

Increasing stand age enhances temperature sensitivity of soil microbial respiration across a mulberry plantation chronosequence

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Abstract

Aims

Stand development can modify soil substrate properties and microbial community composition, thereby regulating soil microbial respiration and its temperature sensitivity (Q_{10}). However, these responses remain poorly understood in mulberry (*Morus alba*) plantations, as an important component of agricultural ecological systems.

Methods

Here, we investigated a 0–100 year mulberry plantation chronosequence in Suzhou, China, combining measurements of soil physicochemical properties, microbial community composition, and laboratory incubations across multiple temperatures.

Results

The results showed that stand age significantly increased soil microbial respiration rate and enhanced its temperature sensitivity. Specifically, the Q_{10} value in 100-year-old stands decreased from a maximum of 1.81 under the 15–25°C incubation regime to 1.05 under the 25–35°C regime, indicating a marked reduction in temperature sensitivity at higher temperatures. Across all stand ages, Q_{10} consistently decreased with increasing incubation temperature. Variations in Q_{10} were closely associated with soil available nitrogen (AN), available phosphorus (AP), soil organic carbon (SOC), soil water content, as well as the structure and diversity of soil microbial communities.

Conclusions

These findings suggest that long-term mulberry cultivation enhances the temperature sensitivity of soil microbial respiration primarily through changes in substrate availability and associated shifts in microbial community structure during stand development. Our study highlights the critical role of soil biogeochemical processes in regulating carbon cycling in managed plantation ecosystems. The results also provide a scientific basis for maintaining soil functionality to support the long-term sustainability of sericulture systems and the conservation of traditional mulberry-associated cultural landscapes.

Introduction

Soil is the largest carbon pool in the terrestrial ecosystem and plays a critical role in regulating global climate (Sanderman et al. 2017). Soil microbial respiration is a major pathway of carbon release from soil to the atmosphere, and even small changes in this flux can substantially affect atmospheric CO_2 concentrations and climate feedbacks (Kuzyakov 2006; Schindlbacher et al. 2009). Because temperature strongly regulates microbial respiration, its temperature sensitivity (commonly expressed as the Q_{10} value) is considered a key indicator of soil carbon emissions (Davidson et al. 2006a; Zhou et al. 2009; Chen et al. 2010). Under the scenario of climate warming, understanding the factors controlling microbial respiration and its temperature sensitivity is therefore essential for predicting ecosystem carbon dynamics.

Climate warming is expected to stimulate soil microbial respiration and accelerate soil carbon loss, potentially creating positive feedbacks between terrestrial carbon cycling and climate change (García-Palacios 2021). Increased temperature can enhance microbial metabolic activity and extracellular enzyme production, thereby increasing the decomposition of soil organic matter (Adekanmbi et al. 2023). Warming can also alter plant carbon allocation, root exudates, microbial community composition, and substrate availability, further influencing microbial carbon-use strategies and carbon turnover (Davidson et al. 2006a; Domeignoz-Horta et al. 2023). Yet, the responses of soil microbial respiration to warming can vary considerably among ecosystems, largely because of differences in soil physicochemical properties, substrate quality, and microbial community composition (Chen et al. 2020; Meyer et al. 2018; Bahn et al. 2010). For example, warming-induced reductions in soil moisture can constrain microbial activity and root growth, thereby decreasing the temperature sensitivity of soil respiration (Byun et al. 2021; Gomez et al. 2020). In addition, variations in litter inputs, substrate availability, microbial community composition, and root activity have been shown to modify microbial metabolic processes and contribute to spatial and temporal differences in respiration rates and Q_{10} values across ecosystems (Shabaga et al. 2015; Tian et al. 2025).

Stand development can substantially influence soil microbial respiration and its temperature sensitivity by altering soil properties and microbial community characteristics. As plant density and age change, plant community composition and structure undergo corresponding shifts, leading to variations in litter inputs, root exudation, and rhizosphere processes (Bormann and DeBell 1981; Huang et al. 2021). These changes can further modify substrate availability and soil physicochemical conditions, including soil organic carbon content, C/N ratio, soil moisture, and pH (Lucas-Borja et al. 2016). Simultaneously, changes in resource availability and soil environments can drive microbial community succession and reassembly (Liu et al. 2020; Zhang et al. 2024; Dang et al. 2017; Zhou et al. 2017). Despite the recognized importance of stand development in shaping soil conditions and microbial communities, how these changes regulate soil microbial respiration and its temperature sensitivity across stand-age gradients remains poorly understood.

As plantation ecosystems continue to expand globally and contribute increasingly to terrestrial carbon sequestration, understanding the mechanisms regulating carbon cycling in managed forests has become increasingly important (Gao et al. 2023; Ni et al. 2022). Mulberry (*Morus alba*) plantations represent a unique long-term managed ecosystem that underpins traditional sericulture systems in China (Jiao et al. 2020). Owing to their long cultivation history and lifespan, mulberry plantations encompass a wide range of stand ages, providing an ideal chronosequence for examining the ecological effects of stand development. Although mulberry plantations play important economic, ecological, and cultural roles, particularly in traditional agricultural heritage systems such as the Mulberry-dyke Fish-pond system, their belowground carbon dynamics remain poorly understood. In particular, the effects of stand age on soil microbial respiration and its temperature sensitivity have received little attention.

