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Contrasting first-year and tenth-year responses of soil CO₂ efflux and soil carbon storage indicators to biochar addition in plantation forests

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Introduction: Soil–atmosphere carbon exchange is central to global carbon budgets and forest-based climate mitigation. Although biochar has been proposed as a potential approach to improve soil carbon storage, its effects on soil CO₂ efflux and associated soil–plant responses remain uncertain, especially in subtropical plantation forests and over longer observation periods.

Methods: A 10-year field trial from 2014 to 2024 was conducted in three subtropical evergreen plantations dominated by *Cinnamomum subavenium*, *Phoebe chekiangensis*, and *Pseudolarix amabilis*. Biochar was applied once at three rates: 0, 5, and 10 t ha⁻¹. Soil carbon fractions, annual cumulative soil CO₂ efflux, tree growth indicators, and major soil environmental factors were quantified during the first and tenth years after application to compare short- and decadal-term responses to biochar addition.

Result: After 10 years, biochar-induced accumulation of organic and microbial C in soil became more evident than in the first-year observation period ($p < 0.01$), whereas dissolved organic carbon and annual cumulative soil CO₂ efflux showed no significant differences among biochar treatments ($p > 0.05$). Biochar also promoted plant growth, with clear species-specific responses. Under the 10 t ha⁻¹ treatment, *C. subavenium* showed the strongest growth response. The variables statistically associated with soil CO₂ efflux differed between the two observation periods. In the first year, biochar showed a weak negative association with soil CO₂ efflux, mainly related to increased soil pH (−0.18). In the tenth year, soil moisture (0.32), total nitrogen (0.16), and photosynthetic rate (0.19) showed stronger positive associations with soil CO₂ efflux.

Discussion: Overall, a single biochar application may enhance soil carbon storage indicators, particularly SOC and MBC, without significantly increasing annual soil CO₂ efflux in the studied subtropical plantations. However, these findings remain site- and system-specific and require further validation across different forest types, soil conditions, biochar properties, and management contexts.

KEYWORDS

biochar addition, soil CO₂ efflux, structural equation model, subtropical evergreen plantations, temporal effects

1 Introduction

Soil carbon pools are central to the carbon cycle in terrestrial ecosystems, with an estimated stock of 1,500–2,400 Pg, approximately two to three times the size of the atmospheric carbon pool, and play a critical role in maintaining the global carbon balance (Lal, 2004; Schmidt et al., 2011). Forest soil carbon cycling is primarily regulated by organic matter decomposition, a process driven fundamentally by the coupled metabolic activities of heterotrophic soil microbes and autotrophic plant roots (Raich and Schlesinger, 1992; Bond-Lamberty and Thomson, 2010). Soil respiration, driven by microbial decomposition and root activity, is an important component of the global carbon cycle, contributing more than 60% of carbon released from land ecosystems (Luo et al., 2011; Hashimoto et al., 2015). Due to the large carbon stocks and high rates of carbon release from the soil, even a slight increase in emissions can significantly raise atmospheric CO₂ concentrations, leading to substantial climate responses (Melillo et al., 2002; Davidson and Janssens, 2006; Crowther et al., 2016). Therefore, understanding how soil CO₂ efflux and soil carbon storage indicators respond to soil amendments is important for evaluating forest carbon cycling under a changing climate.

Biochar is generally produced through the thermal treatment of biomass in a low-oxygen environment, forming a C-rich material with strong aromaticity and high stability. Its production process converts unstable organic matter into stable and highly aromatized carbon structures (Woelf et al., 2010; Lehmann and Joseph, 2015). Previous studies have indicated that biochar amendment enhances soil carbon retention in agricultural soil systems, particularly through stabilization of labile organic matter and suppression of microbial mineralization (Major et al., 2010b; Zimmerman et al., 2011; Zhang et al., 2012). Forest ecosystems represent the largest active carbon reservoir in terrestrial environments, containing nearly half of the world's terrestrial carbon and strongly influencing carbon exchange between land and the atmosphere. In forest ecosystems, converting biomass residues such as litter and thinning by-products into biochar has been proposed as a potential approach for residue recycling and soil carbon management (Pan et al., 2011). However, the effects of biochar in forest soils remain highly context-dependent. Although some studies have reported reduced soil CO₂ efflux after biochar addition (Li et al., 2018b), others have observed stimulatory effects (Liu et al., 2013) or no detectable responses (Zhou et al., 2017). These inconsistent findings suggest that biochar effects on forest soil C release may depend strongly on ecosystem type, soil properties, biochar characteristics, and observation duration.

