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Short-Term Response of the Soil Microbial Abundances and Enzyme Activities to Experimental Warming in a Boreal Peatland in Northeast China

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Abstract: Global warming is likely to influence the soil microorganisms and enzyme activity and alter the carbon and nitrogen balance of peatland ecosystems. To investigate the difference in sensitivities of carbon and nitrogen cycling microorganisms and enzyme activity to warming, we conducted three-year warming experiments in a boreal peatland. Our findings demonstrated that both *mcr*A and *nir*S gene abundance in shallow soil and deep soil exhibited insensitivity to warming, while shallow soil archaea 16S rRNA gene and *amo*A gene abundance in both shallow soil and deep soil increased under warming. Soil *pmo*A gene abundance of both layers, bacterial 16S rRNA gene abundance in shallow soil, and *nir*K gene abundance in deep soil decreased due to warming. The decreases of these gene abundances would be a result of losing labile substrates because of the competitive interactions between aboveground plants and underground soil microorganisms. Experimental warming inhibited β-glucosidase activity in two soil layers and invertase activity in deep soil, while it stimulated acid phosphatase activity in shallow soil. Both temperature and labile substrates regulate the responses of soil microbial abundances and enzyme activities to warming and affect the coupling relationships of carbon and nitrogen. This study provides a potential microbial mechanism controlling carbon and nitrogen cycling in peatland under climate warming.

Keywords: peatlands; climate warming; microbial abundance; soil enzyme; dissolved organic carbon; available nitrogen

1. Introduction

Soil microorganisms, which mediate carbon and nitrogen cycling, are key drivers of biogeochemical processes in terrestrial ecosystems [1] and play a vital role in terrestrial carbon and nitrogen cycling [2,3]. Temperature is an important factor for niche space competition among physiologically similar organisms [4], and has been known to be a key limiting factor for microbial metabolism [5]. Changes in temperature may affect microbial growth and lead to changes of microbial community structure and composition, decomposition rate, as well as enzyme activities [6,7]. Soil microorganisms can adjust their community composition or alter their carbon utilization strategies to adapt to warming [8]. Especially in colder regions, warming has stronger impacts on microbial abundance than in warm regions [9].

Soil bacterial, methanogen, and methanotroph communities play important roles in greenhouse gas emissions [10,11]. Previous studies have indicated that warming could affect the abundances of microbial functional genes mediated in carbon decomposition [2,12]. Moreover, little changes of microbial abundances could significantly affect soil organic carbon (SOC) decomposition, due to the fact that microbial communities and their ability to degrade SOC have different temperature sensitivities [5]. An increase in the bacterial abundance could stimulate the soil respiration [13]. Kotroczó et al. [14] found that increasing temperature could enhance soil respiration dependent on soil microbial activity. However, not all microbial groups/populations increased under warming [2]. Peltoniemi et al. [11] found that methanogen abundance decreased, but methanogenic communities remained similar after three-year experimental warming in boreal fens. These different responses of soil microorganisms and the carbon process depend on ecosystem types and would be influenced by geographical position, soil types, and vegetation characteristics [15–17].

Climate warming could change gene abundances involved in the microbial nitrogen cycling, leading to changes of soil nitrogen cycling processes and production of N_2O gas [18]. The *amo*A gene encodes the ammonia monooxygenase small alpha subunit and participates in the nitrification pathway [19,20]. The *nir*S and *nir*K genes encode nitrite reductase and participate in the denitrification pathway [21]. Warming could increase abundance of *amo*A and *nir*K genes and increase nitrification and denitrification processes [12]. However, Waghmode et al. [3] found that warming decreased the denitrifier (*nir*K and *nir*S genes) abundance in an agricultural ecosystem. Alterations in these nitrogen cycling functional genes are very important because changes of available nitrogen can influence the microbial degradation ability of soil organic matter (SOM) and affect plant productivity [22].

Variations in microbial functions in response to changes in environmental conditions are commonly determined using soil enzyme activity assays because soil enzyme activity indicates changing of microbial community structure and nutrient demand [23,24]. β-Glucosidase (EC 3.2.1.21), invertase (EC.3.2.1.26), urease (EC 3.5.1.5), and acid phosphatase (EC 3.1.3.2) are important enzymes involved in SOM decomposition and carbon and nutrient cycles. The effects of warming on enzyme activity could alter ecosystem carbon, nitrogen, and phosphorus balance [25]. Nevertheless, the response of soil enzyme activity to climate warming is considerably important but poorly understood. In particular, we currently lack the understanding of the enzyme decomposition of soil organic matter in boreal peatlands.

Boreal peatlands contain about 15–30% of the global soil carbon pool [26,27] and 9–16% of the global soil nitrogen pool [28] and play important roles in the global carbon and nitrogen cycles. The accumulation of SOM in peatlands is due to the effects on microbial metabolism of low available oxygen, low pH, low available nutrients, and low temperatures [29]. Global climate change will influence the microbial communities' function and structure and alter the carbon balance of peatland ecosystems [30], and it will have greater impact on boreal peatlands in the future [10]. Therefore, it is very important to improve our knowledge of how carbon, nitrogen, and phosphorus cycling microbes in boreal peatland systems respond to global warming. Understanding the response of soil microbial abundances and enzyme activities to warming will provide insights into the carbon and nitrogen cycles in peatlands. However, this topic is still under high debate.

