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Effect of Soil Acidification on Temperature Sensitivity of Soil Respiration

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Abstract: Soil pH significantly impacts microbial activity and community assembly, which in turn determines the temperature sensitivity (Q_{10}) of soil respiration. Due to the high soil acidification in China, it is necessary to understand how soil acidification impacts Q_{10}. Here, the Q_{10} of soil respiration was examined in a long-term field experiment (1982–present) with different soil pH caused by fertilization management. In this experiment, we selected treatments with neutral pH: (1) no crops and fertilization (CK); (2) crops without fertilization (NF); low pH with (3) crops with chemical fertilization (NPK); and (4) crops with chemical fertilization combined with wheat straw incorporation (WS). Under natural soil temperature changes, we observed that soil acidification lowered the Q_{10} value of soil respiration. Considering only temperature changes, the Q_{10} of soil respiration was strongly associated with microbial community composition, alpha diversity, and soil ammonium nitrogen. Considering the interaction between soil pH and temperature, warming strengthened the negative effect of soil pH on the Q_{10} of soil respiration, and the pathway through which soil pH mediated Q_{10} included not only microbial community composition, alpha diversity, and biomass but also the soil's available phosphorus. This work enhanced our insights into the relationships between Q_{10}, temperature, and soil pH by identifying important microbial properties and key soil environmental factors.

Keywords: soil pH; warming; Q_{10} of soil respiration; soil microbial biomass; microbial community composition; alpha diversity

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1. Introduction

Soil respiration can cause the emission of CO_2 into the atmosphere, driving carbon flux in land ecosystems [1]. As reported previously, global soil respiration generally increases with temperature and ranges from 82 to 98 Pg C yr^{-1}, which is about 10-fold more than the amount from the combination of fossil fuel combustion and deforestation [1–3]. Consequently, slight variations in soil respiration can significantly increase or decrease the CO_2 in the atmosphere and the carbon stored in the soil [4]. The response of soil respiration to temperature changes is represented by the temperature sensitivity of soil respiration (Q10), which can vary depending on a number of environmental factors such as soil physicochemical and microbial properties and temperature [5–8]. However, some ecosystem models, like CASA and TEM, define Q_{10} as a constant value of 2.0; a value that may overrate or underrate the scale of C-climate feedback [2,9]. Therefore, several studies on spatial and temporal changes in Q_{10} have been conducted in forest and pasture ecosystems to accurately estimate the amount of carbon released from specific locations [8,10].

Land use type potentially modulates soil respiration and its Q_{10} variation in regional carbon cycling [11,12]. Different from forest and pasture ecosystems with less disturbance, croplands contribute 0.18% of the total soil respiration in China and have suffered the strongest artificial disturbance [13]. Consequently, a higher value of Q_{10} than in natural ecosystems is generally observed [14,15]. Beyond the climate warming effects, soil microbes that contribute to more than half of soil respiration are stimulated by the disturbance and nutrient enrichment induced by agricultural practices [16–18]. The Q_{10} values of soil respiration in croplands can be greatly affected by the properties of soil microbes, including soil microbial biomass, community structure, and function [19,20]. Thus, a close relationship exists between the Q_{10} and microbial trophic guilds; the copiotrophic strategists (r-strategists) respond positively to high Q_{10} values, while there is a negative response by oligotrophic strategists (K-strategists) [19,21].

Soil pH is widely recognized as a crucial factor in shaping microbial communities in terrestrial ecosystems [22–24]. It not only impacts soil microbial activities but also serves as the key determinant for bacterial diversity and community structure [25,26]. When the pH is low, the concentration of H^+ and Al^{3+} in the soil increases dramatically and damages the absorption and transportation of nutrients in crops. It also destroys the community structure of soil microorganisms, which in turn affects the soil micro-ecosystem and jeopardizes soil health [27,28]. Generally, low soil pH depresses the activity of soil bacteria, reduces the diversity of bacteria, and decreases the relative abundance of the copiotrophic bacterial groups while increasing that of the oligotrophic bacterial groups [29–32]. Accordingly, a strong correlation exists between soil pH and microbial respiration, with soil pH emerging as the primary determinant governing the response of microbial respiration to fluctuating temperatures. For agricultural systems in China, the heavy input of nitrogen fertilization ensures intensive crop production, which also causes the release of redundant fertilizer N (more than 50% into the environment), significantly inducing soil acidification [33,34]. The average soil pH of cropland decreased by about 0.5 units from 1980 to 2000, and the depth of soil acidification reached 150 cm over the last 60 years [35,36]. Because of the serious and widespread soil acidification, soil pH should be taken into account when examining the microbial mechanisms of Q_{10} variation in soil respiration in agricultural systems. Rational fertilization, reduction of continuous cropping, and addition of conditioners to acidified soils are considered to be effective measures that can alleviate soil acidification [37]. As soil pH increased, a significant increase in microbial load and relative microbial abundance was observed. Zhang et al. [38] observed that the negative effects of acidification on soil health were attenuated after the addition of lime. It reduced the abundance of methanogens and, thus, CH_4 emissions.

Several studies have determined the Q_{10} of soil respiration and uncovered the potential mechanisms [8,39,40]. However, numerous Q_{10} values of soil respiration have been estimated using laboratory experiments over the last several decades, and this may have resulted in inaccurate Q_{10} estimations [40,41]. Compared to field experiments, laboratory experiments do not consider the continuous input of organic material and the intact soil structure, both of which are important factors mediating soil respiration [39,40,42,43]. As reported previously, respiration from the new input of plant-derived carbon is more sensitive to temperature than that from soil-derived carbon [44], and isolated soil samples destroy the establishment of the temperature gradient-based movement of soil nutrients [43]. Moreover, pre-incubation of laboratory experiments can result in the loss of labile substrate, which is a key factor in the determination of the Q_{10} of soil respiration [45]. Consequently, the measured Q_{10} value under laboratory conditions is considered lower than that under natural conditions [39]. To accurately determine the Q_{10} value of soil respiration, there is a need for field or natural conditions-based research. Since previous and current environments are continuously shaping soil microbial properties, a long-term experiment employing stable agricultural management was proposed, as this could strengthen the variations in soil microbial properties caused by agricultural management and lessen the impacts of unstable environmental factors [46]. Moreover, a long-term experiment gives the opportunity

to investigate the effects of soil acidification caused by fertilization management on soil respiration and related microbial mechanisms, thereby overcoming the limits of research based on the short-term addition of diluted sulfuric acid to the soil [47,48]. Compared with some simulation experiments, it can more realistically reflect the changes in the soil environment in the natural state. Short-term acidification experiments may significantly reduce soil activity, but it may not be so obvious in long-term acidified soil [49]. Nutrients required by soil microorganisms and soil respiration, such as nitrogen and organic matter, need to be present for a long time before significant changes occur [50,51]. In long-term acidified soils, soil microbial communities may have adapted to the local environment and may differ from changes in microbial dominant species and community composition in short-term acidified soils [27,28].