Such context-dependent responses may arise from complex interactions among biotic and abiotic components in forest ecosystems (Lehmann and Joseph, 2015). Biochar effects on soil carbon mineralization may be shaped by forest type, stand development stage, soil physicochemical and biological properties, and environmental conditions (Yang et al., 2025). Based on previous studies, three non-exclusive response pathways may occur: (1) Potential soil carbon retention pathway: Biochar may be associated with reduced soil CO₂ efflux or enhanced soil carbon retention because pyrolysis converts biodegradable biomass into relatively stable aromatic carbon forms that are more resistant to decomposition (Kuzakov et al., 2014). Owing to its porous structure and chemical stability, biochar-derived C may persist in soil and contribute to soil carbon storage. In addition, biochar may

influence native SOC stabilization by altering soil aggregate stability, pore structure, and the physical protection of organic matter. Biochar-induced changes in soil aeration and pore structure may also affect microbial habitat and metabolic processes, potentially modifying the decomposition of native SOC (Gul et al., 2015). (2) Potential soil CO₂ efflux promotion pathway: Previous studies have also suggested that biochar may increase soil CO₂ efflux under certain conditions. For example, biochar can modify soil pH, porosity, moisture retention, and nutrient availability, which may improve microbial habitat and potentially stimulate microbial metabolism and organic C decomposition (Smith et al., 2010; Jeffery et al., 2011; Lehmann et al., 2011). In addition, biochar produced at relatively low to moderate pyrolysis temperatures may retain labile surface compounds, which can provide readily available substrates for microbial decomposition and induce a positive priming effect (Luo et al., 2011). Biochar may also influence root growth and root-related CO₂ release under specific soil and plant conditions (Major et al., 2010a). (3) Potential neutral response pathway: Biochar may show no detectable effect on soil CO₂ efflux when positive and negative processes offset each other under specific soil, vegetation, and temporal conditions (Castaldi et al., 2011; Karhu et al., 2011). These contrasting findings highlight the context-dependent nature of biochar effects on soil CO₂ efflux. Although individual response pathways have been reported in different forest types, the factors underlying contrasting biochar responses remain unclear. In particular, empirical evidence regarding biochar-mediated regulation of soil C release in forest ecosystems remains largely limited to short-term observations (≤ 3 years, Supplementary Table S1) (Ge et al., 2020; Walkiewicz et al., 2020; Lehmann et al., 2021; Xue et al., 2023; Wei et al., 2024; Yang et al., 2025; Zu et al., 2025). Therefore, it remains unclear whether biochar effects observed in short-term studies are consistent with those observed at the decadal scale in plantation forests.

Plantation forests account for 10%–20% of forest soil carbon stocks (FAO, 2020). They represent an important system for evaluating biochar-based soil carbon management in forest ecosystems (Malhi et al., 1999). However, the effectiveness of biochar amendment in plantation forests remains uncertain and may depend on tree species, soil conditions, biochar properties, and stand development (Lehmann and Joseph, 2015; Zhou et al., 2017; Sarauer et al., 2019; Li et al., 2023). To compare first-year and decadal-term responses, a 10-year field experiment was established at Tiantong Mountain. This study compared soil CO₂ efflux, soil C fractions, plant photosynthesis, growth responses, and soil physicochemical properties between the first and tenth years after a single biochar application. Because intermediate years were not continuously monitored, the study aimed to identify contrasting short- and decadal-term responses. Specific research questions include: (1) Does biochar addition affect soil C fractions and annual soil CO₂ efflux differently in the first and tenth years after application? (2) Do the measured soil physicochemical variables and plant-related variables associated with soil CO₂ efflux differ between the two observation periods? (3) Are plant growth and photosynthetic responses to biochar addition species-specific? By addressing these questions, this study aims to provide preliminary evidence for understanding short- and decadal-term biochar responses in subtropical plantation systems and to inform future long-term evaluations of biochar-based soil carbon management.