In the boreal peatland of northeast China, climate warming significantly stimulated the plant growth [31]. Our previous study has reported that field warming significantly increased N_2O emissions [32]. However, it is important to clarify the microbial mechanisms controlling soil carbon and nutrient cycles in this peatland under warming. The objective of our study is to access the effect of warming on soil carbon and nitrogen cycling microbial gene abundances and enzyme activities in a boreal peatland in northeast China. Based on the previous studies, we hypothesized that (1) climate warming could increase the soil microbial gene abundances via rising soil temperature, considering temperature is a key limiting factor for microbial metabolism [5], (2) experimental warming could inhibit soil enzyme activities by decreasing the available substrate [33,34]. We collected soil samples from the ongoing warming experiment for three years on the northwest slope of the Greater Khingan

Mountains. We assessed the abundances of bacterial 16S rRNA, archeal 16S rRNA, *mcr*A, *pmo*A, *amo*A, *nir*K, and *nir*S genes by quantitative polymerase chain reaction, and soil β-glucosidase, invertase, urease, and acid phosphatase activities. Additionally, we investigated soil carbon and nitrogen contents, which will clarify the mechanism of how climate change will affect soil carbon and nutrient cycling in boreal peatlands.

2. Materials and Methods

2.1. Study Sites and Experimental Design

Our experiment was conducted in a boreal peatland on the northwest slope of the Greater Khingan Mountains in northeast China (52°56′ N, 122°51′ E). The maximum melting depth ranges from 50 cm to 60 cm. The annual average air temperature and precipitation are −3.9 °C and 450 mm from 1991 to 2010, respectively. January is the coldest month, whereas July is the warmest, and the annual average precipitation is with 45% falling as rain from July to August. The vegetation is dominated by *Betula fruticosa* Pall., *Ledum palustre* L., *Chamaedaphne calyculata* L., *Vaccinium uliginosum* L., *Rhododendron parvifolium* Adams, *Eriophorum vaginatum* L., and *Sphagnum spp.* The soil type in this site is classified as Glacic Historthels according to the classification system of USDA.

Four cuboidal open-top chambers (OTCs) (1.5 m length, 1.5 m width, and 1.5 m height) and control plots were placed in 2013 [35]. We used the cuboidal OTCs without top instead of hexagonal OTCs in order to avoid the wind control effect on plant growth and provide a valid method for comparing the effect of warming in a remote field situation. These plots remained in place for three years between 2014 and 2016. The air temperature at a height of 15 cm above ground and soil surface temperature at 5 cm and 15 cm belowground were continuously recorded during the three growing seasons, using temperature logger (TidbiT V2, UTBI-001, Onset, USA). The mean air temperature in the OTCs increased by 0.41 °C (0.27–0.58 °C) during the three growing seasons. The soil temperature also increased by 0.77 °C (0.55–0.98 °C) and 0.61 °C (0.53–0.72 °C) at depths of 5 and 15 cm, respectively. Soils from 0–10 and 10–20 cm below the plant litter layer were collected by using an 8 cm diameter soil drill from four OTCs. Four soil cores were sampled in each OTC and in the control plots on 9 September 2016. The soil samples were mixed thoroughly. The soil samples for NH_4^+–N, NO_3^-–N, microbial biomass carbon (MBC), and dissolved organic carbon (DOC) contents, as well as soil enzyme activities and soil moisture, were stored at 4 °C. The soil samples for microbial gene abundance analysis were stored at –80 °C. In addition, the soil samples were air dried, then sieved through 0.25 mm sieves prior to total carbon (TC) and total nitrogen (TN) analyses.

2.2. Chemical Analyses

We assayed soil MBC using the chloroform fumigation incubation method [36]. The total soluble carbon from fumigated and nonfumigated soils was determined using a Multi N/C 2100 TOC analyzer (Analytik Jena, Germany). MBC content was calculated by dividing the fumigation flush by a K_{EC} factor of 0.45. The content of soil DOC was measured according to the method of Ghani et al. [37]. Fresh soils were extracted with deionized water. Supernatants were then filtered using a 0.45 μm filter. Total carbon and inorganic carbon were measured with a Multi N/C 2100 analyzer (Analytik Jena, Germany). DOC content was calculated as the difference between total carbon and inorganic carbon.

Soil inorganic nitrogen was extracted with 2 mol L^{-1} KCl. NH_4^+–N and NO_3^-–N in extract were analyzed with the AA3 Continuous Flow Analyzer (Seal Analytical, Germany). Soil TC was measured using a Multi N/C 2100 analyzer (Analytik Jena, Germany). TN content in the soils was determined with an AA3 Continuous Flow Analyzer (Seal Analytical, Germany) after wet digestion with sulfuric acid. Soil moisture was determined as weight loss after drying at 70 °C for 48 h to a constant weight. Soil pH in air-dried soils was determined by using a 1:10 soil–water ratio.