Herein, we performed a field experiment that started in 1982 and consisted of neutral soils and acidified soils caused by long-term mineral fertilization. The Q_{10} values of soil respiration were determined from field-based measurements of soil respiration in January, February, June, and late May, corresponding to periods of winter wheat seedling and harvesting. Based on a previous study [52], soil microbes made a major contribution to soil respiration during winter wheat seedling and harvest. Thus, we measured the soil microbial properties accompanying the soil physicochemical characteristics and climatic factors to explore the mechanism through which soil pH mediates the Q_{10} of soil respiration. The following questions were answered: (1) How does the temperature sensitivity of soil respiration change due to soil acidification? (2) What are the microbial mechanisms responsible for Q_{10} variation in acidified soils? We hypothesized that a lower Q_{10} would occur in acidified croplands.

2. Materials and Methods

2.1. Study Site and Soil Sampling

Our study was performed as a long-term field experiment that started in 1982 in Mengcheng County, Anhui Province, in the southern region of the North China Plain (32°14′ N, 116°37′ E), which has the longest history of farming activity in China. The soil is a vertisol with a pH of 7.0. The study system consisted of neutral and acidified croplands. For neutral soil, the two treatments were (1) no crops and fertilization (CK), and (2) crops without fertilization (NF). The acidified cropland had experienced long-term chemical fertilization and consisted of two treatments: (3) crops with long-term chemical fertilization (NPK) and (4) crops with long-term mineral fertilization combined with wheat straw incorporation (WS). Winter wheat and soybeans have been planted in rotation since 1982, except from 1993 to 1997 when winter wheat and summer corn were planted.

Soil samples were obtained from a depth of 5–15 cm, with three replicates per treatment, from 15 to 19 January, 12 to 16 February, 25 to 29 May, and 10 to 14 June in 2015. The temperature ranged from 4.1 to 6.5 °C in January and February, and from 20.8 to 27.1 °C in May and June (Figure S1). Upon collection, soil samples were cleaned by removing plant debris and stones, and then, the collected soil samples were homogenized and sieved through a 2 mm mesh, and then divided into three subsamples. An air-dried subsample was used for the analysis of soil physicochemical properties; another was stored at 4 °C for the measurement of microbial biomass and soil physicochemical properties; the third subsample was used for total microbial DNA extraction, which was subsequently stored at −80 °C.

2.2. Soil Respiration Measurement and Q_{10} Calculation

Soil respiration (CO_2) measurements were conducted twice weekly using the static closed chamber method [53,54] during the seedling stages and winter wheat harvest seasons of 2014 and 2015. For soil respiration measurements, a PVC rectangular chamber base was installed in the cropland at a depth of 10 cm, and wheat seedlings were removed within and around the chamber base. Soil air samples were collected using a sampling chamber sized 30 cm × 15 cm × 30 cm. After air samples were collected using 60 mL airtight syringes,

they were immediately transferred into pre-evacuated vials (40 mL) and analyzed using a gas chromatograph (Agilent 7890A, Agilent Technologies, Wilmington, DE, USA) equipped with a flame ionization detector for CO_2 detection.

The formula for calculating the temperature sensitivity of soil respiration (Q_{10}) is:

$$Rs = \alpha e^{\beta T}$$

where Rs represents the soil respiration at a specific soil temperature (mg m^{-2} h^{-1}), T denotes the soil temperature (°C), and α and β are the exponential fitting parameters.

The Q_{10} of soil respiration was calculated as

$$Q_{10} = e^{10*T}$$

2.3. Soil Physicochemical Properties

The measurement of soil physicochemical properties followed the method by Bao (2005). In summary, the soil water content (SWC) was determined after drying for 24 h at 105 °C. Soil pH was determined using a Mettler Toledo Instrument electrode (Shanghai, China) with a water:soil (*w*/*v*) ratio of 2.5:1. The content of soil organic carbon (SOC) was determined using dichromate oxidation, and the Kjeldahl method was used to determine the total nitrogen (TN) (Kieltec Foss 2200, Denmark). Soil dissolved organic carbon (DOC) and nitrogen (DON) were analyzed using a TOC analyzer following extraction with 0.5 M potassium sulfate (K_2SO_4). For soil nitrate-N (NO_3^-) and ammonium-N (NH_4^+) analyses, 5 g of fresh soil was first extracted with 50 mL of 2 M KCl and then measured using a continuous flow analytical system. Soil microbial biomass carbon (MBC) was assessed via fumigation-extraction post-soil sampling [55]. Air-dried soil was used for soil available nitrogen (AVN), available phosphorus (AVP), and available potassium (AVK) analyses using the alkali distribution method, the molybdate colorimetric method [56,57], and the flame photometry method (Cany Precision Instrument Co., Ltd., Shanghai, China), respectively.