To address this knowledge gap, we investigated a chronosequence of mulberry plantations ranging from 0 to 100 years old. Specifically, our objectives were to: (1) characterize changes in soil physicochemical properties and microbial community attributes across different stand ages; and (2) determine the variation patterns and key drivers of soil microbial respiration and its temperature sensitivity (Q_{10}) during stand development.

Materials and Methods

The study area is located in Wujiang District, Suzhou City, Jiangsu Province, China, situated in a humid subtropical monsoon climate zone with four distinct seasons, mild climate, and sufficient sunshine. The average annual precipitation is 1151.3 mm, the average sunshine duration is 1912.7 hours, the average temperature is 16.3°C, and the frost-free period is 221 days. The soil in this area is loose and fertile, and it is a typical mulberry producing area in China with a long history of mulberry cultivation and mature planting models. The Wujiang silkworm area has suitable temperature and humidity, high accumulated temperature, and a long growing season. Over hundreds of years of long-term selection, the leading variety "Hu Sang" has been cultivated, resulting in extensive mulberry orchards.

Experimental Design

A randomized block design was used, with 7 treatment groups set based on the mulberry planting duration: (a) 5-year-old mulberry (T1); (b) 10-year-old mulberry (T2); (c) 15-year-old mulberry (T3); (d) 30-year-old mulberry (T4); (e) 70-year-old mulberry (T5); (f) 100-year-old mulberry (T6); (g) no mulberry cultivation soil (Control, CK). Three 20 m × 20 m plots were established as biological replicates, with a spacing of 50 m between adjacent plots. Within each plot, seven 2 m × 2 m subplots were deployed (Fig. S1). A 1 m wide isolation belt was set between adjacent plots to avoid cross-contamination. All field management measures in the mulberry planting areas (fertilization, irrigation, weeding, etc.) were consistent and followed local conventional planting standards.

Soil Sampling

Soil sampling was conducted in May 2025 (vigorous growth period of mulberry). A five-point sampling method was used in each plot: a stainless steel soil auger with a diameter of 5 cm was used to collect 0–20 cm surface soil. Five soil cores were collected from each plot and thoroughly mixed into one sample. After removing stones, plant residues, roots, and other impurities, the soil samples were thoroughly mixed. Each mixed sample was divided into two parts: one part was stored at 4°C for the determination of soil physicochemical properties, nutrient content, microbial culture, and enzyme activity; the other part was stored in a -80°C ultra-low temperature freezer for high-throughput sequencing analysis of soil bacterial 16S rRNA genes and fungal ITS regions.

Determination of Soil Physicochemical Properties

Soil water content: Determined by the oven-drying method. 10 g of fresh soil sample (accurate to 0.001 g) was weighed into a known-mass aluminum box and dried to constant weight in a constant temperature oven at 105°C. The soil water content (%) was calculated as: (mass before drying - mass after drying) / mass after drying × 100%.

Soil pH value: Determined by the potentiometric method. Air-dried soil samples passed through a 2 mm sieve were mixed with CO₂-free distilled water at a ratio of 1:2.5 (soil:water, mass-volume ratio). After stirring evenly and standing for 30 min, the potential value of the supernatant was measured using a calibrated pH meter (Leici PHS-3C). Each sample was measured 3 times, and the average was taken.

Soil organic carbon content: Determined by the potassium dichromate volumetric method-external heating method: 0.3 g of air-dried soil sample passed through a 0.149 mm (100 mesh) sieve was weighed into a hard glass test tube. 10.00 mL of 0.8000 mol/L potassium dichromate-sulfuric acid solution was added, shaken well, and placed in an oil bath at 170–180°C for digestion for 5 min (timing started when the solution boiled). After digestion, the tube was cooled, the contents were transferred to a 250 mL Erlenmeyer flask, water was added to about 150 mL, 3–5 drops of o-phenanthroline indicator were added, and the solution was titrated with 0.2 mol/L ferrous sulfate standard solution until the color changed from orange-yellow through blue-green to brick red at the endpoint. A blank test was conducted simultaneously, and the soil organic carbon content was calculated based on the volume of ferrous sulfate consumed.

Soil nitrogen content: Determined by the potassium chloride extraction-flow injection analysis method. 10 g of fresh soil was weighed, 50 mL of 2 mol·L⁻¹ potassium chloride solution was added, and it was shaken on a reciprocating shaker for 1 h (oscillation frequency 180 r·min⁻¹), then filtered

with quantitative filter paper. The contents of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ in the filtrate were measured using a flow injection analyzer (Skalar SAN++), and the total inorganic nitrogen content was the sum of the two. The sum of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ is defined as available nitrogen (AN).

Soil available phosphorus content: Determined by the sodium bicarbonate extraction-molybdenum-antimony colorimetric method: Air-dried soil samples passed through a 20-mesh sieve were weighed, extracted with 0.5 M sodium bicarbonate solution, decolorized with activated carbon, and filtered. The filtrate was mixed with molybdenum-antimony reagent for color development, and the absorbance was measured at a wavelength of 880 nm.