2.3. Real-time PCR Assay of Functional Genes

We used 0.3 g of frozen soil to extract DNA with a FastDNA Spin Kit (MPbio, USA) following the manufacturer's instruction. DNA extracts were purified by 0.5% low-melting-point agarose gels, followed by phenol–chloroform–butanol extraction. The abundance of bacteria, archaea, *mcr*A, *pmo*A, *amo*A, *nir*K, and *nir*S genes were determined by real-time PCR. An ABI StepOne instrument (Applied Biosystems, USA) using SYBR green detection chemistry was used, and each sample replicated three times. The primers and PCR procedures are shown in Table S1. For real-time PCR, each 25 μL reaction mixture contained 12.5 μL of SYBR Buffer (TaKaRa, Japan), 0.4 μL of each primer (10 mM), 0.5 μL of ROXII (TaKaRa), 0.875 μL 3% BSA, 0.625 μL of DMSO, and 10 ng of template DNA. For standard curve generation, the amplicon products of phylogenetic and functional markers were purified by a cyclic purification kit (Omega Bio-Tek, USA), ligated to the vector pMD18-T (TaKaRa), and then transformed into Escherichia coli TOP 10 competent cells. The plasmids were extracted by plasmid mini kit (Omega Bio-Tek). The specificity of plasmids was detected by the basic local alignment search tool [38]. The standard curve was obtained by the continuous dilution of known copy number plasmids.

2.4. Soil Enzyme Activities Analysis

We determined soil β-glucosidase, invertase, urease, and acid phosphatase activities, which are involved in carbon, nitrogen, and phosphorus cycling. Soil β-glucosidase activity was measured with *p*-Nitrophenyl-β-D-glucopyranoside as substrate; the result was expressed as μg *p*NP g^{-1} h^{-1} [39]. We measured soil invertase and urease activities by using sucrose and urea as substrates [40], expressed as mg glucose g^{-1} 24 h^{-1} and mgNH_4^+–N g^{-1} 24 h^{-1}, respectively. We analyzed soil acid phosphatase activity by using *p*-nitrophenyl phosphate as substrate according to the method of Zhao and Jiang [41] and the result was expressed as mg *p*NP g^{-1} 12h^{-1}.

2.5. Statistical Analyses

All the statistical analyses were conducted using an SPSS 16.0 with an accepted significance level of $\alpha = 0.05$. The significant differences between warming and control treatments were analyzed using the one-way analysis of variance (ANOVA) followed by the Duncan's test. All data were normally distributed and met the assumptions of the ANOVA (data not shown). In addition, the Pearson's correlation coefficients between soil microbial abundances, soil enzyme activities and soil carbon, nitrogen contents were calculated.

3. Results

3.1. Soil Carbon and Nitrogen Contents, Soil Moisture, and Soil pH

Three years of experimental warming significantly decreased the soil MBC concentration from 1421.6 μg g^{-1} to 1197.2 μg g^{-1} in shallow soil ($p < 0.05$) and from 1478.7 μg g^{-1} to 841.6 μg g^{-1} in deep soil ($p < 0.01$) (Figure 1A). Warming also significantly decreased the DOC contents from 338.4 μg g^{-1} to 230.3 μg g^{-1} in deep soil ($p < 0.05$, Figure 1B). Ammonia nitrogen contents in soils decreased significantly from 66.6 μg g^{-1} to 22.5 μg g^{-1} in shallow soil and from 47.9 μg g^{-1} to 19.6 μg g^{-1} in deep soil under warming ($p < 0.01$, Figure 1C). Nitrate contents ranged from 0.53 μg g^{-1} to 0.67 μg g^{-1} and were not affected by warming ($p > 0.05$, Figure 1D). Significant difference of TC contents of both soil layers was not observed between the warming plots and the control plots ($p > 0.05$, Figure 2A). We observed significant decreases of TN in deep soil under warming ($p < 0.05$, Figure 2B). C/N ratio significantly increased from 20.61 to 24.71 in deep soil under warming ($p < 0.05$, Figure 2C). Three-year warming tended to decrease the soil moisture in deep soil (from 82.66% to 78.98%), but the difference did not reach the 0.05 significance level (Figure 2D). Soil pH ranged from 5.18 to 5.32, and there were no significant differences between warming treatment and control ($p > 0.05$, Figure 2E).

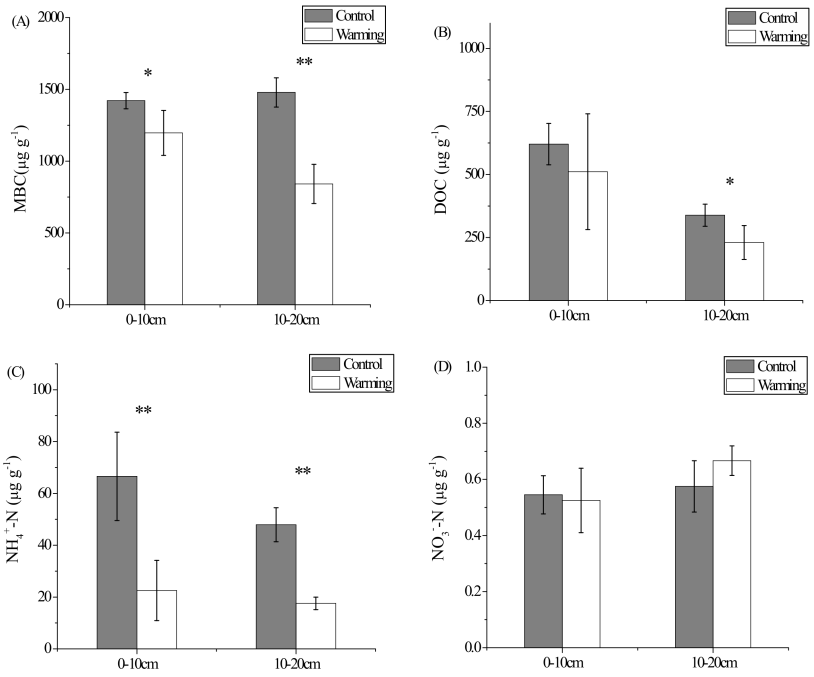
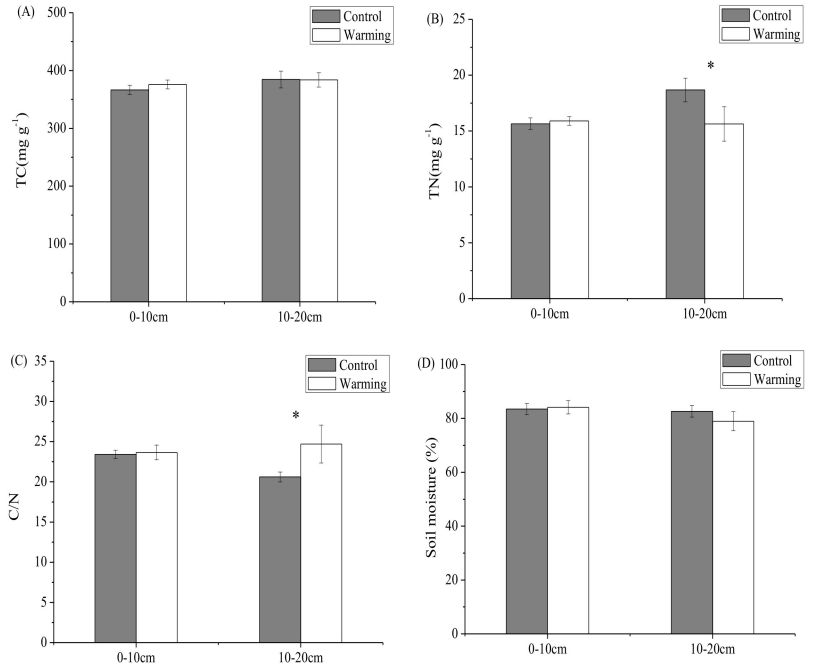