2.4. Analysis of Soil Microbial Community

Soil DNA was isolated from 0.5 g of fresh soil preserved at −80 °C using an MP Fast DNA™ SPIN Kit (MP Biomedicals, Solon, OH, USA) according to the manufacturer's guidelines. The bacterial and fungal community diversity and composition were evaluated by amplifying the V4–V5 regions of the 16S rRNA gene using primer pairs 515F/806R and the ITS gene using primers ITS5/ITS2. The purified amplicons with different barcodes were blended in equal proportions, and sequencing libraries of 16S and ITS1 were constructed and sequenced on an Illumina HisSeq Platform at Novogene Bioinformatics Technology Co., Ltd. (Beijing, China). Raw sequence data underwent processing via QIIME [58], and chimeras were generated using UCHIME [59]. Sequences with ≥97% similarity were clustered into operational taxonomic units (OTUs) using the UPARSE software [60]. Bacterial and fungal α-diversity (Shannon index) and community compositions were determined based on 97% OTU similarity. Taxonomic classification of bacterial and fungal taxa was performed using the Ribosomal Database Project (RDP) classifier and the UNITE database, respectively [61,62]. The bacteria and fungi sequence data were archived in NCBI under the BioProject accession numbers PRJNA909425 and PRJNA909421. Based on previous research, the prevalent bacterial and fungal taxa were classified into r- and K- strategists, corresponding to the bacterial and fungal strategies associated with copiotrophic and oligotrophic categories [22,63]. To calculate the bacterial r/K ratio, the Actinobacteria, Betaproteobacteria, Bacteroidetes, Gammaproteobacteria, and Gemmatimonadetes were classified as r-strategists, and Acidobacteria, Alphaproteobacteria, Deltaproteobacteria, Firmicutes, Verrucomicrobia, and Chloroflexi were classified as K-strategists [8,22]. For fungi, the r-strategists included Ascomycota and Zygomycota, while members of Basidiomycota were K-strategists [8].

2.5. Statistical Analysis

The impacts of soil pH on soil respiration, Q10, MBC, and α-diversity (Shannon index) across four treatments with three replicates and four sampling times (see Section 2.1) were analyzed using ANOVA followed by an LSD test for comparing means using SAS 9.4 software (SAS Institute, Cary, NC, USA). Spearman's correlation analyses of the selected variables were conducted and visualized using the "linkET", "ggplot2", and "dplyr" packages in R 4.1.0, with significance set at $p < 0.05$. Permutational multivariate analysis of variance (PERMANOVA) was employed to examine the variation in bacterial and fungal community structure within the soil microbial community using the "vegan" package in R 4.1.0. A two-way ANOVA was carried out to assess the impacts of temperature and agriculture management on bacterial and fungal community compositions.

We conducted Structural Equation Modeling (SEM) to assess the direct and indirect connections between soil temperature, pH, soil microbial properties, and Q_{10} using Amos 17.0 (IBM, SPSS, Armonk, NY, USA). Two goodness-of-fit parameters were employed to evaluate the adequacy of the SEM: (1) the chi-squared test (χ^2; indicating a good fit when $0 \leq \chi^2/df \leq 2$, and $0.05 < p \leq 1.00$), and (2) the root mean square error of approximation (RMSEA; the model showed a good fit when $0 \leq$ RMSEA ≤ 0.05 and $0.10 < p \leq 1.00$ and an acceptable fit when $0.05 <$ RMSEA ≤ 0.08 and $0.05 < p \leq 0.10$) [64]. The final model encompassed the standardized overall impacts of soil temperature, pH, microbial biomass, Shannon diversity, and the microbial r/K ratio on the Q_{10} values. Drawing on prior knowledge, which indicates that t (1) soil temperature drives the Q_{10} by impacting soil microbial properties [8,65]; (2) soil pH can change the Q_{10} of soil respiration [66,67], and (3) the impacts of soil pH on the soil microbial community structure are contingent upon temperature [68], we constructed a conceptual model of hypothetical relationships.

3. Results

3.1. pH and Temperature Sensitivity of Soil Respiration

Fertilization management changed soil pH, and long-term mineral fertilization, alone or combined with wheat straw, resulted in significant cropland acidification (Figure 1a). Consistent with our hypothesis, cropland acidification lowered the temperature sensitivity of soil respiration (Q_{10}) (Figure 1b,c, $p < 0.05$).

3.2. Soil Microbial Biomass and Community

Soil microbial biomass carbon (MBC) is a vital factor indicating soil microbe activity. Both fertilization management and temperature had a substantial impact on MBC (Figure 2a and Table S1: $p < 0.05$). As shown in Figure 2a, higher MBC occurred at higher temperatures, and the positive effect of temperature on MBC was closely associated with fertilization management. The Shannon index was chosen to assess the alpha diversity of the microbial community. Compared to the fungi, which were not significantly different at low and high temperatures between the fertilization treatments, the Shannon index for bacteria in soils with lower pH differed significantly from those in non-acidified soils (Figure 2b).

According to the PERMANOVA test results ($p < 0.05$), fertilization management and temperature were both significant factors in structuring the bacterial and fungal communities (Tables 1 and S2 and Figure S3). For the microbial community composition, the bacterial r-strategists, i.e., Gammaproteobacteria, Actinobacteria, and Gemmatimonadetes, were significantly affected by the fertilization treatment, while the K-strategists, i.e., Alphaproteobacteria, Deltaproteobacteria, and Chloroflexi, were significantly affected by the fertilization treatment, the temperature, and their interaction. Both fungal r- and K-strategists such as Zygomycota and Basidiomycota were notably influenced by fertilization treatment and the interaction between fertilization management and temperature (Figure 3 and Table S3). Moreover, the bacterial and fungal r-/K- strategist ratios were significantly different between all treatments and between acidified and non-acidified soils, with no significant variances between low and high temperatures within each treatment (Figure 3b,d). Acidification tends to reduce concentrations of soil alkaline cations, speeding up soil H^+

pressure and Al^{3+} toxicity, leading to loss of bacterial diversity [27,28]. Secondly, soil pH serves an essential role in membrane-bound proton pumps and protein stabilization, affecting microbial physiological activities [27]. In addition, soil enzyme activities are dependent on pH, and low pH damages their activities, which in turn affect microbial activities [69,70]. Different microbial species have different sensitivities to soil pH changes during acidification, leading to different changes.