Soil Microbial Community Structure Analysis

0.5 g of fresh soil was weighed, and microbial total DNA was extracted using the soil DNA extraction kit (Omega Bio-tek E.Z.N.A.® Soil DNA Kit) according to the manual using the CTAB method. The integrity of the extracted DNA was detected by 1% agarose gel electrophoresis, and the concentration and purity of DNA (ratio controlled between 1.8-2.0) were measured using a nucleic acid quantifier (Thermo NanoDrop 2000). Qualified DNA samples were stored at -20°C for later use.

For the V3-V4 variable region of the bacterial 16S rDNA gene, primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') were used for PCR amplification; for the fungal ITS1 region, primers ITS1F (5'-CTTGGTCATTAGAGGAAGTAA-3') and ITS2R (5'-GCTGCGTTCTTCATCGATGC-3') were used. The PCR reaction system (25 μL) included: 2.5 μL 10 $\times$ PCR buffer, 2 μL dNTPs (2.5 mmol $\cdot\text{L}^{-1}$), 0.5 μL upstream and downstream primers (10 $\mu\text{mol}\cdot\text{L}^{-1}$), 0.2 μL Taq DNA polymerase (5 U $\cdot\mu\text{L}^{-1}$), 1 μL template DNA, and 18.3 μL sterile H_2O . PCR conditions were: pre-denaturation at 95°C for 5 min; denaturation at 95°C for 30 s, annealing (bacteria 55°C , fungi 52°C) for 30 s, extension at 72°C for 45 s, totaling 35 cycles; final extension at 72°C for 10 min. After verification by agarose gel electrophoresis, PCR products were sent to Hangzhou Lianchuan Biotechnology Co., Ltd., Hangzhou, Zhejiang, China for paired-end sequencing on the Illumina MiSeq platform.

Determination of Soil Microbial Respiration

Fresh soil equivalent to 50 g dry soil was weighed and placed in a 500 mL sealed culture bottle. Free distilled water was added using the weighing method to adjust the soil moisture content to 60% of the maximum field water capacity (maximum field water capacity was determined by the pressure plate/cutting ring method). The culture bottles were placed in a 25°C constant temperature incubator for 7 days of dark pre-incubation to activate microbial activity; if soil moisture evaporated during pre-incubation, a small amount of distilled water was supplemented to keep moisture stable. The entire experimental process used the weighing method for soil moisture adjustment.

After pre-incubation, the culture bottles were placed in constant temperature incubators at 15°C , 25°C , and 35°C for 28 days of formal dark culture. Gas collection was performed at 6 h, 1 d, 5 d, 9 d, 14 d, 21 d, and 28 d after the start of culture. Gas was collected between 10:00–12:00 AM each time. Before each sampling, the bottle stopper was opened, and a gas pump equipped with quicklime was used to ventilate the bottle for 30 min to completely remove residual CO_2 ; then the bottle was quickly sealed, and 10 mL of gas was drawn from the bottle with a syringe and discarded to update the gas environment. After putting the bottles back into the incubators for another 3 h, a 20 mL sterile syringe was used to pull the piston back and forth 10 times to fully mix the gas, then a 10 mL gas sample was extracted, injected into a pre-evacuated sealed serum bottle, and stored in a 4°C refrigerator for testing. After each sampling, the culture bottle was weighed, and distilled water was added to the initial mass to maintain soil moisture stability.

A gas chromatograph (Agilent 7890A) was used to measure CO_2 concentration. Chromatographic conditions: thermal conductivity detector (TCD), detector temperature 200°C ; Porapak Q column (2m $\times$ 3mm), column temperature 60°C ; carrier gas was high-purity nitrogen (purity $\geq 99.999\%$), flow rate 30 mL $\cdot\text{min}^{-1}$. The concentration was calculated based on the CO_2 standard curve (concentration gradient: 100, 500, 1000, 5000, 10000 $\mu\text{mol}\cdot\text{mol}^{-1}$), and combined with bottle volume, culture time, and dry soil mass, converted to soil microbial CO_2 emission rate (unit: $\mu\text{gCO}_2\text{g}^{-1}\text{h}^{-1}$).

Data analyses

Linear mixed-effects models were applied to explore the effects of tree age treatments on microbial respiration rate across multiple incubation temperatures (15°C , 25°C , and 35°C). In these models, plot was specified as a random effect to eliminate the interference of inherent differences among sampling plots. Additionally, an autoregressive correlation structure was incorporated to address temporal autocorrelation originating from repeated measurements during the incubation experiment. We calculated Q_{10} values on the basis of respiration rates at two different measurement temperatures T_1 and T_2 as:

$$Q_{10} = \left(\frac{R_{T2}}{R_{T1}} \right)^{\frac{10}{(T2-T1)}}$$

where R_{T2} and R_{T1} are respiration rates in the two incubation temperatures. Here, we calculated temperature sensitivity of microbial respiration (R_h) by using $15^\circ\text{C}/25^\circ\text{C}$ and $25^\circ\text{C}/35^\circ\text{C}$ respectively. Hence, we have both Q_{10} ($15^\circ\text{C}/25^\circ\text{C}$) and Q_{10} ($25^\circ\text{C}/35^\circ\text{C}$). All statistical analyses were performed with R 4.5.2 (R Development Core Team).

Results

Soil Physicochemical Properties of Mulberry at Different Ages

With increasing mulberry tree age, soil pH value increased slightly, being lowest at 5 years (T1) and highest at 70 years (T5), at 6.61 and 7.14, respectively (Fig. S2). Soil water content differed significantly among ages, with the 100-year-old (T6) reaching the highest at 33%, significantly higher than the 30-year-old (T4) and the control group (CK).

