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Effects of Soil Warming on Soil Microbial Metabolism Limitation in a *Quercus acutissima* Forest in North Subtropical China

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Abstract: In order to explore the influence of climate warming on soil microbial metabolism in the ecosystem and reveal the relationship between soil microbial metabolism limitation and environmental factors, in this study, the effects of warming on soil enzyme activities and nutrient availability were investigated by setting underground heating cables at 2 °C and 4 °C soil warming in a typical *Quercus acutissima* forest in the northern subtropics, and enzyme stoichiometric models were used to evaluate the limits of soil microbial metabolism. The results showed that soil warming significantly increased the activities of β-1,4-glucosidase (BG) and L-leucine aminopeptidase (LAP), and significantly increased the contents of nitrate nitrogen (NO_3^--N) and available phosphorus (AP) in soil. The soil warming increased soil microbial C limitation and alleviated soil microbial P limitation. Our study showed that the change of soil microbial C and P limitation caused by warming may cause a large amount of SOM decomposition in a short period, leading to a large fluctuation of soil carbon turnover, which is not conducive to the stability of the soil C pool. This study provides important insights linking microbial metabolism to soil warming and improves our understanding of C cycling in forest systems.

Keywords: soil warming; soil enzyme activities; nutrient availability; microbial C; P limitation; microbial metabolism

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1. Introduction

Global climate change is one of the global issues that society is concerned about, and its most direct manifestation is global climate warming. Over the last century of observations, the average global temperature has risen by 1.07 °C [1]. According to the simulation results of the CMIP 5 model, by the end of the 21st century, the global average surface soil temperature may rise as high as 2.6~4.8 °C compared with the period from 1986 to 2005, which is most serious environmental problem facing mankind at present. As key biological factors in the terrestrial ecosystem, soil microorganisms play an important role in regulating soil nutrient cycling and are sensitive to soil temperature [2]. Soil microorganisms influence nutrient cycling by producing various enzymes that regulate the decomposition and mineralization of soil organic matter [3,4], which in turn provide energy and available nutrients for soil microbial metabolic activities [5,6]. Therefore, soil enzymes are key participants in microbial metabolism and soil organic matter decomposition, and soil enzymatic stoichiometry can be used as an important index to evaluate soil nutrient availability and soil microbial metabolism [7]. The carbon-metabolizing enzyme β-1,4-glucosidase (BG), nitrogen-metabolizing enzymes β-1,4-*N*-acetylglucosidase (NAG) and L-leucine aminopeptidase (LAP), and phosphorous-metabolizing enzyme acid phosphatase (ACP) are usually the main research objects [8]. Because these four enzymes can catalyze the generation of bioavailable terminal monomers [9], this process is closely related to

nutrient cycling in the ecosystem. At present, there is no uniform conclusion on the response of soil enzyme activities to soil temperature change. Melillo et al. [10] showed that increasing soil temperature would enhance the activities of microorganisms and enzymes, accelerate the decomposition of soil organic matter, enhance soil respiration, and positively affect global change. Liu et al. [11] found that changes such as the decrease of soil water caused by the increase in soil temperature would reduce the availability of soil water and affect the metabolic activities and enzyme activities of soil microorganisms by limiting the diffusion loss of reaction substrates. Many studies showed that the soil enzyme activity was significantly affected by many temperature-related factors, including temperature increase amplitude, soil moisture content, and temperature increase duration [12–14].

Given that temperature is an important driver of ecosystem processes [15], climate warming is bound to affect soil enzyme activities and change ecosystem C, N, and P cycles, which in turn change the nutrient limitation of soil microorganisms [16]. Zheng et al. [17] found short-term soil warming decreased microbial C limitation and increased microbial P limitation by affecting soil available nutrients and soil water content. Therefore, with the background of global warming, it is of great significance to elucidate the variation characteristics of soil enzyme activities for studying soil nutrient availability, microbial nutrient metabolism, and soil C pool stability in specific regions. Moreover, Moorhead et al. [16,18] proposed quantifying soil microorganisms' relative C limitation by vector length and the relative N and P limitation of soil microorganisms by vector angle, which can directly reflect the relative nutrient requirements of soil microorganisms. This method can help us intuitively understand the soil microbial metabolism in the ecosystem with the background of global warming [19] and reveal the relationship between soil microbial metabolism limitation and environmental factors [4]. It is helpful to elucidate the mechanism of soil C, N, and P cycling and thus improve our ability to predict soil C stocks under climate warming [20].

The existing soil warming experiments focus on temperate and tropical ecosystems, and few studies have been conducted in the northern subtropical region, which is particularly sensitive to climate change. To comprehensively assess the effects of climate warming on soil enzyme activities, soil nutrient availability, and soil microbial metabolism characteristics in the north subtropical forest ecosystem, this study took a *Quercus acutissima* forest in the Northern subtropical region as the research subject, and soil climate warming to increase soil temperature (warming by 2 °C, 4 °C) by burying heating cables. Therefore, we hypothesized that: (1) soil warming would have a positive effect on soil enzyme activities; (2) soil warming would decrease microbial C limitation and increase microbial P limitation; and (3) soil water content would be the key factor affecting microbial metabolism limitation.

