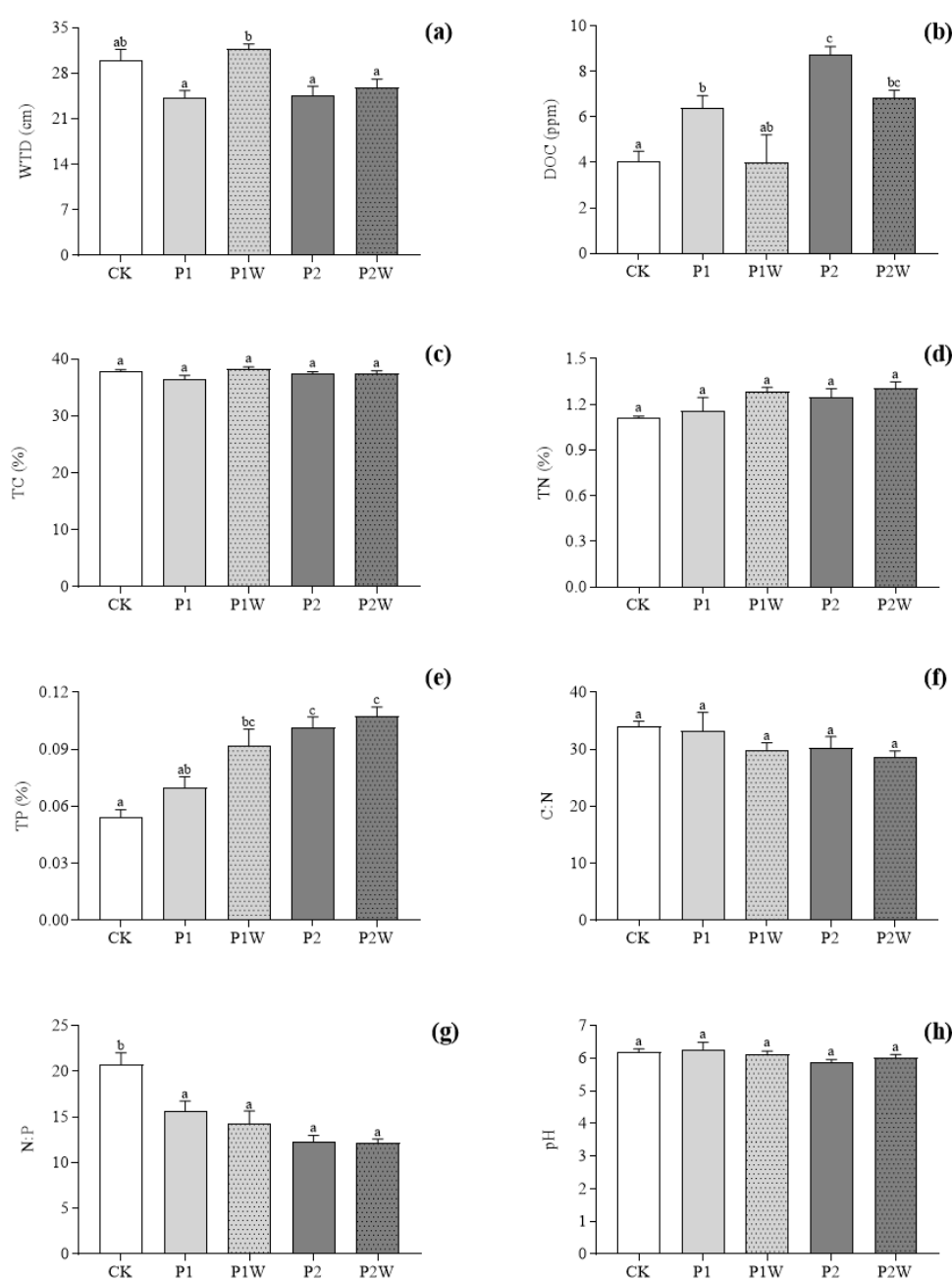


Figure 2. Abiotic factors among the different treatments in Hani peatland in 2019 (mean $\pm$ SEM, $n = 4$). (a) WTD; (b) DOC; (c) TC; (d) TN; (e) TP; (f) C:N; (g) N:P; (h) pH. CK, control; P1, P addition with 5 kg P ha⁻¹ yr⁻¹; P2, P addition with 10 kg P ha⁻¹ yr⁻¹; P1W, P1 + warming; P2W, P2 + warming. WTD, water table depth; DOC, dissolved organic carbon; TC, total carbon; TN, total nitrogen; TP, total phosphorus. Different lowercase letters represent significant differences ($P < 0.05$) between the treatments.