A significant positive correlation was found between SOC content and tree age ($r = 0.231$, $P < 0.05$). The SOC content of 100-year-old soil was highest, with an average content of $14.16 \text{ g}\cdot\text{kg}^{-1}$.

Soil ammonium nitrogen ($\text{NH}_4^{+}\text{-N}$) content showed a significant linear positive correlation with tree age ($r = 0.454$, $P < 0.001$), with the 100-year-old (T6) soil showing the highest $\text{NH}_4^{+}\text{-N}$ content at $1.97 \text{ mg}\cdot\text{kg}^{-1}$, followed by the 70-year-old (T5). Soil nitrate nitrogen ($\text{NO}_3^{-}\text{-N}$) and soil available phosphorus showed no significant correlation with tree age (Table S1).

Soil Microbial Community Structure at Different Ages

Soil bacterial richness and diversity in young forests (T1, T2, T3) decreased with tree age, recovered in middle-aged forests (T4), and remained at lower levels in mature forests (T5, T6) (Fig. S3). Similar to bacterial α -diversity, fungal richness and diversity in young forests (T1, T2, T3) decreased with age, recovered in middle-aged forests (T4), but differently, mature forests (T5, T6) maintained higher levels (Fig. S4).

Weighted analysis revealed significant differences in the beta diversity of soil bacteria among mulberry plantations of different stand ages. Along with the succession of plantations, soil bacterial communities experienced not only species turnover but also pronounced shifts in the relative abundance structure. By contrast, no obvious variation was observed in the beta diversity of soil fungi (Fig. S5).

Changes in Soil Microbial Respiration and Temperature Sensitivity (Q_{10})

Both stand age and incubation time significantly affected soil microbial respiration rates (Table 1). Soil microbial respiration generally increased with stand age, although some fluctuations were observed among intermediate-aged stands (Fig. 1). During the 28-day incubation, the 100-year-old mulberry plantation (T6) consistently exhibited the highest cumulative carbon release across all incubation temperatures (Fig. 2). Specifically, cumulative carbon release in T6 was significantly greater than that of the other stand ages at 25°C , whereas the 30-year-old stand (T4) showed the lowest cumulative carbon release at 35°C . Across all stand ages, respiration rates increased with incubation temperature, following the order of $35^{\circ}\text{C} > 25^{\circ}\text{C} > 15^{\circ}\text{C}$.

Table 1. Linear mixed-effects models to detect the effects of tree age treatments on the microbial respiration rate under different incubation temperatures (15°C , 25°C , 35°C). In the models, plot was treated as a random effect and autoregressive correlation structure was used to account for repeated measurements over time.

Source	Microbial respiration rate											
	15 °C				25 °C				35 °C			
	numDF	denDF	F ratio	P	numDF	denDF	F ratio	P	numDF	denDF	F ratio	P
Treatment	6	14	1.4233	0.2736	6	14	3.0944	0.0382	6	14	2.2014	0.1050
Time	6	84	41.1501	<0.0001	6	84	56.3413	<0.0001	6	84	9.6900	<0.0001
Treatment×Time	36	84	1.3862	0.1122	36	84	1.203	0.2425	36	84	0.9004	0.6295

Q_{10} values also exhibited clear stand-age effects (Fig. 3). In both the $15\text{--}25^{\circ}\text{C}$ and $25\text{--}35^{\circ}\text{C}$ intervals, Q_{10} increased significantly with increasing stand age, indicating greater temperature sensitivity of microbial respiration in older mulberry plantations. Meanwhile, within each stand age, Q_{10} values were consistently lower in the $25\text{--}35^{\circ}\text{C}$ interval than in the $15\text{--}25^{\circ}\text{C}$ interval, demonstrating a decline in temperature sensitivity at higher temperatures. LMM and ANCOVA analyses revealed a significant interaction between stand age and incubation temperature for Q_{10} calculated over the $15\text{--}25^{\circ}\text{C}$ interval ($P = 0.0116$), whereas the interaction was not significant in the $25\text{--}35^{\circ}\text{C}$ interval ($P = 0.1041$).

Correlation Analysis

RDA results (Fig. 4a) showed that with the increase of stand age, Q_{10} is strongly correlated with soil available nitrogen (AN), available phosphorus (AP), soil organic carbon (SOC), soil water content, as well as the bacterial and fungal beta diversity of microbial communities.

The mean soil respiration rate was closely associated with AN, SOC, and soil water content, and such correlations become stronger with rising temperature (Fig. 4b). At low temperatures, soil respiration (R_s) strongly correlated with fungal alpha diversity of microbial communities, while at high temperatures, it showed a strong correlation with bacterial beta diversity.

Discussion

The present study demonstrates that stand development exerts significant impacts on soil microbial respiration and its temperature sensitivity in mulberry plantations. Soil microbial respiration increased with stand age, accompanied by an overall increase in Q_{10} . Under higher incubation temperatures, Q_{10} values are lower reflecting the non-linear nature of microbial thermal responses. Furthermore, variations in soil microbial respiration and Q_{10} were closely associated with changes in soil physicochemical properties, particularly SOC, AN, AP, and soil moisture, as well as shifts in bacterial and fungal community composition. These findings indicate that long-term mulberry cultivation alters the mechanisms governing soil carbon turnover and its response to temperature, highlighting the importance of stand development in regulating soil carbon dynamics and potential climate feedbacks in managed ecosystems.