Figure 1. Soil microbial biomass C (**A**), dissolved organic C (**B**), NH_4^+–N (**C**), and NO_3^--N (**D**) in 0–10 cm and 10–20 cm layers received warming treatment from 2014 to 2016. Error bars represent the standard error of the means (n = 4). Significant differences between the control and warming treatment are indicated by * $p < 0.05$, ** $p < 0.01$.



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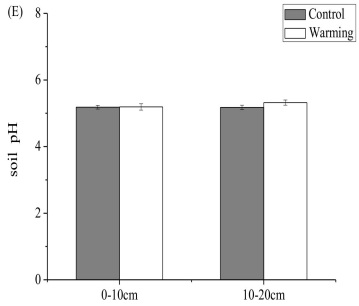
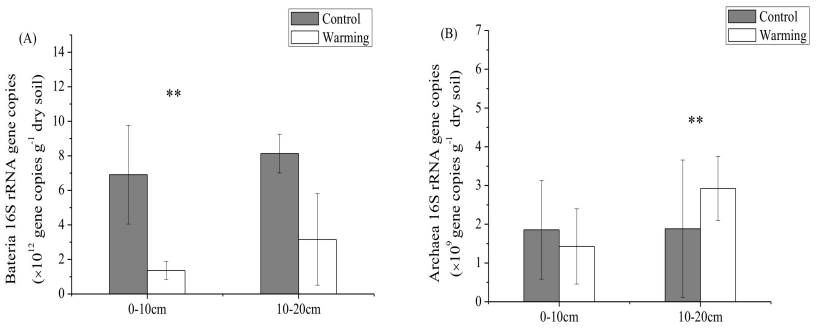


Figure 2. Soil total C (**A**), total N (**B**), C/N (**C**), soil moisture (**D**), and pH (E) in 0–10 cm and 10–20 cm layers received warming treatment from 2014 to 2016. Error bars represent the standard error of the means (n = 4). Significant differences between the control and warming treatment are indicated by * $p < 0.05$, ** $p < 0.01$.

3.2. Soil Microbial Abundance

Real-time, PCR-based determination of soil bacterial and soil archaeal 16S rRNA gene abundances indicated that bacterial gene copy numbers were about 1000 times greater than the archaea numbers (1.36×10^{12}–8.13×10^{12} gene copies g^{-1} dry soil for bacteria versus 1.43×10^{9}–2.92×10^{9} gene copies g^{-1} dry soil for archaea). Warming significantly decreased the bacteria 16 S rRNA gene abundance from 6.91×10^{12} gene copies g^{-1} dry soil to 1.36×10^{12} gene copies g^{-1} dry soil in shallow soil ($p < 0.05$, Figure 3A). However, in the warming plots, significantly higher archaeal 16 S rRNA gene abundance (2.92×10^{9} gene copies g^{-1} dry soil) was detected in deep soil (Figure 3B). The *mcr*A gene abundance ranged from 5.71×10^{8} gene copies g^{-1} dry soil to 7.30×10^{8} gene copies g^{-1} dry soil and were not changed by warming ($p > 0.05$, Figure 3D). Warming significantly decreased the *pmo*A gene abundance from 7.34×10^{7} gene copies g^{-1} dry soil to 3.83×10^{7} gene copies g^{-1} dry soil in shallow soil and from 7.73×10^{7} gene copies g^{-1} dry soil to 3.97×10^{7} gene copies g^{-1} dry soil in deep soil ($p < 0.05$, Figure 3D). The correlation analysis revealed that *pmo*A gene abundance was significantly correlated with MBC, NH_4^+–N, and TN contents ($p < 0.05$, Table 1).