3.3. Vegetation

Phosphorus addition significantly reduced *Sphagnum* cover but increased vascular plant cover ($P < 0.009$, Figure 3). However, the interaction between warming and P addition did not significantly affect total plant cover ($P > 0.05$). No significant difference in total plant cover among treatments with P addition was found (Figure 3).

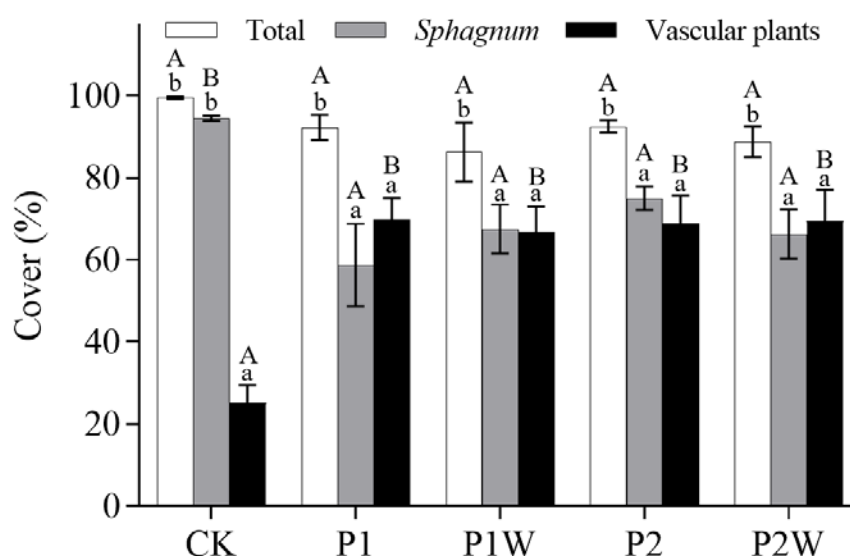


Figure 3. Plants cover (mean $\pm$ SEM, $n = 4$). CK, control; P1, P addition with 5 kg P ha⁻¹ yr⁻¹; P2, P addition with 10 kg P ha⁻¹ yr⁻¹; P1W, P1 + warming; P2W, P2 + warming. Different capital letters represent significant differences between different treatments of the same plant cover, and different lower-case letters represent significant differences between different plants under the same treatment. $P < 0.05$.

3.4. Enzyme activities

Almost all enzyme activities were promoted under warming condition. There was no significant difference in BDG, NAG and POX activities among the treatments with P addition and they were all significantly higher than CK ($P < 0.05$ for all, Figure 4a, b and d). The PHO activity in the treatment with P addition was significantly lower than that in CK ($P < 0.001$), but increased with P addition (Figure 4c).