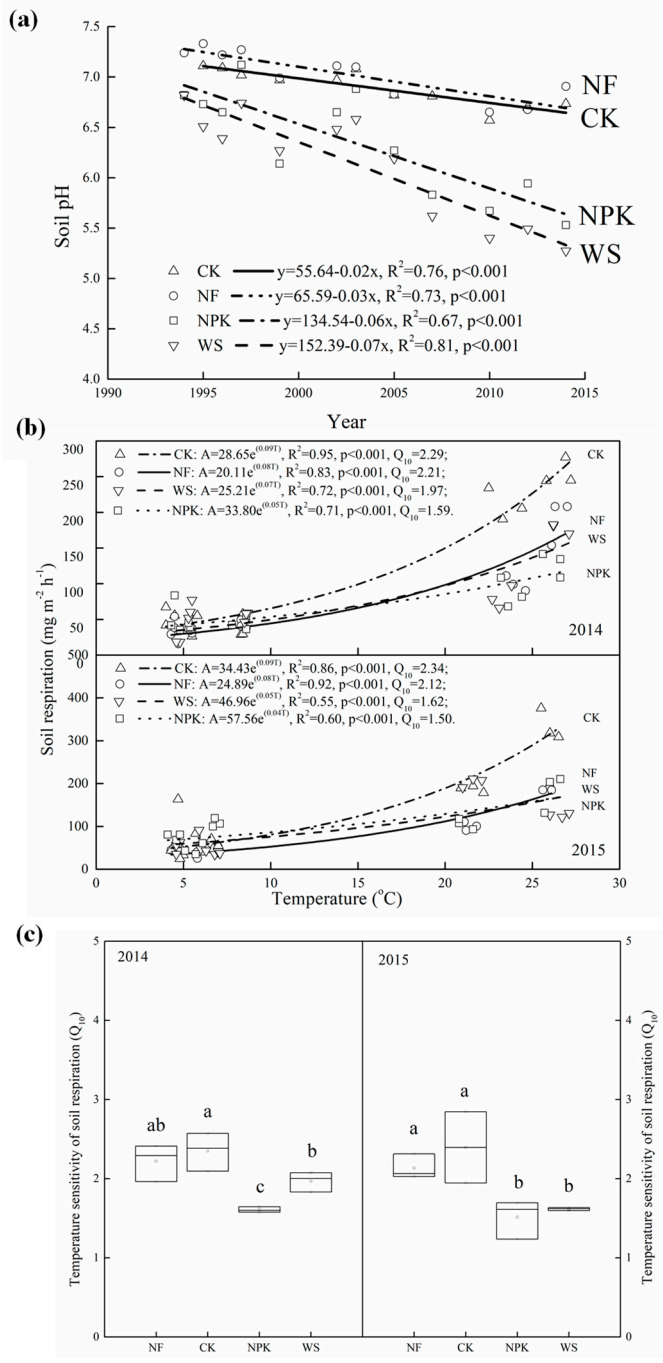

Figure 1. Soil pH from 1994 to 2014 (**a**); the temperature sensitivity of soil respiration (**b**); and ANOVA (**c**) under NF, WS, NPK, and CK treatments in 2014 and 2015. The different lowercase letters in the figure indicate significant differences at the $p < 0.05$ level.

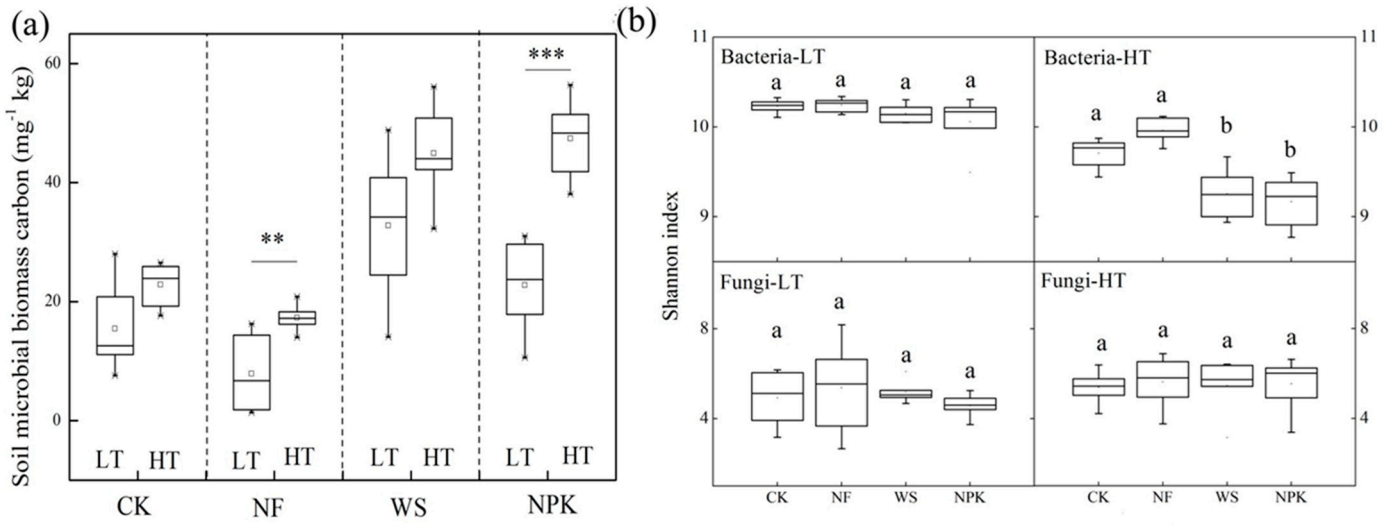

Figure 2. Soil microbial biomass carbon (mg kg^{-1}) (**a**) and the Shannon index (**b**) of soil bacterial and fungal communities at low (LT: <10 °C) and high (HT: >20 °C) temperatures. The symbols *** and ** and the different lowercase letters denote significant differences at $p < 0.001$, $p < 0.01$, and $p < 0.05$ levels, respectively.