For the nitrogen-cycling gene, warming significantly increased the *amo*A gene abundance from 7.30×10^{7} gene copies g^{-1} dry soil to 14.65×10^{7} gene copies g^{-1} dry soil in shallow soil and from 6.76×10^{7} gene copies g^{-1} dry soil to 11.05×10^{7} gene copies g^{-1} dry soil in deep soil ($p < 0.01$, Figure 4A). The *nir*S gene abundance ranged from 1.94 to 3.08×10^{9} gene copies g^{-1} dry soil, which were unaffected by three-year warming ($p > 0.05$, Figure 4B). In the control plots, the *nir*K gene abundance was 6.09×10^{9} gene copies g^{-1} dry soil in shallow soil and 5.81×10^{9} gene copies g^{-1} dry soil in deep soil. In the warming plots, the *nir*K gene abundance was 2.23×10^{9} gene copies g^{-1} dry soil in shallow soil and 1.57×10^{9} gene copies g^{-1} dry soil in deep soil. Warming caused significant negative effects on *nir*K gene abundance in deep soil ($p < 0.01$, Figure 4C). We found that *amo*A gene abundance was negatively correlated with MBC and NH_4^+–N contents, but *nir*K gene abundance was positively correlated with MBC and NH_4^+–N contents ($p < 0.05$, Table 1).



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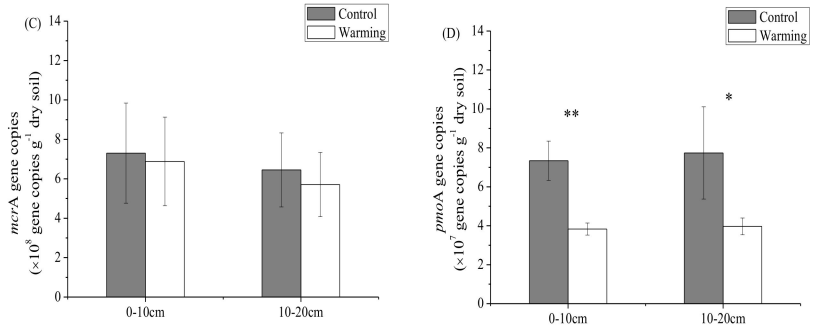

Figure 3. Total soil bacteria (**A**), archaea (**B**), *mcr*A gene (**C**), and *pmo*A gene (**D**) abundances in 0–10 cm and 10–20 cm layers received warming treatment from 2014 to 2016. Error bars represent the standard error of the means (n = 4). Significant differences between the control and warming treatment are indicated by * $p < 0.05$, ** $p < 0.01$.

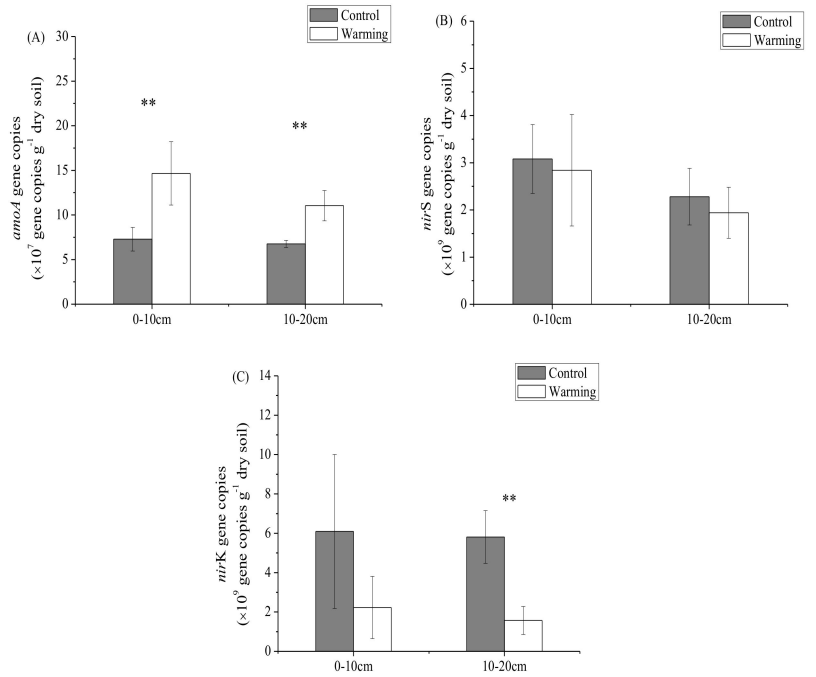

Figure 4. Soil *amo*A (**A**), *nir*S (**B**), *nir*K (**C**) gene abundances in 0–10 cm and 10–20 cm layers received warming treatment from 2014 to 2016. Error bars represent the standard error of the means (n = 4). Significant differences between the control and warming treatment are indicated by * $p < 0.05$, ** $p < 0.01$.

Table 1. Pearson's correlations (R value) between soil microbial abundances and soil carbon and nitrogen contents.