Stand development enhances soil microbial respiration through changes in substrate availability and microbial community structure

Our results showed a clear increase in soil microbial respiration with increasing mulberry stand age, indicating that long-term stand development enhances microbial metabolic activity and further increases the decomposition of soil organic carbon. As mulberry stands mature, increased litter inputs, root biomass, and rhizosphere carbon deposition may gradually cumulate soil organic carbon pools and nutrients availability, thereby providing greater substrate resources for soil microbial growth. Here, we found that soil organic carbon and available nitrogen increased significantly with stand age and were positively associated with soil microbial respiration (Fig. S2). This finding suggests that aged mulberry plantations have enhanced substrate supply, which is important for microbial activity. This interpretation was also supported by evidence from *Robinia pseudoacacia* plantations of different stand ages, where soil organic carbon was reported as the dominant factor controlling microbial metabolism (Zhao et al. 2025). In this case, higher SOC levels substantially alleviated microbial carbon limitation and enhanced microbial functioning (Zhao et al. 2025). Similar age-related patterns have also been observed in Chinese fir (*Cunninghamia lanceolata*) plantations, where stand development from young to mature stages (7–25 years) significantly altered soil organic carbon and nutrient availability as well (Lei et al. 2023). It was similarly reported that soil nutrient concentrations and enzyme activities were greater in mature forest stands, highlighting the strong linkage between stand age and soil properties (Lei et al. 2023).

Besides of substrate quantity, changes in substrate quality may represent another important mechanism regulating soil microbial respiration during stand development. Long-term mulberry cultivation can modify the chemical composition of litter and root-derived carbon inputs, altering the proportion of labile compounds relative to recalcitrant fractions such as lignin and cellulose. Such shifts in substrate quality can enhance the accessibility of soil carbon for microbes, stimulate extracellular enzyme activity, and accelerate the decomposition processes of SOC. A previous study in citrus orchard chronosequences (3–22 years) showed that soil respiration increased with stand age, which was largely driven by changes in substrate stoichiometry (e.g., soil C/N ratio) (Wu et al. 2020). In the present study, we did not directly quantify the chemical composition of soil organic carbon in this study, which limits our ability to directly resolve changes in substrate quality. Nevertheless, given that stand development is widely recognized to alter litter inputs and root-derived carbon chemistry, it is reasonable to infer that shifts in the balance between labile and recalcitrant carbon components likely contributed to the observed patterns in soil microbial respiration.

The shifts of microbial community may also contribute to the observed increase in soil microbial respiration with stand age. Long-term stand development can alter soil resource availability and microenvironmental conditions, thereby driving microbial community shifts and reassembly (Liu et al. 2020; Zhang et al. 2024; Yang et al. 2025; Wu et al. 2025; Qu et al. 2020; Zhu et al. 2019). Here, we found that both bacterial and fungal community composition varied significantly across the stand-age gradient, and microbial respiration was closely associated with changes in microbial β -diversity. Mature plantations may favor copiotrophic taxa with greater metabolic capacities for soil organic carbon decomposition. Although microbial richness showed a temporary recovery in 30-year-old stands, it generally declined with stand age (Fig. S3 & S4). Similar findings were previously reported. For example, reduced microbial diversity lowered community-level carbon-use efficiency, resulting in a greater proportion of assimilated carbon being released through respiration rather than incorporated into microbial biomass (Li and Qi 2025; Pan et al. 2026).

Drivers of increased temperature sensitivity (Q_{10}) during stand development

Stand age significantly enhanced the temperature sensitivity of soil microbial respiration, as reflected by the higher Q_{10} values observed in older mulberry plantations. The age-related increase in Q_{10} was likely driven by changes in soil substrate properties (Gershenson et al. 2009; Wetterstedt et al. 2010; Liu et al. 2021). In the present study, Q_{10} was positively correlated with SOC, available nitrogen, available phosphorus, and soil moisture, suggesting that greater substrate and nutrient availability enhanced the temperature responsiveness of microbial activity. Higher resource availability can stimulate microbial growth and extracellular enzyme production, thereby increasing the sensitivity of SOC decomposition processes to elevated temperature (Gomez et al. 2020; Adekanmbi et al. 2023). In addition to substrate quantity, changes in substrate quality may also contribute to the observed pattern. According to the carbon quality-temperature sensitivity hypothesis, the decomposition of more recalcitrant organic compounds requires higher activation energy and therefore exhibits greater temperature sensitivity (Davidson and Janssens 2006b). Because long-term mulberry cultivation substantially increased soil organic carbon accumulation, older stands may contain a larger pool of chemically complex organic substrates. Under such conditions, carbon quality may also play a critical role in regulating Q_{10} (Zhang et al. 2024).