2 Materials and methods

2.1 Site description

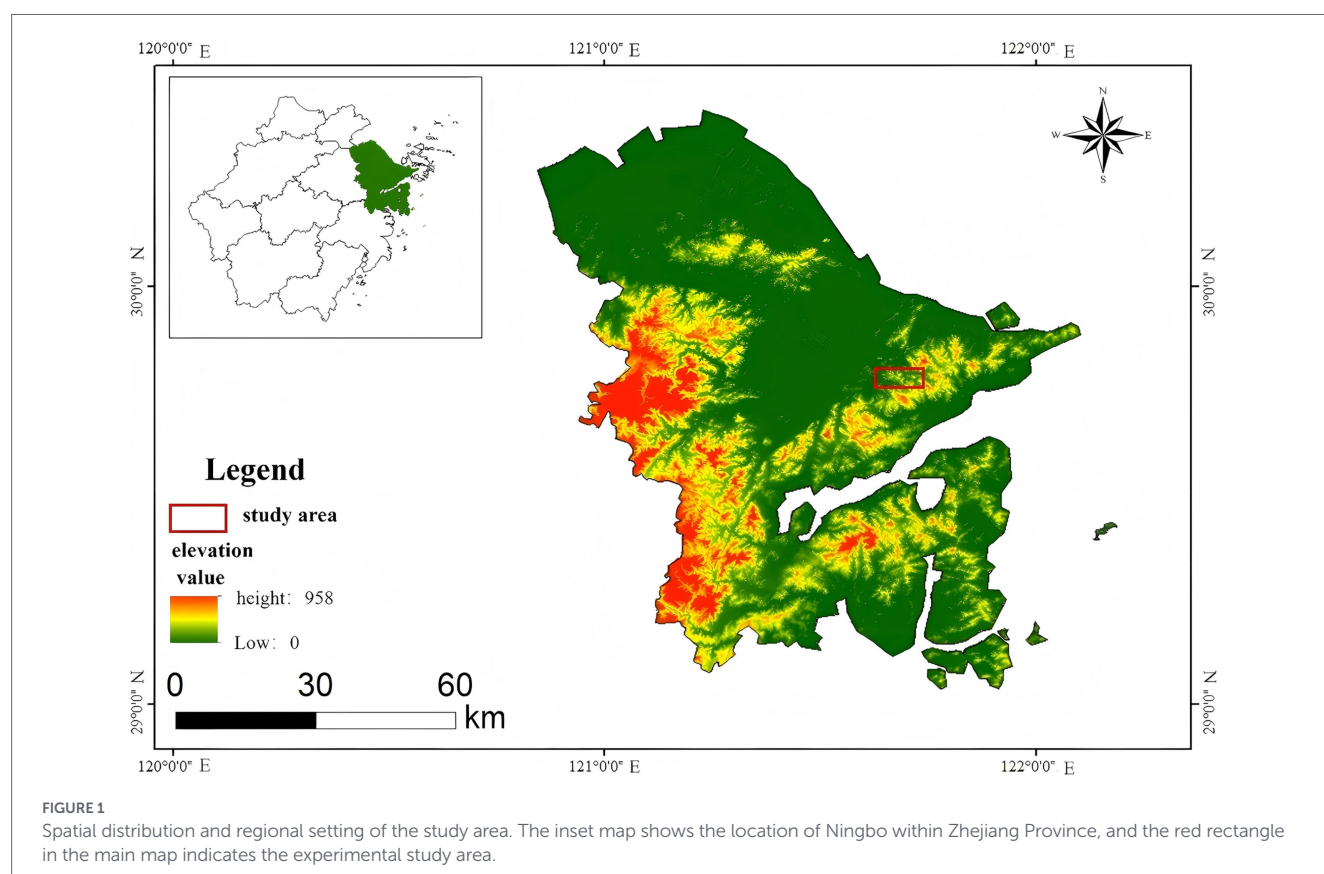
The experimental plots were established in the hilly forest of Tiantong Mountain, Ningbo City, Zhejiang Province (29°46'N, 121°48'E) (Figure 1). The study site features a low-mountain and hilly landscape, with its vertical profile spanning an altitudinal range of 310 to 510 m. Annual sunshine amounts to roughly 1,515 h alongside a mean temperature of 16.2 °C, reflecting the typical hydrothermal profile of this subtropical monsoon region. It is also characterized by high relative humidity (84%) and a frost-free period lasting 230–240 days. Annual precipitation averages about 1,480 mm, exhibiting marked seasonal variation. The summer months (June–August) account for approximately 28% of the annual rainfall total, reflecting distinct dry and wet seasons, the annual evaporative demand fluctuates between 1,300 and 1,500 mm. The soil is classified as mountain yellow-red soil, which is acidic in nature. The native flora is dominated by subtropical evergreen broad-leaved forests, primarily composed of *Fagaceae* species. Species belonging to *Machilus*, *Castanopsis*, and *Cyclobalanopsis* constitute the primary vegetation cover within the region, which are evergreen trees, with associated species such as Chinese sweet gum, Chinese fir, and Masson pine.