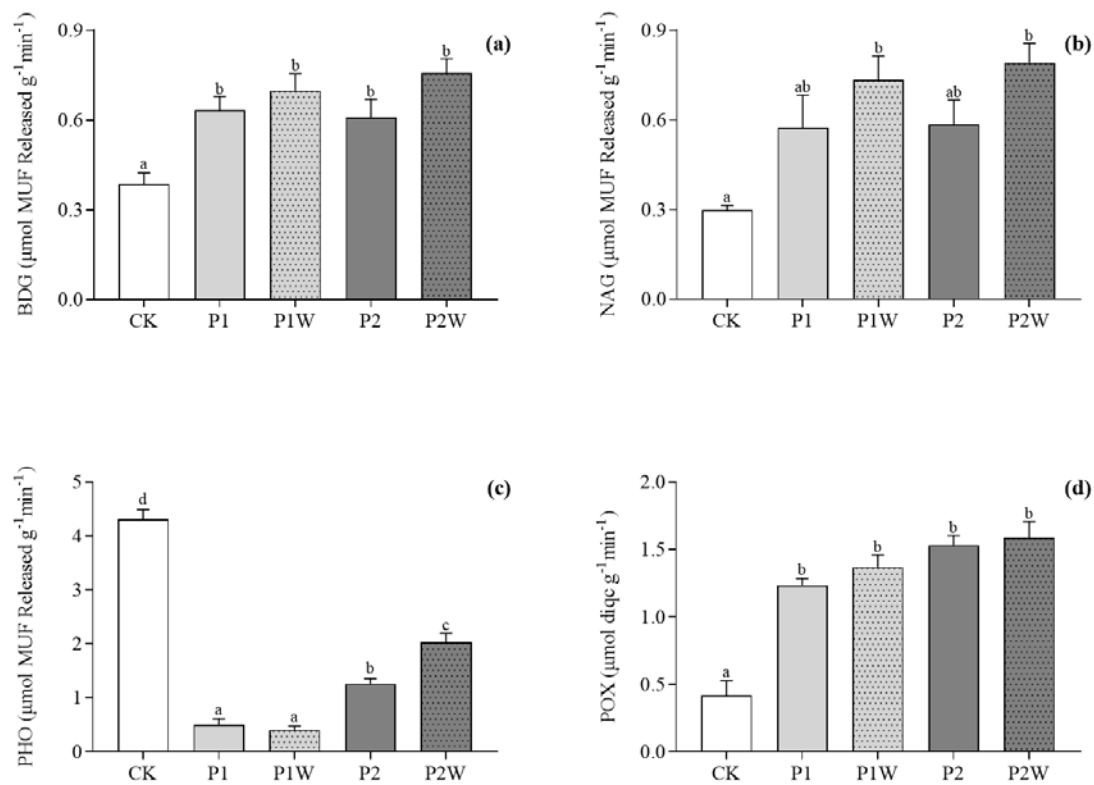


Figure 4. Soil enzyme activities (mean $\pm$ SEM, $n = 4$). (a) BDG; (b) NAG; (c) PHO; (d) POX. CK, control; P1, P addition with 5 kg P ha⁻¹ yr⁻¹; P2, P addition with 10 kg P ha⁻¹ yr⁻¹; P1W, P1 + warming; P2W, P2 + warming. BDG, β -D-glucosidase activity; NAG, N-acetyl- β -glucosaminidase activity; PHO, phosphatase activity; POX, phenol oxidase activity. Different lowercase letters represent significant differences between treatments. $P < 0.05$.

3.5. PCA and correlation

PCA (Figure 5) showed that the cumulative percentage of variance of PC1 and PC2 reached 65.41%. The control, P addition alone (P1 and P2), and P addition under warming treatments (P1W and P2W) showed a clear cluster, indicating that P addition and warming significantly affected N₂O fluxes and biotic/abiotic factors in Hani peatland. DOC was correlated with vascular plant cover, and BDG, NAG, POX, TN and TP had high correlation (Figure 5, Table S1).

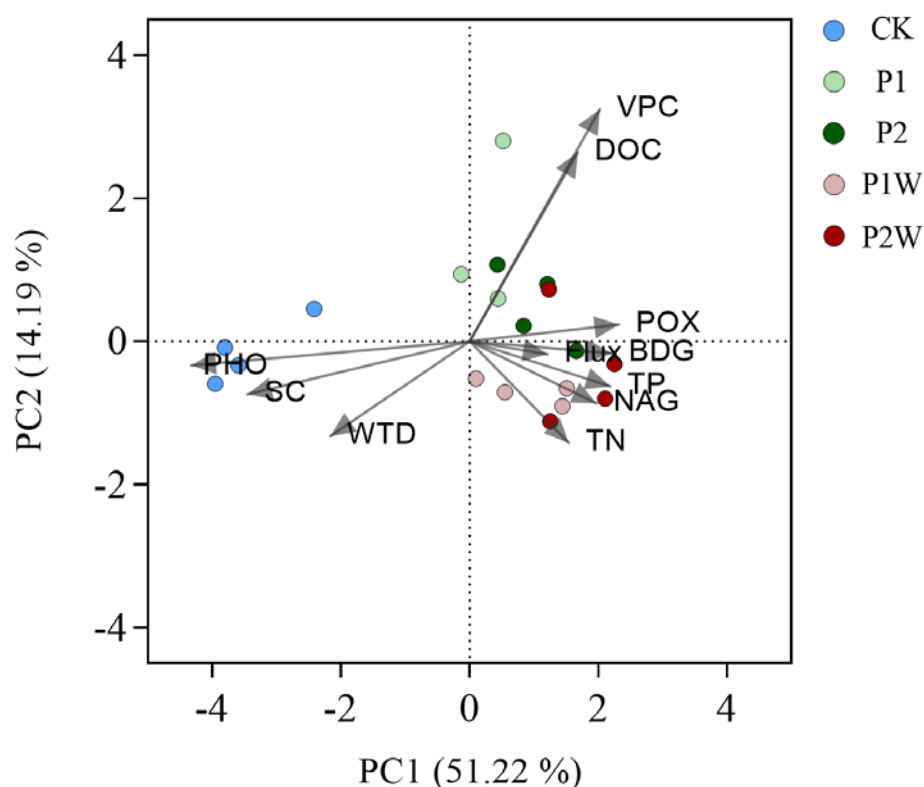


Figure 5. Principal component analysis. DOC, dissolved organic carbon; TP, total phosphorus; TN, total nitrogen; WTD, water table depth; VPC, vascular plant cover; SC, *Sphagnum* cover; BDG, β -D-glucosidase; NAG, N-acetyl- β -glucosaminidase, PHO, phosphatase, POX, phenol oxidase.