	Bacteria 16S rRNA	**Archaea 16S rRNA**	***mcr*A**	***pmo*A**	***amo*A**	***nir*S**	***nir*K**
MBC	0.359	–0.427	0.288	0.653 **	−0.529 *	0.146	0.608 *
DOC	0.084	–0.262	0.395	0.229	0.134	0.558 *	0.477
NH_4^+–N	0.417	−0.046	0.422	0.726 **	−0.609 *	0.321	0.607 *
NO_3^-N	0.150	0.321	0.146	−0.043	0.201	−0.009	−0.095
TC	0.121	−0.135	-0.051	−0.026	0.022	−0.265	−0.344
TN	0.164	0.141	0.299	0.512 *	−0.402	0.008	0.297

Significance level: $p < 0.01$ (**), $p < 0.05$ (*)

3.3. Enzyme Activities

After three years of field warming, soil β-glucosidase activity was significantly reduced from 2215.3 μg *p*NP $g^{-1}h^{-1}$ to 1290.5 μg *p*NP$g^{-1}h^{-1}$ in shallow soil ($p < 0.01$, Figure 5A) and from 2732.1 μg *p*NP $g^{-1}h^{-1}$ to 1434.7 μg *p*NP $g^{-1}h^{-1}$ in deep soil ($p < 0.01$, Figure 5A). Warming also significantly decreased the invertase activity from 214.0 glucose $g^{-1}24\ h^{-1}$ to 106.2 mg glucose $g^{-1}24\ h^{-1}$ in deep soil ($p < 0.05$, Figure 5B). By contrast, warming significantly increased the acid phosphatase activity from 12.6 μg *p*NP $g^{-1}12\ h^{-1}$ to 24.2 μg *p*NP $g^{-1}12\ h^{-1}$ in shallow soil ($p < 0.01$, Figure 5D). The shallow soil urease activity in control and warming treatments was 4.88 and 5.52 mg NH_4^+–N $g^{-1}24\ h^{-1}$, respectively. The deep soil urease activity in the control and warming treatments was 3.20 and 3.21 mg NH_4^+–N $g^{-1}24\ h^{-1}$, respectively. No difference of the urease activity was observed between the warming treatment and the control in both soil layers ($p > 0.05$, Figure 5C). Soil β-glucosidase activity positively correlated with MBC, NH_4^+–N, and TN contents, and soil invertase activity was significantly positively correlated with MBC, DOC, and NH_4^+–N contents ($p < 0.05$, Table 2).

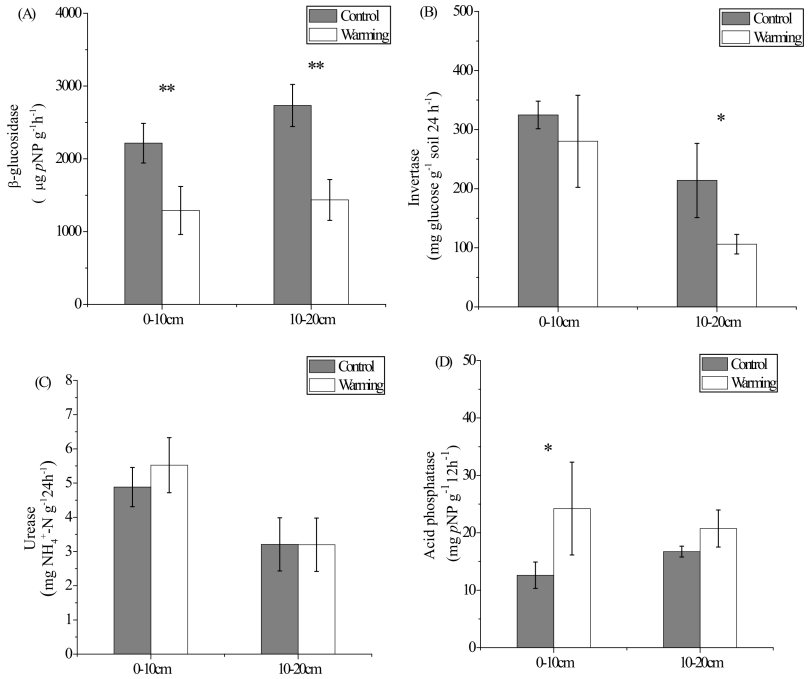

Figure 5. Soil β-glucosidase (**A**), invertase (**B**), urease (**C**), and acid phosphatase (**D**) activities in 0–10 cm and 10–20 cm layers received warming treatment from 2014 to 2016. Error bars represent the standard error of the means (n = 4). Significant differences between the control and warming treatment are indicated by * $p < 0.05$, ** $p < 0.01$.

Table 2. Pearson's correlations (R value) between soil enzyme activities and soil carbon and nitrogen contents.

	β-Glucosidase	Invertase	Urease	Acid Phosphotase
MBC	0.791 **	0.633 **	0.204	–0.370
DOC	0.059	0.848 **	0.690 **	–0.238
NH_4^+–N	0.735 **	0.597 *	0.164	–0.470
NO_3^-–N	−0.016	–0.199	−0.155	0.424
TC	0.153	–0.426	−0.377	0.366
TN	0.595 *	–0.077	−0.273	0.005

Significance level: $p < 0.01$ (**), $p < 0.05$ (*)

4. Discussion

4.1. Response of Soil C and N Contents to Experimental Warming

SOC is used as substrate for microbial respiration and is therefore vital to the process [42]. We found that soil MBC and DOC decreased under three years of warming in the boreal peatland. The decrease of DOC was consistent with a previous report that soil warming stimulated DOC decomposition in the Zoige peatland [43]. We previously found that increasing temperature significantly hastens the cumulative CO_2 emission from the decomposition of peat soil MBC and DOC by an incubation experiment [44]. Rinnan et al. [45] and Hou et al. [46] also found that soil warming negatively affects the soil MBC. One possible explanation for this result is that the cell reserves and labile substrates for microbes are depleted under warming [46]. The results of our study indicated that warming exerted no evident effect on soil TC, which is consistent with the results of Chen et al. [47]. They found that 5 and 15 years of warming did not affect the SOC pool because of high soil carbon levels. Although warming influences MBC and DOC, the proportions of these labile organic carbon (LOC) in the total SOC pool are low; thus, the variation in labile carbon is insufficient to influence the total carbon storage [47]. In addition, warming could decrease microbial decomposing capacity to recalcitrant carbon, and then ameliorate soil carbon loss [48].