Microbial community might also affect the response of Q_{10} to stand development (Li et al. 2021; Qin et al. 2024). In the current study, we found that Q_{10} was significantly associated with both bacterial and fungal β -diversity, indicating that shifts in microbial community composition accompanied changes in temperature sensitivity of SOC decomposition. Long-term resource enrichment in soil can favor microbial taxa with distinct metabolic strategies and enzyme systems, thereby driving community-level responses to increased temperature (Domeignoz-Horta et al. 2023). We also found that microbial diversity generally declined with stand age in our study. Reduced diversity may lower functional redundancy within microbial communities, making ecosystem processes more sensitive to environmental fluctuations (Allison and Martiny 2008; Delgado-Baquerizo et al. 2016). Conversely, more diverse communities can buffer temperature effects through complementary resource use and functional compensation among taxa (Dang and Morrissey 2024).

Our results also showed that Q_{10} declined with increasing incubation temperature across all stand ages. This pattern is consistent with numerous studies showing that the temperature sensitivity of soil microbial respiration is generally greater at lower temperatures (Jian et al. 2018; Han and Jin 2018; Qi et al. 2002). One possible explanation is that microbial metabolic processes become less temperature-limited at higher temperatures, resulting in a smaller proportional increase in respiration rate for each additional degree of warming. In addition, the activity of extracellular enzymes may approach its optimum temperature range, reducing the relative stimulatory effect of further warming on SOC decomposition. Substrate depletion during incubation may also contribute to lower Q_{10} values at higher temperatures because readily available carbon may be consumed more rapidly, leaving a larger proportion of less accessible substrates. These findings suggest that the response of microbial respiration to warming is inherently nonlinear, with progressively weaker temperature sensitivity under warmer conditions, consistent with thermal adaptation of microbial respiration across biomes (Bradford et al. 2019).

Ecological implications of stand-age effects on soil organic carbon dynamics

Long-term mulberry cultivation promoted soil organic carbon accumulation, but the higher soil microbial respiration rates and Q_{10} values observed in older stands suggest that these carbon stocks may become increasingly vulnerable to warming-induced losses. As a result, stand development may enhance both carbon storage and carbon turnover in response to warming temperature, highlighting a potential trade-off between carbon sequestration and carbon stability in aging mulberry plantations. These findings further indicate that stand age should be considered when evaluating soil carbon dynamics and predicting soil carbon–climate feedbacks in managed ecosystems (Bradford et al. 2016).

This study also provides new insights into the sustainable management of mulberry-based agroecosystems. As the foundation of traditional sericulture systems, mulberry plantations support not only agricultural production but also important cultural heritage landscapes. Our results suggest that long-term cultivation substantially alters soil properties, microbial communities, and carbon cycling processes. Understanding these belowground changes can improve the management of soil fertility and ecosystem functions, thereby contributing to the long-term sustainability and resilience of mulberry-based production systems under ongoing climate change (Paustian et al. 2016).

Declarations

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Conflict of interest statement

The authors have no relevant financial or non-financial interests to disclose.

Data accessibility statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

CRediT Author Contributions

Jingyun Xing: Conceptualization, Methodology, Formal analysis, Investigation, Writing-original draft, Visualization. Yaping Deng: Investigation, Data curation, Formal analysis. Yiheng Zhang: Investigation, Data curation. Hui Guo: Conceptualization, Methodology, Resources, Writing-review and editing. Ying Liu: Investigation, Resources. Yuhui Gao: Investigation, Resources. Hongkai Qie: Methodology, Supervision, Project administration, Funding acquisition.

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Figures

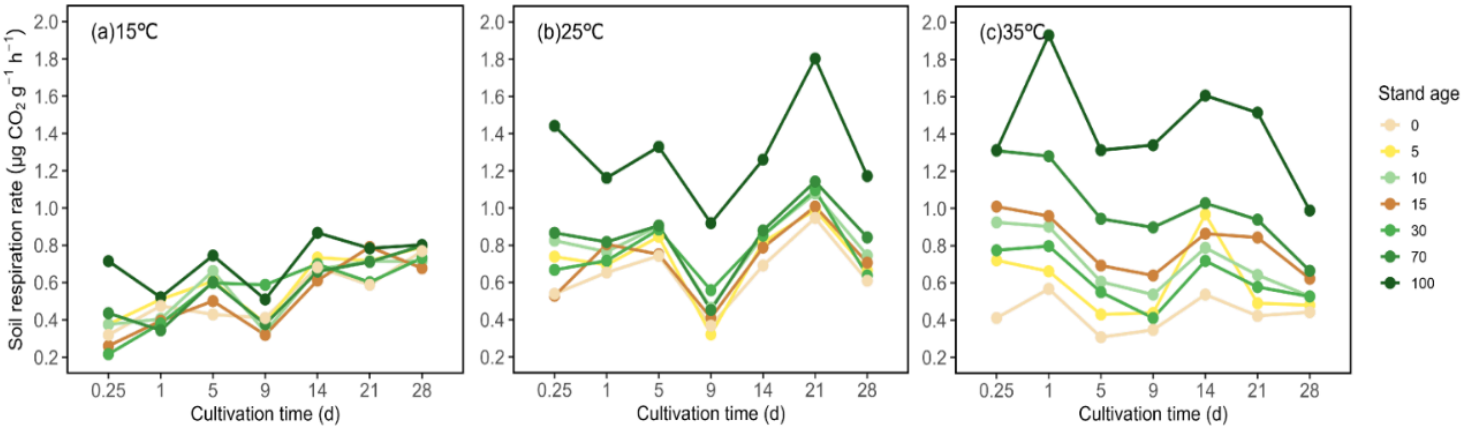


Figure 1

The respiration rate of soil microbes at a cultivation temperature of 15 °C, 25 °C, and 35 °C. Different colors denote mulberry plantation with different age. Age 1, 5-year-old mulberry (T1); age 2, 10-year-old mulberry (T2); age 3, 15-year-old mulberry (T3); age 4, 30-year-old mulberry (T4); age 5, 70-year-old mulberry (T5); age 6, 100-year-old mulberry (T6); ck, soil without mulberry cultivation.