2. Materials and Methods

2.1. Study Site

The study area is located in Zhenjiang, Jiangsu Province, which belongs to the north subtropical monsoon climate zone with four distinct seasons and sufficient light. The annual average temperature is 15.2 °C, the highest temperature is 39.6 °C, and the lowest temperature is −16.7 °C. The average annual precipitation is 1055.6 mm, with a great inter-annual variation. The average annual relative humidity is 79%. The study area is located in the hilly region of Jianghuai, and the terrain is mostly hilly and gentle. The soil layer thickness is generally 40~60 cm, and the soil pH is 4.5~5.0. The forest vegetation belongs to the east of the north subtropical region of China, and the zonal vegetation is a deciduous broad-leaved mixed forest with evergreen components. The test site is based on the *Quercus acutissima* forest. Shrubs mainly include *Rosa multiflora*, *Fortunearia Sinensis*, *Callicarpa cathayana*, *Ilex cornuta*, *Symplocos paniculata*, etc. Herbaceous plants mainly include *Parthenocissus tricuspidata* and *Adiantum capillus-veneris* [21].

2.2. Experimental Treatments

The study plots were set in Jurong City, Jiangsu Province, and three sample plots were arranged in a *Q. acutissima* forest with relatively uniform topography and slope, with a distance of more than 10 m from each other. Four 3 × 3 m subplots were set in each sample plot, a total of 12 subplots, with a spacing of more than 3 m between the subplots. Four treatments (soil temperature increase of 2 °C, soil temperature increase of 4 °C, disturbed control, and blank control) were randomly applied to four subplots in each sample plot. In December 2019, the same heating cables were laid in parallel in each plot. The heating power per unit area of the soil was calculated according to the plot area, which was arranged in parallel with a depth of 10 cm and a spacing of 20 cm and surrounded in the outermost circle to ensure the uniformity of warming in the plot. The temperature sensor buried at 10 cm was used to collect and compare the soil temperature data of the control field and the warming sample field. The temperature controller scanned every 10 min, and the relay switch was controlled by the temperature controller to warm the soil. The cables were arranged in the same way as before but without heating. Three months after the cable was laid out, it was powered on for warming (March 2020) to reduce the impact of soil disturbance.

2.3. Soil Sample Collection

In March 2021 (1 year of warming), soil samples ranging from 0 to 10 cm were collected in each subplot after removing surface litter according to the 5-point sampling method. Soil samples from 5 points in each plot were evenly mixed to collect a total of 12 bags of soil samples. The samples were quickly frozen with dry ice and brought back to the laboratory. Gravel and plant roots were picked out and divided into two parts after a 2 mm sieve. One part was stored in a −20 °C refrigerator for long-term determination of soil enzyme activities and available nutrients, and the other part was dried naturally for determination of soil physical and chemical properties.

2.4. Determination of Soil Physical and Chemical Properties and Enzyme Activities

Soil TN and SOC contents were determined by an elemental analyzer (Vario EL iii, Elementar, Frankfurt, Germany). Soil TP content was prepared by H_2SO_4-H_2O_2 de-boiling solution and measured by molybdenum-antimony anticolorimetric method. Soil water content was determined after oven-drying 10 g of fresh soil at 105 °C for 48 h. Soil pH was measured by a pH meter (soil and water ratio 2.5:1, Sartorius Gmb H: Gottingen, Germany). Soil DOC concentration was extracted with 0.5 M K_2SO_4 and shaken for 60 min at 200 rpm on a reciprocal shaker. The extracts were filtered through a Millipore 0.45-μm filter and then measured using a TOC analyzer (Shimadzu TOC-L, Kyoto, Japan). Soil NO_3^--N concentration was quantified by spectrophotometry, and soil NH_4^+-N was determined by (indophenol blue) colorimetric method [22]. The activities of BG, NAG, LAP, and ACP in soil were tested with the kit produced by Keming Biology (http://www.cominbio.com/index.html, accessed on 28 March 2022).

2.5. Analysis Methods

Based on the vector theory of enzyme chemistry proposed by Moorhead et al. [18,23], vector length (vector L) and vector angle (vector A) calculated by enzyme stoichiometric ratio were used in this study. Soil microbial nutrient limitation was analyzed by calculating vector length (vector L) and vector angle (vector A) of all data:

$$X = (BG)/(BG + AP)$$

$$Y = (BG)/(BG + NAG + LAP)$$

$$\text{Vector L} = SQRT(X^2 + Y^2)$$

$$\text{Vector A} = DEGREES[ATAN2(X,Y)]$$

where X represents the relative activity of C and P metabolic enzymes and Y represents the relative activity of C and N metabolic enzymes. Vector L is the straight-line distance from the origin to the point (x,y), representing the degree of soil microbial C limitation. Vector A is the arctangent function of the extension line from origin to point (x,y), representing the limitation degree of soil microorganisms N and P. Vector angles greater than 45° indicate microbial P-limit, while vector angles less than 45° indicate N-limit.