Previous studies observed that warming manipulations result in high inorganic nitrogen in tundra, grassland, and forest soils because of the high nitrogen turnover rate due to warming [25,49,50]. By contrast, our results showed that soil NH_4^+–N and TN in warming treatments were decreased, which indicated their negative responses to warming. Similar to our results, Sardans et al. [51] reported that warming causes soil NH_4^+–N loss, which is in accordance with the increased N uptake by plants. Alatalo et al. [52] also reported that warming results in reduced TN content in soil. We found an increase in foliar N concentration under warming in the same experimental plots (data not shown). Thus, our results indicated the high demand, assimilation, and immobilization of N by plants under warming. Consequently, low soil NH_4^+–N and TN contents were observed. However, after multiple years of warming, high biological N fixation may increase the soil nitrogen stocks [53].

4.2. Response of Soil Carbon and Nitrogen Cycling Genes Abundance to Experimental Warming

Although we hypothesized that warming would increase soil microbial gene abundance, we found that different soil carbon and nitrogen cycling genes abundances respond differentially to warming. The response of soil microbial functions to different temperatures can be mediated by a combination of enzyme response within microbial communities and changes in the microbial taxa abundances adapted to different temperatures [54,55]. The response mechanisms of soil microbial communities to changing of temperature include acclimatization, genetic adaptation, and sorting of species [56,57]. Contrary to our first hypothesis, we found that warming significantly decreased bacterial 16S rRNA gene copy numbers in shallow soil. Wang et al. [56] and Hayden et al. [58] also observed that bacterial abundance decreased in response to increasing temperature. Aillson et al. [25] found that bacterial abundance declined by over 50% in boreal forest soils under warming. Increasing temperature can change soil bacterial physiology and activity [59]. Warming can induce changes of microbial community patterns due to the faster species turnover under higher temperature [56,60]. Moreover, warming decreased the bacterial abundance possibly due to the decreased availability of carbon and nitrogen substrate. Conversely, decrease of bacterial abundance suggested a reduced potential for the microbial community to metabolize carbon and nitrogen. The contents of labile organic carbon (DOC), available nitrogen (NH_4^+–N), and TN decreased, which may have contributed to the decrease of bacterial abundance. Bastida et al. found that DOC could shape the activities of bacterial population in soils [61]. Warmer conditions may alleviate the temperature limitation of microbial activity, but decrease the labile substrate, which then leads to the decline in regulation of soil microbial functions [62]. Liu et al. [63] also found that concentrations of DOC and total organic carbon significantly decreased under warming, while respiration increased. In our study, soil TC content was so large that it did not significantly

change under three years of warming. However, other studies showed that bacterial abundance changed little or did not change under warming [64,65]. The reason is that the responses of soil bacteria depend on ecosystem types and can be affected by geographic position, soil types, and plant traits [15,16].

Archaea can play important roles in carbon [66] and nitrogen cycling [18,67]. Firstly, the archaeal populations play important roles in the methane cycle in soil [66]. In addition, archaea can be directly involved in denitrification processes. Rasche et al. [67] and Martin et al. [68] found that total archaeal and *amo*A gene abundances were correlated with N_2O emissions. In agreement with our hypothesis, we found that warming significantly increased both the total archaea and *amo*A gene abundances in the deep soil, which might stimulate N_2O emissions. Indeed, our previous study showed that warming treatment increased N_2O fluxes by 147% in a boreal peatland [32], consistent with our finding that warming increased total archaea under *Themeda triandra* (a grass with C_4 photosynthetic pathways) [58] and the abundance of *amo*A gene in antarctic soils [69]. In addition, temperature has been shown to be an important driver of AOB distributions [70]. The genes encoding methyl coenzyme M reductase (*mcr*A) and particulate methane monooxygenase (*pmo*A) participate in CH_4 cycling [71]. Changes of temperature can affect methanogenic microbial community structure and function [72]. However, we found no significant difference of *mcr*A gene abundance between the control and warming treatment. Fuchs et al. [71] also found that methanotrophic community size did not change under different incubation temperatures. No temperature effect was also observed on methanogens (*mcr*A) abundance in peatland soil [73]. However, temperature is an important factor regulating methanotrophs abundance in soil. We found that warming resulted in a significant decrease of *pmo*A gene abundance, indicating that less of the CH_4 produced could be oxidized in warmed plots. Liu et al. [63] also observed that the abundance of methane-oxidizing bacteria significantly decreased under higher temperature. The decrease observed in the abundance of methane-oxidizing bacteria may be related to increased methane emission under climate warming [74].