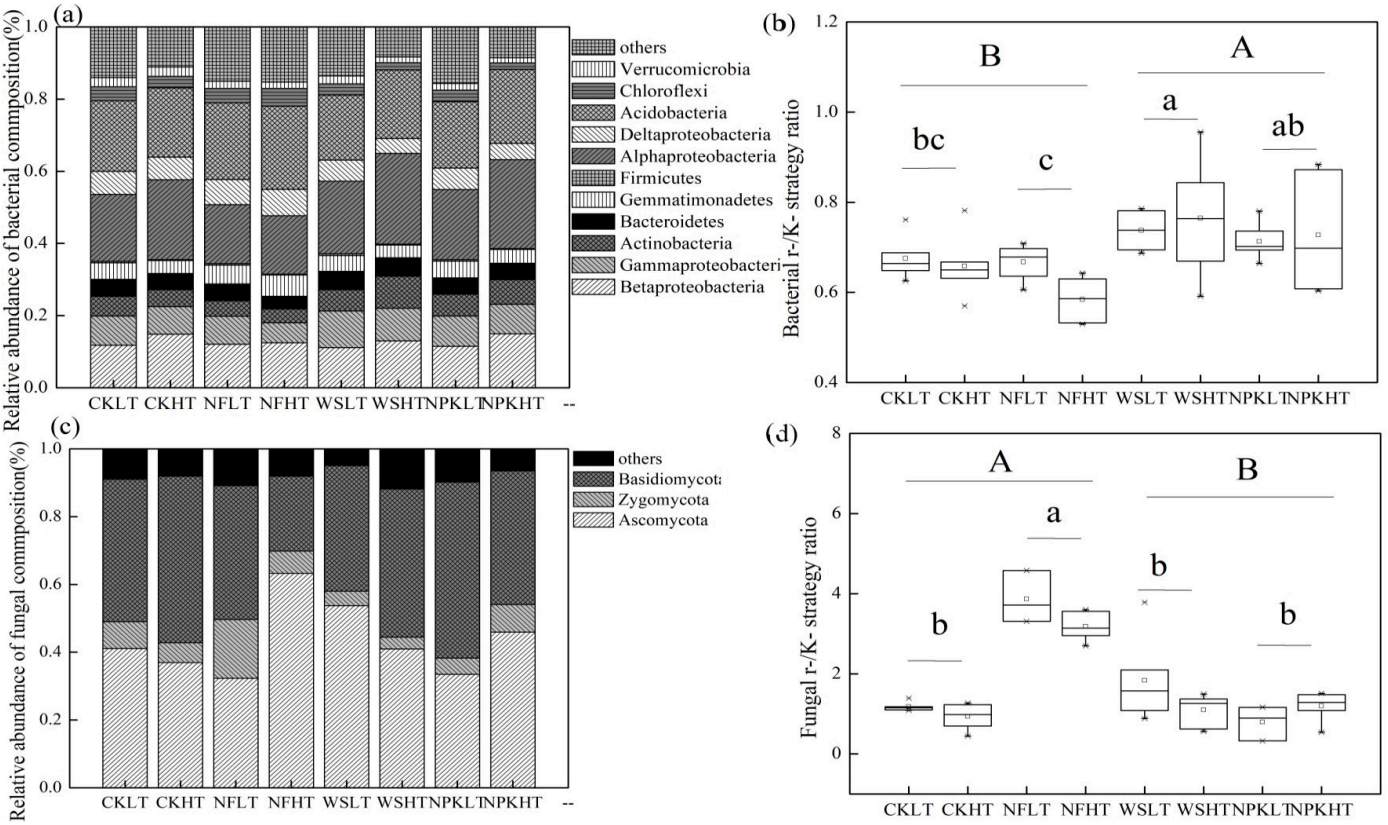

Figure 3. Relative abundances of soil bacterial (**a**) and fungal (**c**) compositions at phylum and class levels, and the ratios of r- and K-strategists (**b**,**d**) under CK, NF, WS, and NPK treatments at low (LT) and high (HT) temperatures. The different upper- and lowercase letters in (**b**,**d**) indicate significant differences between low and high temperatures for each treatment at the $p < 0.05$ level.

Table 1. The PERMANOVA test for soil microbial diversity.

	Treatment		Temperature		Treatment * Temperature	
	F Value	*p*	F Value	*p*	F Value	*p*
Bacteria	1.66	<0.001 ***	4.24	<0.001 ***	1.17	0.04 *
Fungi	2.04	<0.001 ***	1.59	0.02 *	0.95	0.69

The symbols *** and * denote significant differences at $p < 0.001$ and $p < 0.05$ levels.

3.3. Determinants Influencing the Q10 of Soil Respiration

SEM was performed to differentiate between the direct and indirect effects of temperature, soil pH, and microbial properties, including microbial biomass, alpha diversity, and life strategies, on Q_{10} (Figure 4). As shown in Figure 4a,b, the final fits of the two SEM models were good (Figure 5a: $p > 0.05$, $\chi^2/df = 1.81$, RMSEA = 0.07, and GFI = 0.99; Figure 5b: $p > 0.05$, $\chi^2/df = 1.43$, RMSEA = 0.06, and GFI = 0.99). Overall, the total effects of the alpha diversity, microbial community composition, and soil pH on Q_{10} were positive, consistent with the lower Q_{10} in croplands under acidification (Figure 1b).