SPSS 21.0 (SPSS Inc., Chicago, IL, USA) and Canoco 5.0 were used for data analysis. One-way analysis of variance was used to analyze the differences in soil physical and chemical properties, soil enzyme activities, and enzyme stoichiometric ratios, and Pearson correlation analysis and redundancy analysis were performed. GraphPad Prism 9 was used for plotting.

3. Results

3.1. Effects of Soil Warming on Soil Physical and Chemical Properties

Table 1 showed that compared with T0 treatment, T2 and T4 treatments significantly reduced SOC content by 19.3% and 17.9% (both $p < 0.05$), respectively, and decreased SWC by 14.84% and 20.01%, respectively (both $p < 0.05$). Compared with the T0 treatment, T4 treatment significantly increased the contents of soil NO_3^--N and AP by 18.82% and 98.58% (both $p < 0.05$). Soil N/P under T2 and T4 treatments significantly decreased compared with T0 treatment ($p < 0.05$). Moreover, soil TN, TP, NH_4^+-N, DOC, pH, and C/N had no significant response to soil warming ($p > 0.05$).

Table 1. Soil physical and chemical properties contents under different treatments.

Treatments	T0	T2	T4
pH	4.60 ± 0.21 a	4.45 ± 0.09 a	4.46 ± 0.20 a
SWC (%)	21.05 ± 1.08 a	18.18 ± 0.61 b	17.54 ± 0.64 b
SOC ($g \cdot kg^{-1}$)	22.70 ± 3.99 b	18.33 ± 3.13 a	18.63 ± 2.42 a
TN ($g \cdot kg^{-1}$)	1.53 ± 0.35 a	1.13 ± 0.31 a	1.30 ± 0.17 a
TP ($g \cdot kg^{-1}$)	0.34 ± 0.09 a	0.32 ± 0.08 a	0.35 ± 0.05 a
NH_4^+-N ($mg \cdot kg^{-1}$)	17.75 ± 0.43 a	19.45 ± 4.64 a	16.17 ± 1.86 a
NO_3^--N ($mg \cdot kg^{-1}$)	21.95 ± 4.32 b	24.21 ± 5.17 ab	26.08 ± 5.14 a
AP ($mg \cdot kg^{-1}$)	1.41 ± 0.39 b	2.21 ± 0.57 ab	2.80 ± 0.50 a
DOC ($mg \cdot kg^{-1}$)	141.19 ±16.88 a	153.41 ± 19.20 a	150.82 ± 23.97 a
C/N	14.73 ± 0.56 ab	15.97 ± 1.17 a	14.34 ± 0.37 b
C/P	65.99 ±1.01 a	55.87 ± 6.69 a	53.72 ± 6.09 a
N/P	4.48 ± 0.19 a	3.49 ± 0.51 b	3.74 ± 0.34 b

All results were mean ± standard error. Lowercase letters represent significant differences in soil nutrient contents under different treatments ($p < 0.05$). SWC: soil water content; SOC: soil organic carbon; TN: total nitrogen; TP: total phosphorus; NO_3^--N: nitrate nitrogen; NH_4^+-N: ammonium nitrogen; AP: available phosphorus; DOC: dissolved organic carbon; C/N: SOC/TN; C/P: SOC/TP; N/P: TN/TP. T0: control without warming; T2: 2 °C warming treatment; T4: 4 °C warming treatment.

3.2. Changes in Soil Enzyme Activities and Soil Enzymatic Stoichiometry

In general, soil warming changed soil enzyme activities, but it depended on the type of enzyme. Specifically, compared with the T0 treatment, the activity of BG significantly increased under T2 and T4 treatments by 125.74% and 80.55% ($p < 0.05$, Figure 1a), while the activity of LAP only significantly increased under T4 treatment ($p < 0.05$, Figure 1b). Moreover, NAG and ACP activities did not respond to soil warming ($p > 0.05$, Figure 1c,d).

Soil enzymatic stoichiometry reflects the relative requirements of soil microorganisms for C, N, and P acquisition. In this study, BG: (NAG+LAP), BG: AP, and (NAG+LAP): AP increased with soil warming. Specifically, BG: (NAG+LAP) under T2 treatment was significantly higher than that under T0 treatment ($p < 0.05$, Figure 2a). Compared with the T0 treatment, soil warming significantly increased BG: AP ($p < 0.05$), but had no significant effect on (NAG+LAP): AP (Figure 2b,c).