4. Discussion

4.1. Phosphorus addition and source/sink function of N₂O

Hani peatland is a P limited ecosystem [36]. Although P addition alone (P1 and P2) did not significantly affect the N₂O flux (Table 2), it did not drive the peatland to be a significant source or sink of N₂O during the growing season (Figure 1), the response of both N₂O flux characteristics and biotic and abiotic factors to the two levels of P addition were different. In CK plots, PHO activity was high (Figure 4c), and it showed a negative correlation with TP, indicating that microorganisms need to decompose monophosphate esters to obtain available P in the P limited peatland [7]. Since P is an essential nutrient for microbial growth and metabolism, the lack of P will restrict microbial and extracellular enzyme activities, leading to weak denitrification [44-46]. This should be one of mechanisms to explain why N₂O fluxes were low in CK plots. Although P1 treatment was not a significant source of N₂O during the growing season, a significant N₂O emission in August was observed (Figure S1, $P=0.05$), indicating that long-term P addition alleviated the P restriction of denitrification, resulting in increased N₂O fluxes [20,21,47], which proved our first hypothesis, to a certain extent.

Phosphorus addition significantly affected the activities of BDG and NAG (Table 1), and the activity of these two enzymes was positively correlated with TP and DOC concentration (Table S1), indicating that P addition promoted the decomposition of peat, which was consistent with our first hypothesis. The DOC concentration, BDG and NAG activities in P1 and P2 treatment plots was higher than those in CK, implying that more hydrolases are needed for microorganisms to decompose organic matter to meet their requirement for C and N [48]. This process finally will provide substrates and energy sources for the biochemical processes of N₂O production and emissions [49]. Previous studies have shown that the P addition increases the microbial communities in soil [50,51], thereby

accelerating C mineralization, which provide energy sources for nitrification and denitrification [52] to enhance N_2O emissions.

Phosphorus addition with high dose (P2 treatment) tended to result in a N_2O uptake, which may be due to the reduction of N_2O to N_2 . Dissolved organic C (DOC) concentration in P2 plots was the highest among all treatments, which may indicate that the available C and N of P2 treatment were higher than that of P1. As mentioned above, higher nutrient availability and lower C:N [53,54] were more conducive to the reduction of N_2O to N_2 [55-59]. As an electron donor in denitrification, the concentration of DOC is critical for the occurrence of denitrification, and higher DOC concentration can stimulate denitrification to consume N_2O , leading to N_2O uptake [60]. Anderson, et al. [61] found that the high level addition of P (250 kg P ha^{-1}) will greatly promote the mineralization of N in grassland ecosystems, thereby stimulating the occurrence of denitrification. O'Neill, et al. [62] observed that the cumulative N_2O emissions in "low P soil" (with a P addition level of $30 \text{ kg P ha}^{-1} \text{ yr}^{-1}$) were significantly higher than in "high P soil" ($45 \text{ kg P ha}^{-1} \text{ yr}^{-1}$), because the response of microorganisms to P addition was dose dependent. In addition, as we mentioned above, high level of P addition could also lead to the desorption and transfer of available C from the surface soil, which also promoted the denitrification.

4.2. Source/sink function of N_2O under P addition with warming

In our previous study, we found that warming significantly promoted N_2O emissions in Hani peatland [30]. In the current study, the interaction between warming and P addition greatly enhanced both the mean and cumulative N_2O fluxes, which was consistent with our second hypothesis. TN concentration of P1W and P2W was slightly higher than that under P addition alone. Although it is not statistically significant, the slightly more TN can alleviate the N limitation due to the strengthening of decomposition by warming. SEM shows that the addition of P causes an improvement in the activities of BDG and NAG, as well as an increase in DOC and TN concentration, ultimately resulting in an increase in N_2O fluxes. The activities of BDG and NAG are simultaneously affected by warming (Figure S2), and the dual effect of warming and P addition on enzyme activities leads to further enhancement of hydrolase activities and more intense decomposition of organic matter. A close relation between TN concentration and N_2O fluxes showed in PCA further supports our above view. A study in a boreal peatland found a similar mechanism for warming effect on N_2O fluxes [9], and a laboratory experiment demonstrated that warming could transform organic P into inorganic P, which may increase the availability of P in peat soil [63], and further impact N_2O fluxes.