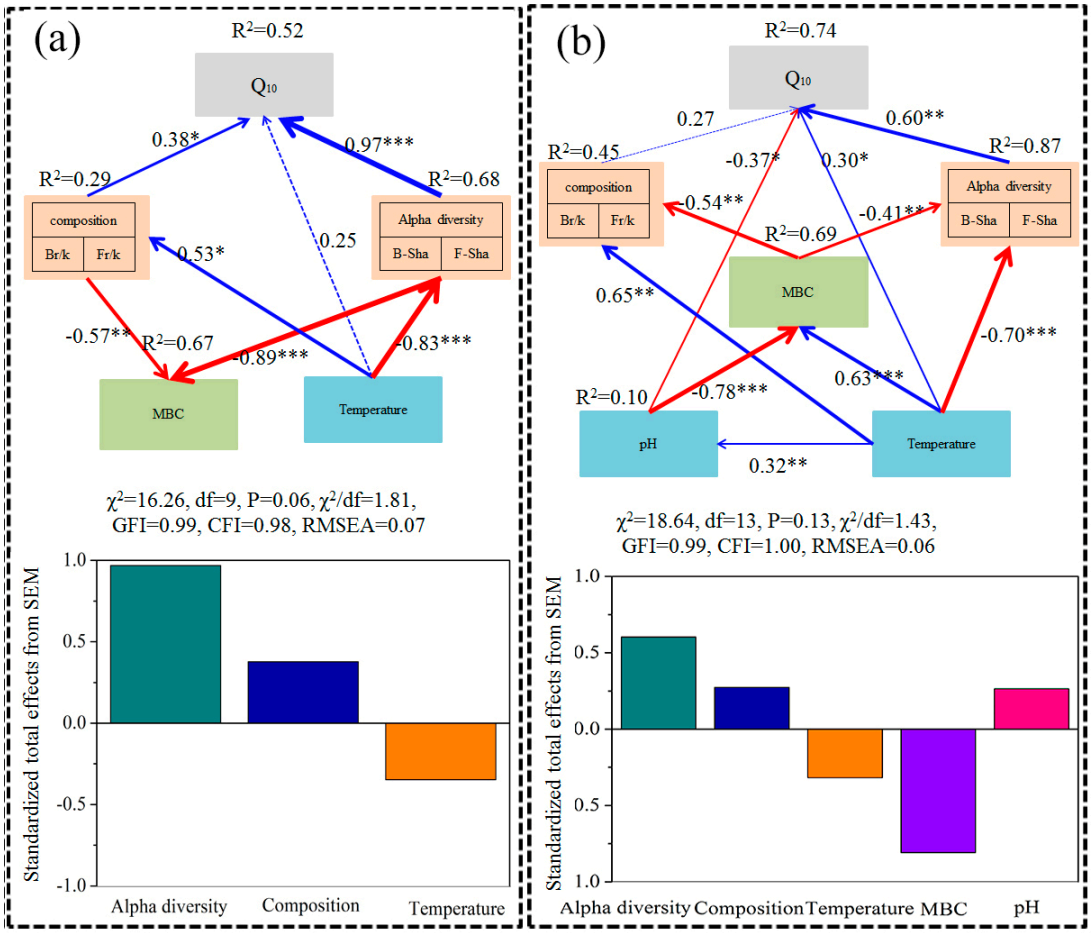

Figure 4. Factors and their total standardized effects on Q_{10} evaluated using SEM (**a**,**b**). The alpha diversity encompasses both the bacterial and fungal Shannon diversity and the bacterial and fungal r-/K-strategist ratios for microbial community composition. The Br/k and Fr/k represent Bacterial r-strategists/K- strategists and Fungal r-strategists/K- strategists, respectively. The colored arrows in the figure denote significant positive (blue) and negative (red) relationships ($p < 0.05$), with arrow thickness representing the strength of the relationship. The numbers adjacent to the arrows and the variances represent standardized path coefficients and the percentage of variance (R^2) explained by the model. ***, **, and * indicate significant differences at the $p < 0.001$, $p < 0.01$, and $p < 0.05$ levels, respectively.

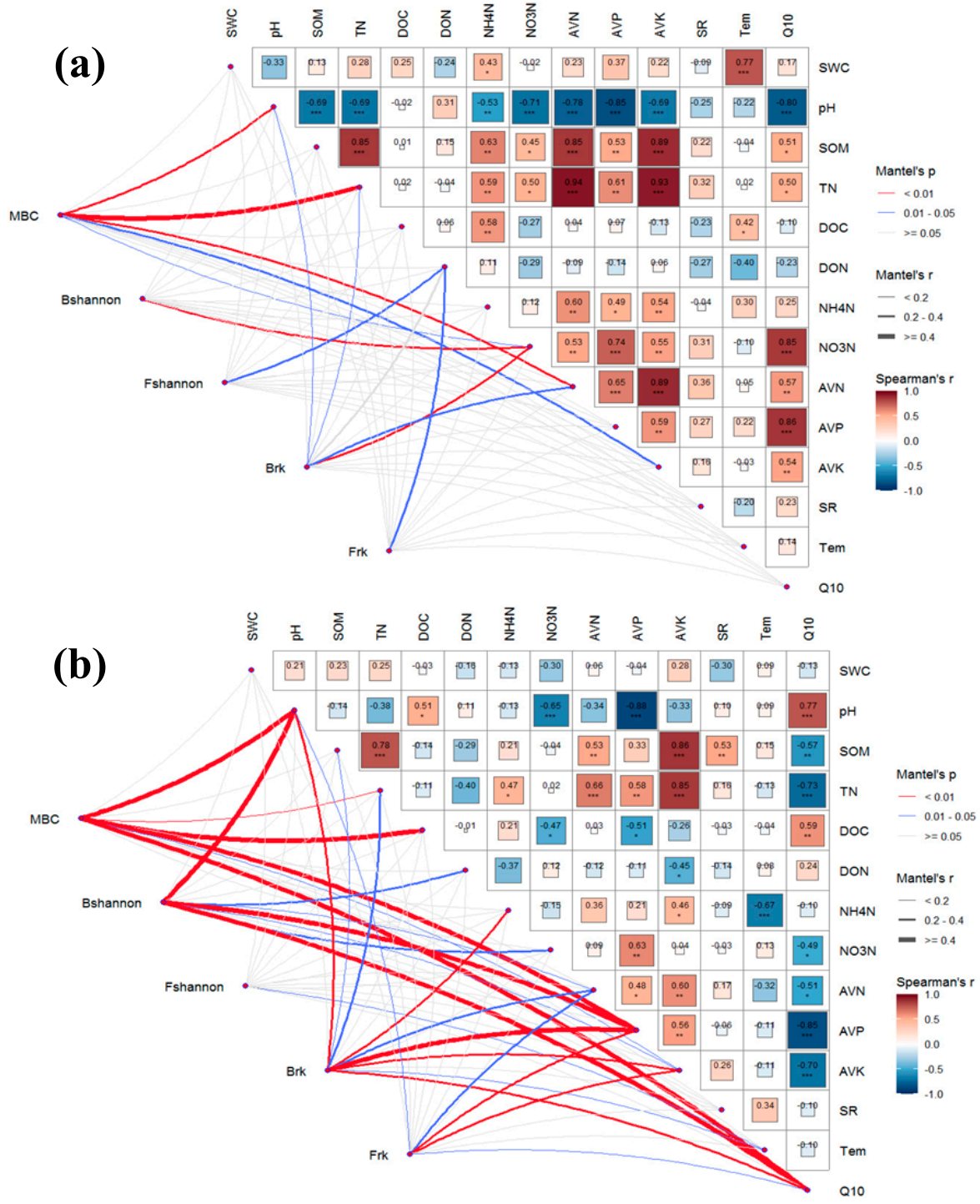

Figure 5. Spearman's correlation analysis of microbial biomass carbon, Shannon diversity, r-/K-strategist ratio, and environmental factors at low (**a**) and high temperatures (**b**). Significance levels are represented by red, blue, and white lines for 0.01, 0.05 levels, and non-significant effects ($p > 0.05$), respectively. The width of the line corresponds to the strength of the relationship, measured by Spearman's r value. Pairwise comparisons of soil properties, soil respiration, and temperature are depicted in the right panel. The color intensity of the correlation coefficients ranging from negative to positive varies from blue to red. Statistical significance is denoted by *** at $p < 0.001$, ** at $p < 0.01$, and * at $p < 0.05$.