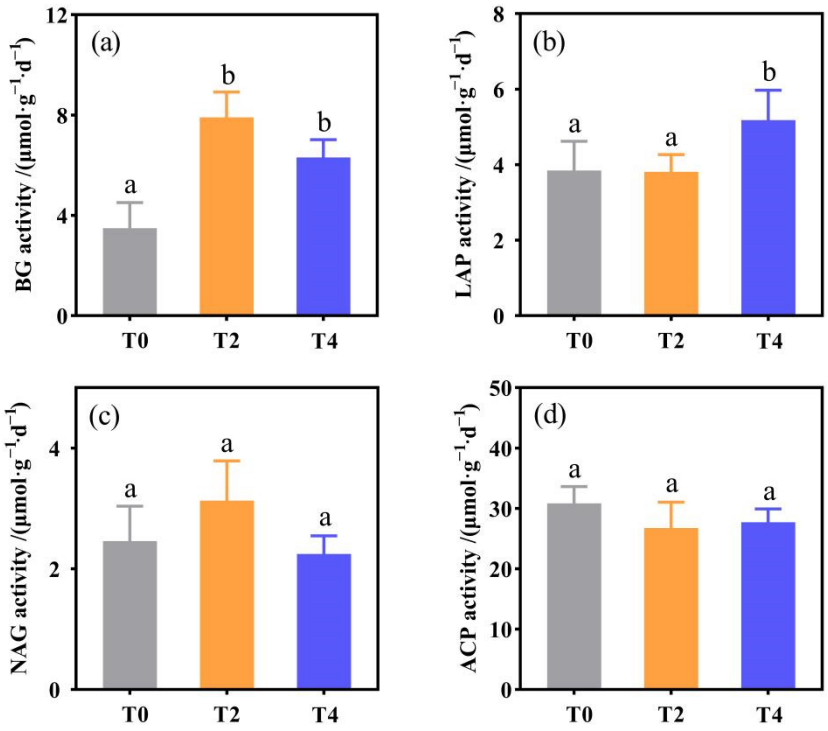

Figure 1. Changes in soil enzyme activities under different treatments: (**a**) the activity of BG (β-1,4-glucosidase); (**b**) the activity of NAG (β-1,4-*N*-acetylglucosidase); (**c**) the activity of LAP (L-leucine aminopeptidase); (**d**) the activity of ACP (acid phosphatase). T0: control without warming; T2: 2 °C warming treatment; T4: 4 °C warming treatment. Lowercase letters represent significant differences in soil enzyme activity under different treatments ($p < 0.05$).

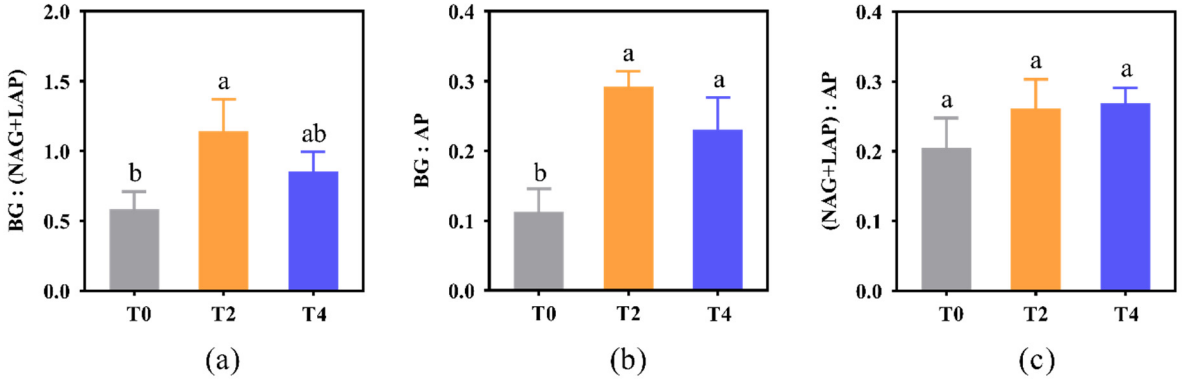

Figure 2. Changes in soil enzymatic stoichiometry under different treatments. (**a**) ratio of C metabolizing enzyme to N metabolizing enzymes; (**b**) ratio of C metabolizing enzyme to P metabolizing enzyme; (**c**) ratio of N metabolizing enzymes to P metabolizing enzyme. BG: β-1,4-glucosidase; NAG: β-1,4-*N*-acetylglucosidase; LAP: L-leucine aminopeptidase; ACP: acid phosphatase. T0: control without warming; T2: 2 °C warming treatment; T4: 4 °C warming treatment. Lowercase letters represent significant differences in soil enzyme activity under different treatments ($p < 0.05$).

3.3. Associations of Microbial C and P Limitation with other Factors

The results of correlation analysis further showed that SOC, SWC, and microbial P limitation were significantly negatively correlated with microbial C limitation ($p < 0.05$, Figure 3a,b,d). AP was positively correlated with microbial C limitation ($p < 0.05$, Figure 3c). Furthermore, AP and NO_3^--N were significantly negatively correlated with microbial P limitation ($p < 0.05$, Figure 3e,f).