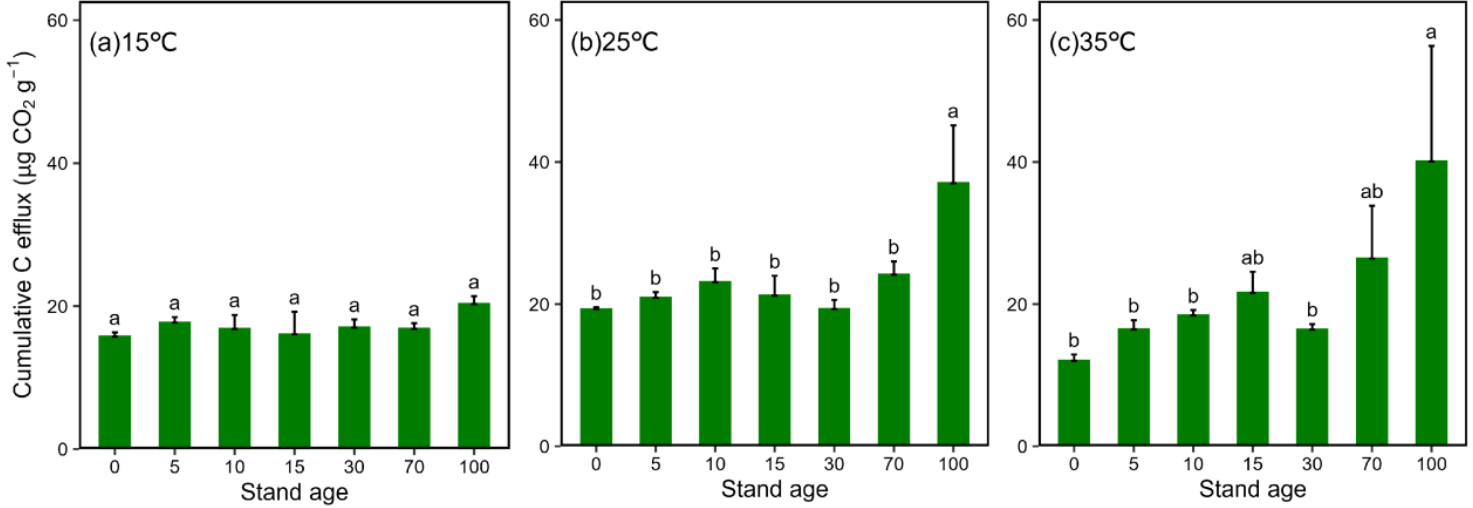


Figure 2

The cumulative carbon emissions after 28 days of cultivation at 15 °C(a), 25 °C(b), and 35 °C(c) of the soils from mulberry plantations of different ages. Error bars are standard errors. The different letters above the bars denoted significant differences among treatments ($P < 0.05$).