2.2 Experimental setup and soil analysis

To minimize interspecific interference, the experiment was conducted in artificial monocultures of three zonal dominant tree species (*Cinnamomum subavenium*, *Phoebe chekiangensis*, and *Pseudolarix amabilis*). We set 9 plots (2 m × 5 m) per tree species, giving 27 total

plots. Perimeter PVC isolation barriers (40 cm buried depth, 20 cm aboveground height) were installed to block biochar displacement by surface runoff or wind. Three biochar addition levels were tested: control (S/LCK), 5 t/ha (S/L5BC), 10 t/ha (S/L10BC). For each species, plots were randomly assigned to biochar treatments, with three independent plot-level replicates per treatment (Figure 2). Each plot was treated as the experimental unit in all statistical analyses. The same plots were repeatedly measured during each observation period. Measurements from multiple collars, trees, leaves, or repeated observations within the same plot were summarized at the plot level before statistical analysis to avoid pseudoreplication. Effects were assessed at two time points: the first year (referred to as the short-term observation period) and the tenth year (referred to as the long-term observation period) after biochar application, corresponding to the treatments SCK, S5BC, and S10BC (referred to as the short-term period controls and biochar amendments) and LCK, L5BC, and L10BC (referred to as the long-term observation period counterparts), respectively. Biochar was applied in summer 2014, marking the initiation of the experiment. Biochar was produced from local woody debris (e.g., *Quercus myrsinifolia*) via pyrolysis at 600 °C for 24 h under nitrogen atmosphere (Supplementary Table S2) (Junna et al., 2016), then sieved (2 mm) and uniformly incorporated into the surface soil (depth ≤ 10 cm).

Before biochar application, five topsoil subsamples were taken from the 0–20 cm layer of each plot following an S-shaped sampling design and combined into one composite sample. For each plot, five soil samples were thoroughly mixed to produce a composite sample for subsequent physicochemical analysis (Supplementary Table S2). All samples were pretreated according to (Li G. et al., 2018). The concentration of SOC was quantified through external



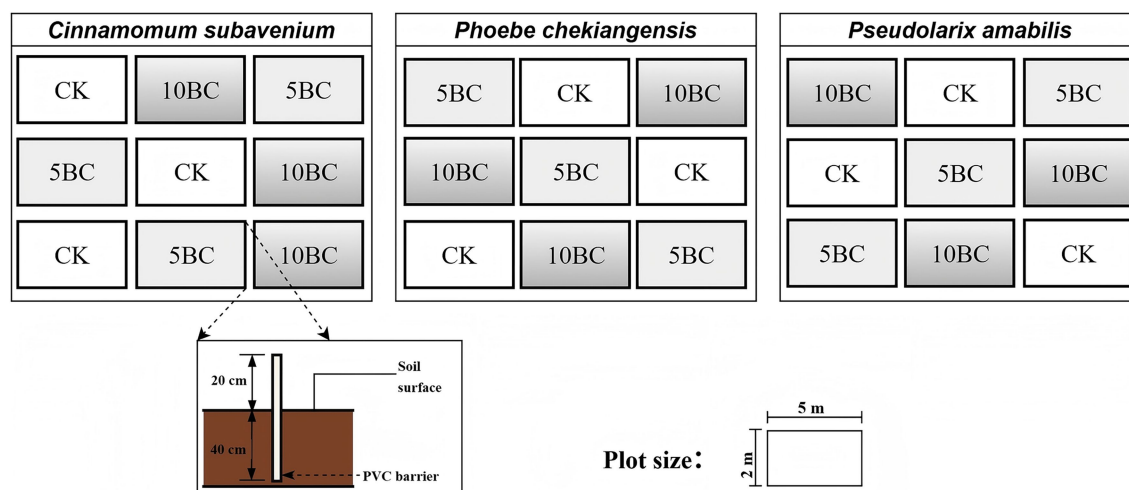


FIGURE 2

Experimental plot schematic diagram. Three plantation species, *Cinnamomum subavenium*, *Phoebe chekiangensis*, and *Pseudolarix amabilis*, were included. For each species, three biochar treatments were established: CK, 5 BC, and 10 BC, corresponding to 0, 5, and 10 t ha⁻¹ biochar addition, respectively. Each treatment included three independent plot-level replicates, and each plot was 2 m × 5 m. Treatments were randomly assigned within each species. PVC barriers were installed around each plot, with 20 cm aboveground and 40 cm belowground, to reduce lateral movement of biochar.

heating-assisted oxidation using potassium dichromate (Wang et al., 2012); soil MBC was extracted using 0.5 mol K₂SO₄ after a 24 h chloroform fumigation (Babur et al., 2021), while DOC was recovered from a 1:5 soil-water extract (Niu et al., 2014); a calibrated precision pH meter was employed to determine edaphic acidity, while soil moisture levels were quantified via the gravimetric oven-drying technique (Li et al., 2025).