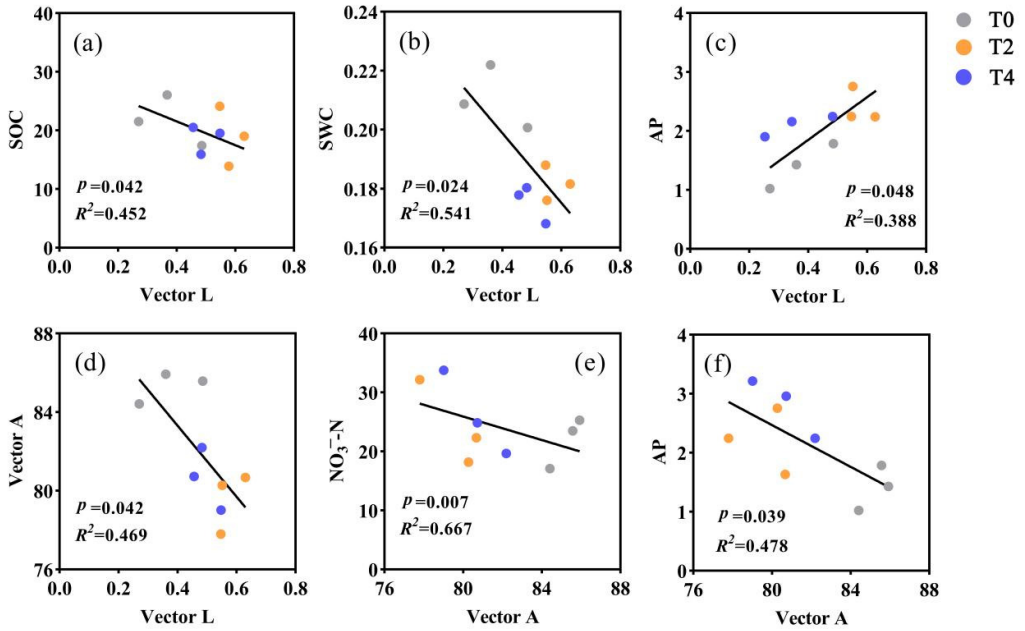

Figure 3. Relationships between microbial C limitation with SOC (**a**), SWC (**b**), AP (**c**), and microbial P limitation (**d**). Relationships between microbial P limitation with NO_3^--N (**e**), AP (**f**). T0: control without warming; T2: 2 °C warming treatment; T4: 4 °C warming treatment.

3.4. Response of Soil Enzyme Activity and Microbial C, P Limitation to Environmental Factors

Dimensionality reduction of the factors involved in this study was carried out by principal components, and the results (Figure 4a) showed that the first two axes of principal components explained 63.70% of the total variance contribution rate. There was a significant difference in PC1 between the T0 treatment and the two warming treatments. SWC, AP, and BG were the main difference factors, but there was no significant difference between T2 and T4 treatment. Among them, SWC, C/P, SOC, C/N, AP, NO_3^--N, NAG, and BG have the highest contribution to the two principal components (Figure 4b).

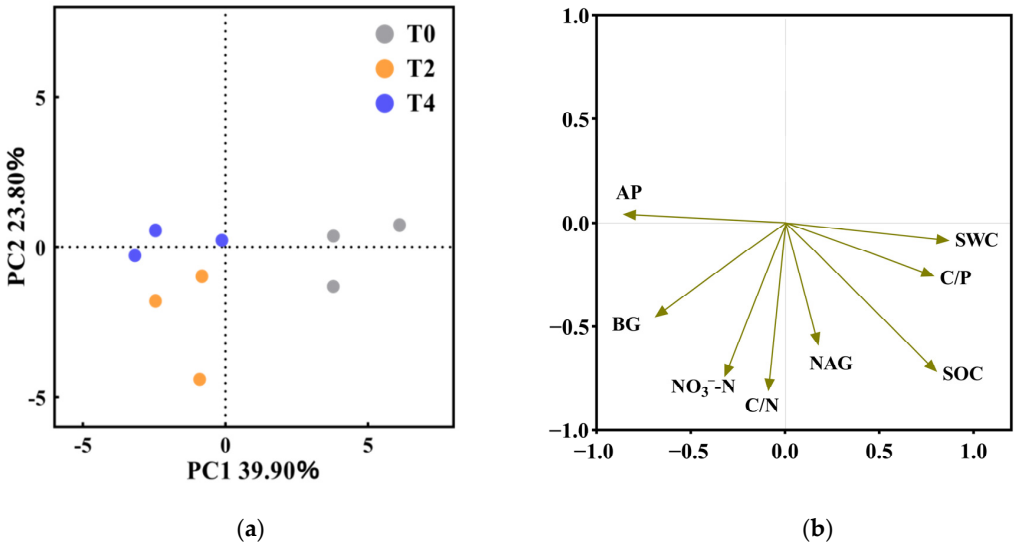

Figure 4. Principal component analysis (PCA) of soil and enzyme activity factors under different treatments. T0: control without warming; T2: 2 °C warming treatment; T4: 4 °C warming treatment. (**a**) Different colors represent different treatments, the farther the distance between points represents the greater the sample difference; (**b**) According to the quality of variables in principal component analysis, eight variables with greater contribution were selected.

RDA analysis was conducted with soil enzyme activity and microbial C, P limitation as response variables, and soil physical and chemical properties as explanatory variables, respectively (Figure 5). The explanation rate of the first two ranking axes reached 77.76%, the first axis explained 57.79% of the variables, and the second axis explained 19.97% of the variables. SWC (p = 0.008) was the significant factor affecting soil, with explanatory rates of 44.6%.