Compared to CK, POX activity clearly increased after P addition and this was related to the increase of vascular plant cover (Figure 5, Figure 6). The litter of vascular plants was rich in lignin, which need high POX activity to break down [64]. According to the "enzyme latch" hypothesis, high POX activity may reduce phenolics to promote the activities of hydrolases, leading to increased peat decomposition and hence DOC concentration to provide an energy source for nitrification and denitrification [6,7,42]. The SEM demonstrates the process how P addition affects N_2O fluxed by affecting vegetation succession, enzyme activity response and then organic matter decomposition. Zeng and Wang [65] found that the effect of P addition on forest plant litter was greater than that on aboveground biomass. We believe that there may be a similar mechanism in peatlands, where the input of plant litter was important factor affecting N_2O production.

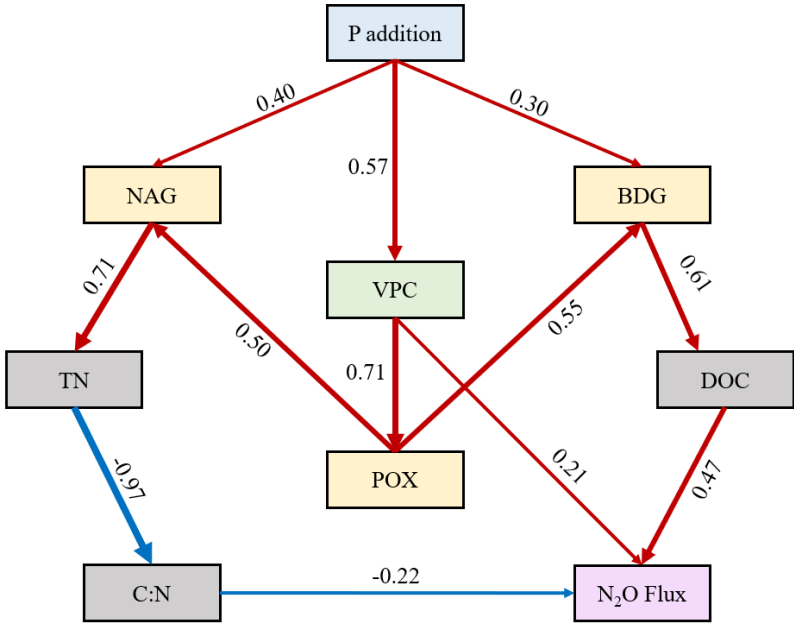


Figure 6. Structural equation model between P addition and parameters (Chi-square = 0.494, CFI = 0.990, TLI = 0.984, AIC = 999.11, BIC = 965.01, RMSEA = 0.049, SRMR = 0.007). The red arrow represents positive correlation and the blue arrow represents negative correlation, the numbers on the arrows represent the standardized parameter estimates. DOC, dissolved organic carbon; VPC, vascular plant cover; BDG, β -D-glucosidase activity; NAG, N-acetyl- β -glucosaminidase activity; POX, phenol oxidase activity; TN, total nitrogen.

5. Conclusions

In summary, our study clarified the effect of long-term P addition, as well as its interaction with warming on peatland net N₂O fluxes. We observed that although long-term P addition did not significantly alter the source/sink function of N₂O in the peatland, it altered the plant composition of the peatland, stimulated enzyme activities, and promoted peat decomposition, resulting in a potential source of N₂O emissions in the peatland. Warming amplified the effect of P addition to increase N₂O emissions by stimulating enzyme activities and changing soil stoichiometry, and even turned the peatland into a significant source of N₂O. Our results suggest that current and future warming and P deposition caused by global change will greatly promote peatland N₂O emissions and seriously threaten the N pool function in peatlands.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

Author Contributions: Conceptualization, A.C. and Z.-J.B.; Methodology, Z.-J.B. and B.Y.; Software, B.Y. and F.L.; Validation, A.C.; Investigation, B.Y., F.L. and X.C; Resources, Z.-J.B.; Data curation, B.Y., J.Z. and J.-X.M.; Writing—original draft, B.Y.; Writing—review & editing, A.C. and Z.-J.B. All authors have read and agreed to the published version of the manuscript.

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