Temperature had an indirect impact on Q_{10} by altering the microbial community composition and alpha diversity (Figure 4a). However, the effects of temperature on Q_{10}

varied based on soil pH. Q_{10} was also positively affected by temperature (Figure 4b). Thus, the direct and indirect negative impacts of soil pH on Q_{10} were closely correlated with temperature. Spearman's correlations between Q_{10}, soil, and microbial properties were analyzed based on low temperature (<10 °C) and high temperature (>20 °C) (Figure 5). In line with the SEM analysis, Spearman's correlation analysis supported the positive correlations between pH and Q_{10} at high temperatures, but a nonsignificant relationship occurred at low temperatures. Furthermore, the negative impacts of soil acidification on Q_{10} were caused by decreasing the r-/K- strategist ratio, microbial biomass, and bacterial Shannon diversity (Figure 6).

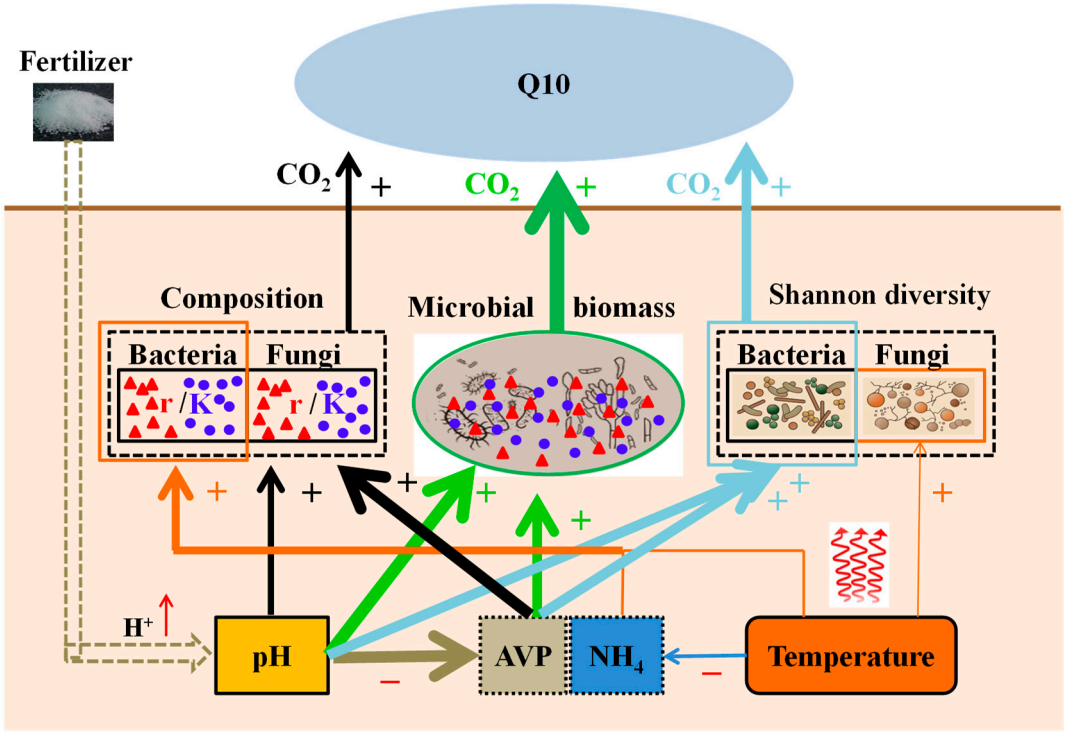

Figure 6. Conceptual model of microbial mechanisms driving the Q_{10} of soil respiration as soil acidification caused by fertilization management. AVP means available phosphorus. The width of the arrow corresponds to the strength of the correlation coefficient. The plus signs indicate positive effects and the minus signs indicate negative effects.

4. Discussion

4.1. Soil pH and the Q_{10} Value of Soil Respiration

Temperature sensitivity (Q_{10}) is a valuable index for describing the changes in soil respiration [71,72], making it crucial for predicting ecosystem-level responses to climate change. Field-based measurements of soil respiration revealed that Q_{10} in neutral soils under CK and NF treatments ranged from 2.12 to 2.34, values consistent with those of a previous study [73], which obtained a value of 2.25 ± 0.28 for the Q_{10} in China. In addition, we found a lower Q_{10} (Q_{10} < 2.0) in acidified soils under the WS and NPK treatments. This indicates an affirmative answer to our first question: will soil acidification decrease the Q_{10} value? This study is the first to clearly demonstrate the impact of long-term mineral fertilization-induced soil acidification on the temperature sensitivity of soil respiration. However, contrary to Yang et al. (2021) [48], who reported a decrease in soil respiration due to lower soil pH, we did not observe a positive relationship between soil respiration and soil pH (Figures 5 and S2). This may be attributed to the short-term addition of diluted sulfuric acid to soils by Yang et al. (2021) [48], a factor that could introduce extra error in the measurement of soil microbial properties caused by interannual variations in climate variables. In contrast, the soil acidification process in this research lasted longer than two

decades, and the climatic effects on soil microbial properties were masked by the effects of cropland management [46].