The nitrogen cycle depends on microbial processes and contains two important processes: nitrification and denitrification [75,76]. Studies of the nitrifiers and denitrifiers are commonly focused on functional genes, such as *amo*A, *nir*K, and *nir*S [75,77,78]. Ammonia oxidation is a rate-limiting step in nitrification, and is regulated by the *amo*A gene, which encodes the ammonia monooxygenase small alpha subunit [19,20]. In agreement with the study of Xue et al. [12], we found that warming increased *amo*A gene abundance (Figure 4A), which may increase ammonia oxidation processes and decrease soil ammonia nitrogen under warming. Our result illustrated that *amo*A gene abundance was negatively correlated with soil ammonia nitrogen content. The *nir*S and *nir*K genes can be used to compare the abundance and diversity of denitrifying communities [76], and these gene abundances correlate with potential denitrification rates [79,80]. In our study, the *nir*K gene abundance in deep soil decreased markedly under warming, and the *nir*K gene abundance in shallow soil and *nir*S gene abundance in the two soil layers tended to decrease under warming, but the changes were not statistically significant. The decreased abundances of the *nir*K gene may result in a decrease in denitrification and restrain the transformation of NO_3^--N into NO, N_2O, and N_2 [81]. We also found a decrease of ammonia nitrogen in the warming plots, indicating the decrease of the denitrification substrate. The significant relationship between *nir*K gene abundance and ammonia nitrogen content suggests that denitrification substrate is an important factor influencing the denitrifier community. Warming enhanced plant nitrogen uptake and increased plant foliar nitrogen content in our experiment plots (data not shown), and decreased soil available nitrogen and TN content. This enhanced plant nitrogen uptake could compete with soil microorganisms and in turn affect nitrogen functional gene abundance. However, previous studies observed that temperature rising did not change soil bacterial *nir*K gene abundance in grassland ecosystems [82], and warming increased *nir*K gene abundance in Antarctic soils [83], suggesting that the shifts in warming on the microbial functional traits may be ecosystem-dependent.

4.3. Response of Soil Enzyme Activities to Experimental Warming

Soil enzymes can catalyze complex organic matter and provide low-molecular-weight substrate for microorganisms [84]. Enzyme activity indicates changes of microbial community structure and nutrient demand. β-Glucosidase catalyzes the final step in cellulose depolymerization, the hydrolysis of cellobiose to glucose [24]. This enzyme is important in liberating the nutrients of organic compounds; such action reduces the molecular size and produces small organic structures, thereby facilitating further microbial enzyme activity [51]. Invertase is also crucial in releasing low-molecular-weight sugars, and provides energy resources for microbes. In agreement with our second hypothesis, we found that experimental warming decreased soil invertase and β-glucosidase activities in boreal peatland. Correlation analysis illustrated that these enzymes are positively correlated with soil microbial biomass. The negative effects may be due to the small active microbial biomass and fast depletion of labile organic matter under warming [85]. Enzyme pool reduction [34,86] and microorganism starvation [7] also occur with substrate depletion. German et al. [87] also found that the enzyme production of many substrates decreases with low substrate concentration.

Urease activity indicates the microbial metabolic activity involved in the soil nitrogen cycle. Given that urease is highly resistant to environmental changes [88], our result indicates that warming did not change soil urease activity. Nevertheless, studies of Sardans et al. [51] have also documented that the soil urease activity is improved with experimental warming. Gong et al. [33] demonstrated that warming significantly inhibits urease activity by reducing the availability of the soil enzyme substrate. Phosphatase can catalyze the hydrolysis of soil organic phosphorus compounds [89]; its activity is important in soil phosphorus cycling [90]. We found that warming significantly increased shallow soil acid phosphatase activity. Increased acid phosphatase activity will enhance the soil organic phosphorus dynamic and increase the available phosphorus for plants. Several studies also demonstrated that increasing temperature often enhances phosphatase activity [25,33,51,90]. Since higher temperature favors vascular plants but not Sphagnum mosses, the root activity stimulates vascular plants and triggers acid phosphatase activity [91]. In our study, the observed changes in invertase, β-glucosidase, and acid phosphatase activities, but not in urease, under warming showed that enzymatic-reaction-induced carbon and phosphorus cycles may be more responsive to temperature than the nitrogen cycle.

5. Conclusions

In this study, we observed that warming resulted in changing of soil LOC, available N, carbon and nitrogen cycling microbial functional gene abundances, and enzyme activities in boreal peatland. Our results showed that three years of warming significantly decreased the MBC and NH_4^+–N contents in both shallow soil and deep soil. DOC and TN contents also decreased in deep soil under warming. The reduced LOC, NH_4^+–N, and TN can be attributed to the increased nutrient uptake by plants under warming. Soil bacterial, *pmo*A, and *nir*K abundances were decreased under warming, which accompanied the decreased soil labile carbon and nitrogen availability. Soil archaea and *amo*A gene abundances were increased under warming, which may have been affected by increased soil temperature. In addition, warming resulted in inconsistent changes in soil carbon and phosphorus cycling enzyme activities. We found a significant decrease in soil β-glucosidase and invertase activities and positive relationships with soil MBC. However, warming exerted a significant positive effect on shallow soil acid phosphatase activity. These soil microbial gene abundance and enzyme activity changes may alter carbon, nitrogen, and phosphorus biochemical processes, and affect CO_2, CH_4, and N_2O emissions under climate warming in the boreal peatland. However, since our experiments were carried out in a peatland and had continued for three years, long-term effects at large spatial scales need to be investigated. Especially, the response of plants to warming had significant relationships with soil microorganisms and enzymes, therefore the association between the plant and soil ecosystems and climate warming should be considered in future research.

Supplementary Materials: The following are available online at http://www.mdpi.com/2071-1050/11/3/590/s1, Table S1: Description of primer, amplification details used for quantitative PCR analysis.

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