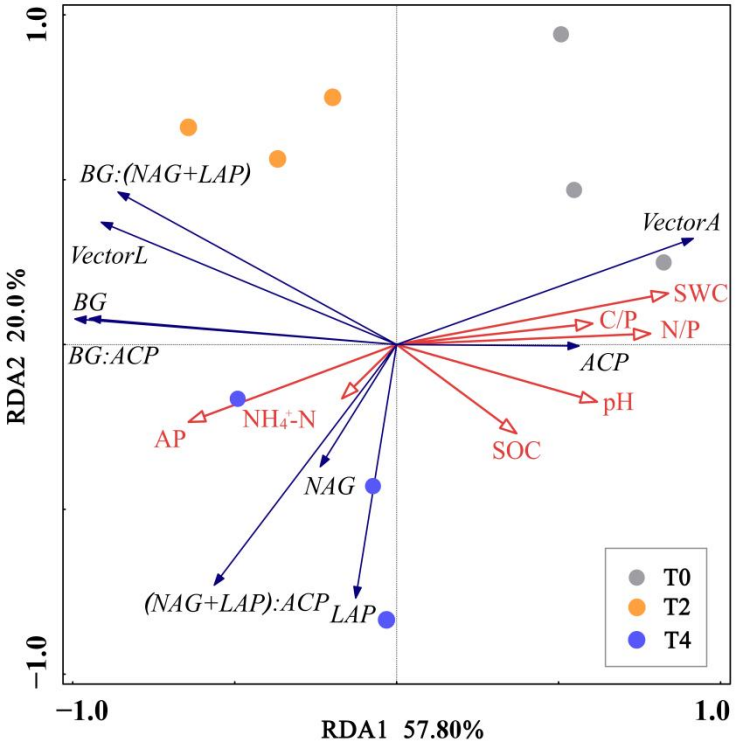

Figure 5. Redundancy analysis (RDA) of soil properties, soil enzyme activities, and microbial C, P limitation. T0: control without warming; T2: 2 °C warming treatment; T4: 4 °C warming treatment. SWC: soil water content; SOC: soil organic carbon; $NH4^+$-N: ammonium nitrogen; AP: available phosphorus; C/P: SOC/TP; N/P: TN/TP; BG: β-1,4-glucosidase; NAG: β-1,4-N-acetylglucosidase; LAP: L-leucine aminopeptidase; ACP: acid phosphatase.

4. Discussion

4.1. Effects of Soil Warming on Soil Enzyme Activities

Soil enzymes are mainly derived from soil microorganisms, plant root exudates, soil animal exudates, and their residues. As reported, soil enzymes are affected by soil temperature [24], soil water content [25], and nutrient availability [26]. The C-metabolizing enzyme BG is associated with a relatively unstable carbon pool and is a major component of cellulase, mainly involved in the hydrolysis of glycosidic bonds between atomic groups in cellulose [27]. In our study, soil warming increased the activity of BG, which is consistent with the results of Caitlin et al. [28]. Moreover, we found that BG activity was significantly negatively correlated with SOC and SWC (Table A1), indicating that the SOC decomposition rate and SWC level may be the main factors affecting its activity under warming conditions [29]. Previous studies have shown that increasing temperature and decreasing SWC will accelerate the cycling rate of unstable carbon and promote the decomposition of SOC [15,30,31]. Qi et al. [32,33] found that temperature change could significantly change soil enzyme activities by affecting the soil water-driven SOC decomposition rate, which was similar to our findings.

The response of soil enzyme activities to different temperature gradients was also different. Specifically, LAP activity increased significantly only under T4 treatment, while NAG and ACP activities did not change significantly. On the one hand, some hydrolases were not sensitive to small temperature increases [33]. On the other hand, since the

samples of this experiment were collected in March, the temperature was relatively low, which would lead to the passivation of some soil enzymes, and the seasonal variation of temperature had a significant impact on enzymes [19,34]. However, these results are in contrast to a recent meta-analysis. Chen et al. [35] studied the effects of soil warming on soil enzymes and found that soil warming only significantly affected oxidase but did not have a consistent and significant effect on hydrolase. One of the author's explanations was that soil hydrolases mainly degrade unstable reaction substrates, and soil warming leads to the decrease of substrate availability, which will gradually inhibit soil enzyme activities [36]. Although warming can directly increase enzyme activity by increasing enzyme kinetics [37], the effects of these two directions cancel each other, resulting in an insignificant response to warming.

Soil pH is also an important factor affecting soil enzyme activities and their stoichiometric ratios [38]. In this study, pH was significantly negatively correlated with BG activity and BG/AP (Table A1), which is consistent with the results of Sinsabaugh et al. [39] on enzyme activities at a global ecological scale. This may be because pH regulates soil enzyme activities by influencing the soil microbial community structure, the binding state between enzymes and soil particles, soil nutrients, and soil functions [40]. In addition, the warming time of this experiment is relatively short, and long-term warming experiments usually exceed 10 years [35,41,42]. In the future, we will focus on exploring the coupling relationship between soil enzyme activities and seasonal dynamic changes in temperature and duration of warming.