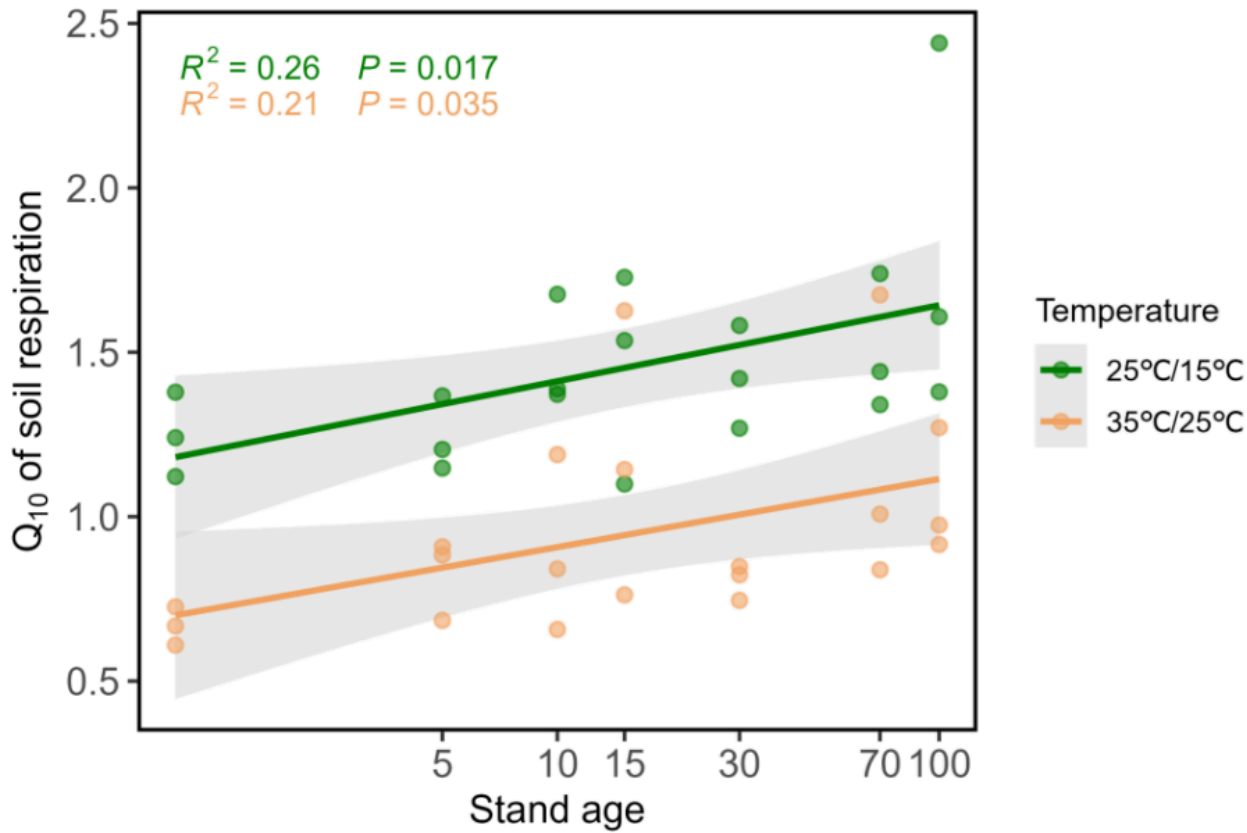


Figure 3

Temperature sensitivity (Q_{10}) of soil from mulberry plantations of different age. The gray area represents the confidence interval. The green line represents temperature-sensitive Q_{10} (15 °C/25 °C), while the orange line indicates temperature-sensitive Q_{10} (25°C/35°C). For compatibility with the logarithmic scale, stand age of the 0-year site was set to 1 for visualization, with the axis label retained as 0 years, without influencing the results.

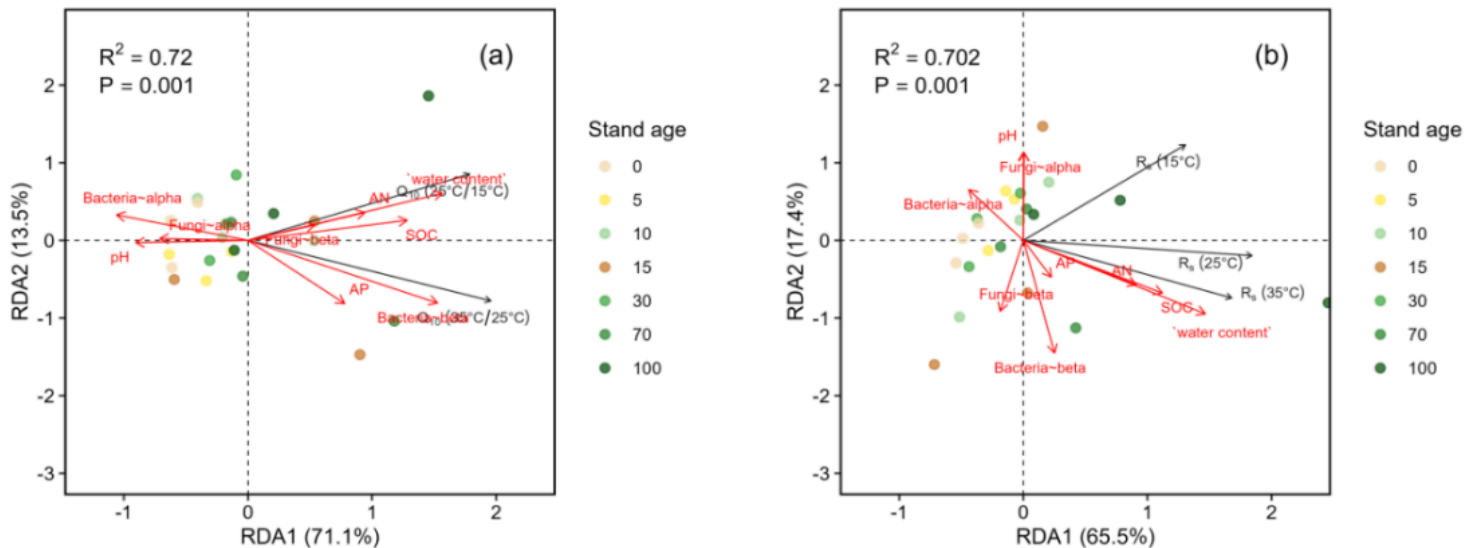


Figure 4

Redundancy analysis among different factors with Q_{10} (a). Redundancy analysis among different factors with R_s (b). Red color arrows represent soil properties, and blue color arrows represent respiration characteristics. Different point colors denote mulberry plantations with different ages. Age 1, 5-year-old mulberry (T1); age 2, 10-year-old mulberry (T2); age 3, 15-year-old mulberry (T3); age 4, 30-year-old mulberry (T4); age 5, 70-year-old mulberry (T5); age 6, 100-year-old mulberry (T6); ck, soil without mulberry cultivation. The figure 4a shows that environmental vectors account for 72% of the

total variation (Adj. R^2), with RDA1 representing the primary successional axis (71.1% explained). The figure 4b shows that environmental vectors account for 70.2% of the total variation (Adj. R^2), with RDA1 representing the primary successional axis (65.5% explained).

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