Furthermore, we found a stronger effect of soil pH than DOC on Q_{10} (Figure 5a) at low temperatures, in contrast to previous studies [74,75], emphasizing the significant importance of available substrate (DOC) on the Q_{10} value. One reason may be related to our experimental design. In comparison with previous research that determined the Q_{10} of soil respiration through laboratory-based incubation [8,75], we quantified the Q_{10} using field-based measurements of soil respiration. This method considers the continuous input of organic material through plant litter or root exudates in the field and lessens the limits of substrates available to soil microbes [39]. Another important reason may be the pre-incubated treatment used in most studies. In general, the pre-incubation period was generally longer than several days, and this may have resulted in a substantial loss of available soil substrates [8,76].

4.2. Soil Microbial Properties and Q_{10} Variations

Soil microbes, as the key participants mediating the global carbon cycle, contribute over half of the total soil respiration [17,18] and microbial properties are considered more important for the Q_{10} than the substrate and soil's chemical properties [20]. Our study revealed a positive correlation between microbial alpha diversity, community composition, and the Q10 of soil respiration under warming conditions, aligning partially with previous findings [6,8] and emphasizing the importance of the microbial r-/K-strategist ratio for Q_{10}. Furthermore, soil microbial biomass had no direct effects on the Q_{10} value in the presence of warming or warming combined with soil acidification conditions (Figure 4a,b). These results indicate that microbial alpha diversity and community composition play a more substantial role in Q_{10} variation than microbial biomass.

Temperature is considered the primary controlling Q_{10} [2], and the Q_{10} value generally decreases as the temperature increases [15]. The reasons could be the lower bacterial diversity at higher temperatures and the warming selection of microbial communities with higher carbon use efficiency, such as K-strategists [68,77]. However, when considering the joint impacts of temperature and soil pH on Q_{10}, we should consider the direct positive influence of temperature on soil pH (Figure 4b). This is an interesting phenomenon, indicating that warming could strengthen the negative impacts of soil pH on the Q_{10}. We speculate that the effects of interaction between treatment and temperature on the soil bacterial community and the warming and soil acidification selection on microbial communities with K-strategists (Table 1) were the main reasons for this [12,30–32,77]. As soil acidification occurs widely in terrestrial ecosystems in China, the effect of temperature on the negative impact of soil pH on Q_{10} may be an important reason for the increase in the Q_{10} with latitude [14,21,33,78,79].

4.3. Soil Acidification, Warming, and The Pathways Controlling the Q_{10} of Soil Respiration

Soil pH and temperature are both important factors that affect the Q_{10} of soil respiration [2]. However, in comparison to temperature, we observed a more complex pathway for soil pH's mediation of Q_{10} (Figure 6) [80]. For example, in addition to the microbial community composition and alpha diversity, pH also mediated Q_{10} through the microbial biomass. This could be attributed to enhanced soil phosphorus availability, while soil acidification occurred in neutral or alkaline soils, and this then supported microbial growth (Figure 2a) [81]. Nonetheless, soil acidification still decreased the respiration of microbes and selected a community with high carbon use efficiency (Figure 6) [82]. Consequently, we observed an interesting phenomenon: the simultaneous soil acidification partly canceled the effects of warming on Q_{10}, and more organic carbon was left in the acidified soils; this supports previous findings [24,83], i.e., that soil acidification can be an important way of promoting the accumulation of soil organic carbon by decreasing microbial mineralization.

Unlike the indirect influences of soil pH on Q_{10} by changing soil phosphorus availability, the "spillover effect" of temperature was confined to soil ammonium nitrogen

(Figure 6). Our findings suggest the importance of nitrogen availability in driving Q_{10} variation under warming conditions, different from previous laboratory results, which emphasized substrate availability [39]. In addition to continuous input from available substrates under natural conditions, we speculated that intact soil profiles in field-based studies may be another reason [43,84]. For example, Sahoo et al. [43] found that low temperatures in winter resulted in significant production of available nitrogen in deeper soil layers and that increasing temperatures during the transition from winter to spring benefited the movement of available nitrogen toward the soil surface. Moreover, considering the greater sensitivity of fast-growing r-strategists to temperature fluctuations compared to slow-growing K-strategists [85], we observed completely different pathways mediating the Q_{10} of soil respiration between soil acidification and warming. Nonetheless, our findings were based on site-level research. Further detailed investigations at the site level are warranted to validate the microbial mechanisms underlying soil acidification-driven changes in Q10.

5. Conclusions

In this investigation, we assessed the temperature sensitivity (Q_{10}) of soil respiration in neutral and acidified croplands during the seedling stage and at winter wheat harvest time. Soil acidification caused by long-term mineral fertilization lowered the Q_{10} of soil respiration. Under warming conditions, the Q_{10} of soil respiration was intricately linked to the microbial community composition, alpha diversity, and soil ammonium nitrogen. Moreover, we observed that the interaction between pH and temperature had an effect on the Q_{10} of soil respiration; warming strengthened the negative impact of soil pH on the Q_{10} of soil respiration. Additionally, the pathway through which soil pH mediates the Q_{10} involved not only microbial community composition, alpha diversity, and biomass but also the soil's available phosphorus. To our knowledge, this study is the first to elucidate the microbial mechanism underlying soil acidification's influence on the Q10 of soil respiration in field settings.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14051056/s1, Table S1. Two-way ANOVA test of soil microbial biomass carbon (MBC) at low and high temperatures under NF, CK, WS, and NPK treatments. Table S2. Beta diversity of soil bacterial and fungal communities under CK, NF, WS, and NPK treatments under low (LT) and high (HT) temperatures based on the Aitchison distance. Table S3. Two-way ANOVA for soil bacterial and fungal community composition under NF, WS, NPK, and CK treatments. Figure S1. Soil temperature in 2014 and 2015 under the NF, NPK, WS, and CK treatments. Figure S2. Soil respiration (mg m^{-2} h^{-1}) in 2014 and 2015 under the NF, NPK, WS, and CK treatments. The different lowercase letters indicate significant differences between months for each treatment, and the different uppercase letters represent significant differences between treatments. Figure S3. Principal component analysis (PCA) for soil microbial diversity: (a and b) soil bacterial diversity, (c and d) soil fungal diversity, (a and c) soil microbial diversity at low temperatures, (b and d) soil microbial diversity at high temperatures.

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