4.2. Effects of Soil Warming on Soil Nutrient Availability

The results of this study revealed that the T2 and T4 treatments increased the contents of AP and NO_3^--N in soil and improved soil N and P availability in the *Q. acutissima* forest in the north subtropical region [14,43]. Many studies found that the availability of soil N increased with the increase of soil temperature, because the increasing temperature increases the activities of soil N metabolizing enzymes and related microorganisms, accelerates the mineralization rate of soil N, and promotes the accumulation of soil available N [44,45], which is consistent with the results of our study. However, during the whole experimental period, the response of AP to soil warming was different from the results of previous studies. In contrast to soil N, P supply in soil is controlled by a combination of biotic and abiotic (adsorption/desorption and dissolution) processes [46]. Usually, soil warming promotes the uptake of phosphorus by surface plants, increases the content of litter phosphorus, and reduces soil available phosphorus [41,47]. However, as the *Q. acutissima* forest in the experimental area of this study belongs to an overmature forest, short-term warming of 1 year may not affect its growth.

The correlation results (Table A2) showed that AP and SWC were significantly negatively correlated, indicating that there was some coupling relationship between them. The decrease of SWC may be caused by warming, which is not conducive to the migration and diffusion of P components in the soil [48] and can reduce the leaching loss of slow-available P in the surface soil [49,50], which provides a possibility for the increase of soil AP.

4.3. Effects of Soil Warming on Soil Microbial Nutrient Limitation

Soil microbes can regulate soil nutrient availability, and the change in soil temperature will affect the soil microbial community and activity, thereby affecting the process of nutrient absorption and release [41]. Therefore, there is a close relationship between soil microbial nutrient limitation and soil nutrient availability. However, the limiting factors of soil microbial nutrients under warming conditions in different regions are still unclear. In this study, the lnBG: ln(NAG+LAP): lnACP of soil in the north subtropical *Q. acutissima* forest was about 1:1.57:2.89, which was different from the global average of 1:1:1 [39,41], indicating that N and P elements available to soil microorganisms in the study area were relatively lacking. A serious north subtropical soil microbial P limit has been confirmed, because the soil P element mainly comes from rock weathering, and the rainfall erosion and

soil erosion soil P element loss [51], and in the north subtropical acid soil metal ion binding ability is strong, and availability P element is easily incorporated into the stability of the closed state storage P. The utilization rate of the P element is reduced [50,51]. The soil lnBG: ln(NAG+LAP): lnACP after warming treatment was about 1:1.02:1.71, which gradually tended to be balanced compared with T0 treatment, indicating that warming alleviated the relative N and P limitations of microorganisms in the north subtropical forest system. The analysis showed that BG: ACP was significantly negatively correlated with TN/TP and soil pH (Table A1), which was consistent with Guan's findings [52]. The decreasing trend of TN/TP was induced by soil warming, which further proved that warming alleviated phosphorus limitation in the study area, However, soil P limitation still exists in the study area (Figure 6).

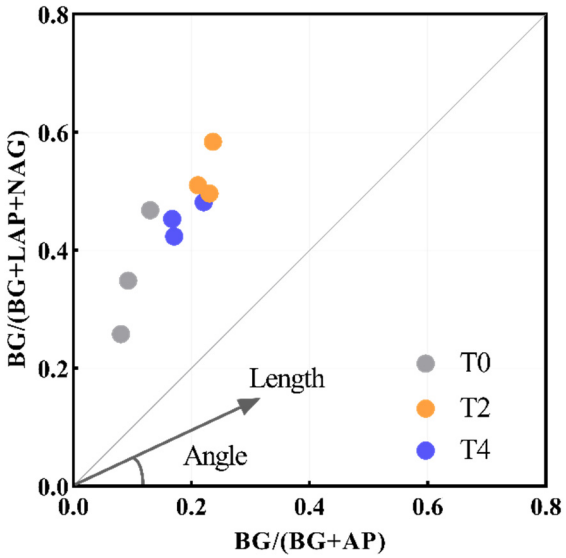

Figure 6. The variation of vector length and angle. T0: control without warming; T2: 2 °C warming treatment; T4: 4 °C warming treatment.

Soil enzymatic stoichiometry can reflect soil microbial nutrient acquisition ability, nutrient resource utilization, and microbial nutrient limitation [5], and sometimes can be used as a biological index to measure soil fertility [53]. Our results revealed that BG: ACP and BG:(NAG+LAP) were significantly increased compared with the T0 treatment. According to the principle of resource allocation, the relative input of soil microorganisms to C metabolic enzymes was greater than that of N and P metabolic enzymes due to warming [54]. In addition, the significant increase of vector L due to warming also indicates the transition of soil microorganisms to the C-limit (Figure 6). Linear regression analysis showed that SOC and SWC were significantly correlated with vector L. The relative increase of soil microbial C limitation may be related to the significant decomposition of SOC caused by warming [32,55]. The results of PCA and RDA showed that SWC is the most important factor affecting soil enzyme activity and soil microbial nutrient limitation among soil physical and chemical properties, and it is also the main factor distinguishing significant differences in soil enzyme activity and soil microbial nutrient limitation under different temperature changes. Therefore, warming may change the decomposition of SOC driven by SWC, thereby indirectly changing soil enzyme activities and microbial metabolic limitation [30,33]. Linear regression analysis showed that AP and NO_3^--N was significantly negatively correlated with vector A, and AP was significantly positively correlated with vector L($p < 0.05$, Figure 3c,e,f), indicating that the limitation of soil microorganisms C and P was strongly affected by the available nutrients of the soil, which was similar to Cui's results [56].

In this study, there were significant changes in soil microbial C and P limitations, and there was a significant negative correlation between them ($p < 0.05$, Figure 3d), reflecting

the coupling relationship between soil microbial C and P limitations under warming conditions, which jointly play an important role in soil nutrient cycling [6]. It is generally believed that soil microbial C and P limitations will accelerate the decomposition of SOM to produce more available nutrients [56], which will stimulate the decomposition of SOC. In this study, soil microbial C limitation was significantly correlated with SOC ($p < 0.05$, Figure 3a), indicating that the limitation of soil microorganisms C may be detrimental to SOC assimilation by soil microorganisms [57–59]. In addition, changes in soil microbial C and P limitations caused by soil warming may cause a large amount of SOM decomposition in a short period and affect soil C sequestration. Under the current global warming, it will be more difficult to accurately estimate global soil carbon storage in the short term.

5. Conclusions

Based on a 1-year field warming experiment, we found that soil warming significantly altered enzyme activity, but it depended on the type of enzyme. Warming significantly increased the activities of BG and LAP, and the changes of these enzymes were directly mediated by warming temperature, seasonal temperature, and SWC. Warming significantly reduced SOC content and soil SWC but significantly increased AP and NO_3^--N contents, and increased soil available N and P. After one year of short-term warming, soil microbial N and P limitations were alleviated due to the improvement of soil available nutrients, but soil microbial P limitation still existed in the north subtropical *Quercus acutissima* forest. The enhancement of soil microbial C limitation was mainly attributed to the decomposition and loss of SOC driven by SWC changes caused by warming, which indirectly changed soil microbial C limitation. In addition, the change of soil microbial C and P limitations caused by soil warming will cause a large amount of SOM decomposition in a short period, which is not conducive to soil C sequestration. Under the current climate warming, accurate estimation of global soil carbon storage will become more difficult.

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Data Availability Statement: The data that support the findings of this study are available from the authors upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Correlation analysis of soil enzyme activities and environmental factors.

	SOC	TN	TP	SWC	PH	NO_3^--N	NH_4^+-N	AP	DOC	C/N	C/P	N/P
BG	−0.84 *	−0.398	−0.021	−0.642 *	−0.717 *	0.436	0.279	0.442	0.046	0.353	−0.356	−0.56
LAP	0.043	0.157	0.148	−0.093	−0.424	0.202	−0.188	0.405	−0.047	−0.506	−0.126	0.116
NAG	0.372	0.158	0.368	0.102	0.143	0.2	0.856 **	−0.249	0.048	0.572	0.001	−0.22
ACP	0.539	0.562	0.101	0.252	0.652	−0.155	0.306	−0.733 *	−0.798 *	0.236	0.903 **	0.881 **
CEs/NEs	−0.439	−0.57	−0.259	−0.61	−0.662	0.256	0.068	0.384	0.053	0.333	−0.332	−0.543
CEs/PEs	−0.365	−0.51	−0.036	−0.642	−0.814 **	0.44	0.15	0.607	0.302	0.241	−0.587	−0.760 *
NEs/PEs	−0.062	−0.121	0.274	−0.183	−0.595	0.379	0.188	0.579	0.474	−0.145	−0.618	−0.572

** and * indicate significant difference at $p < 0.01$ and $p < 0.05$. CEs/NEs: BG/(NAG+LAP); CEs/PEs: BG/ACP; NEs/PEs: (NAG+LAP): ACP.

Table A2. Correlation analysis between environmental factors.

	SOC	TN	TP	pH	SWC	NO_3^--N	NH_4^+-N	AP	DOC	C/N	C/P	N/P
SOC	1	0.961 **	0.844 **	−0.157	0.651	0.431	0.383	−0.408	−0.119	0.394	0.625	0.442
TN		1	0.807 **	−0.085	0.660	0.3736	0.154	−0.387	−0.087	0.124	0.619	0.553
TP			1	−0.405	0.261	0.721 *	0.234	0.050	0.300	0.334	0.122	−0.031
pH				1	0.428	−0.825 **	−0.023	−0.590	−0.141	−0.286	0.231	0.372
SWC					1	−0.240	0.240	−0.832 **	−0.314	0.123	0.763 *	0.705 *
NO_3^--N						1	0.233	0.464	0.221	0.308	−0.146	−0.282
NH_4^+-N							1	−0.322	−0.407	0.861 **	0.376	0.012
AP								1	0.422	−0.187	−0.797 *	−0.710 *
DOC									1	−0.140	−0.692 *	−0.628
C/N										1	0.206	−0.234
C/P											1	0.902 **
N/P												1

** and * indicate significant difference at $p < 0.01$ and $p < 0.05$.

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