2.3 Soil CO₂ efflux, photosynthesis, and growth parameters measurement

The observation period spanned 10 years after a single biochar application. To evaluate short- and decadal-term responses while minimizing potential physical disturbance caused by repeated soil sampling, this study focused on two observation periods: the first year and the tenth year after biochar application. Therefore, the dataset represents a comparison between two key observation years rather than a continuous temporal trajectory. Intermediate years were not sampled, and temporal processes occurring between the first and tenth years could not be directly monitored. Data were collected during a full annual cycle in each observation period using an LI-8100 system (LI-COR Biosciences, USA) to account for variations between growing and dormant seasons. The following protocols were adopted to mitigate measurement bias: (1) Three standardized PVC collars (20.3 cm ID × 15.0 cm height) per plot were randomly positioned (>50 cm from trees) and inserted 5 cm into the soil. The average of these three collars represented the plot-level CO₂ efflux, which encompassed both autotrophic and heterotrophic components. The measured soil CO₂ efflux represents total soil respiration, including both autotrophic and heterotrophic components; however, root-derived and microbial-derived CO₂ fluxes were not separated in this study; (2) Field campaigns were performed on three consecutive clear days per season. Measurements were taken during three specific windows (08:00–11:00, 11:00–14:00, and 14:00–17:00) to account for diurnal fluctuations; (3) Daily efflux rates were calculated as the mean of the three time intervals, and seasonal averages were then

determined from these three-day observation periods. Annual cumulative soil CO₂ efflux was calculated from seasonal mean soil CO₂ efflux rates using the following equation (Gao et al., 2014):

$$R = 12 \times 10^{-6} \times 86400 \times \sum R_i D_i$$

Where (*R*) is annual cumulative soil CO₂ efflux expressed as g C m⁻² yr⁻¹, (*R_i*) is the mean soil CO₂ efflux rate during season (*i*) expressed as μmol CO₂ m⁻² s⁻¹, and (*D_i*) is the number of days in season (*i*).

Concurrent with soil measurements, net photosynthesis rate (*P_n*) was measured using a portable system (LI-6800, LI-COR, USA). *P_n* was analyzed for *Cinnamomum subavenium* and *Phoebe chekiangensis*, the only two species in the dataset with complete, seasonally resolved photosynthetic measurements. Comparable *P_n* data were unavailable for *Pseudolarix amabilis*; consequently, all *P_n*-related analyses and interpretations were confined to these two species. Three plants were selected from each subplot, with 3 to 7 mature leaves collected from the upper canopy. Following a photosynthetic induction period of 15 ± 2 min under saturating irradiance (1,500 μmol·m⁻² s⁻¹), *P_n* was measured. Measurements were conducted under a controlled leaf-chamber microclimate: 25 ± 1 °C, 60 ± 5% relative humidity, and 400 μmol·mol⁻¹ CO₂. To ensure data representativeness, *P_n* values were calculated as the mean of ten consecutive, instantaneous measurements per leaf. Basal diameter, tree height, and crown width were recorded according to the protocol described by Hao et al. (2022).

2.4 Statistical analysis

The first-year and tenth-year data were interpreted as a two-period comparison rather than a continuous temporal trend. All measurements were summarized at the plot level, and each plot was treated as the experimental unit. One-way ANOVA was used to compare soil carbon fractions, soil physicochemical properties, annual soil

CO₂ efflux, net photosynthetic rate, and plant growth indicators among biochar treatments within each observation period. Homogeneity of variance was assessed using Levene's test before ANOVA; LSD *post hoc* tests were used when variance homogeneity was met, whereas Welch's ANOVA and Games-Howell comparisons were used when it was not met (Supplementary Table S3). Repeated-measures ANOVA was used to evaluate the effects of biochar treatment, tree species, and season on soil CO₂ efflux, net photosynthetic rate, and plant growth indicators within each observation period, with season as the within-subject factor and plot identity as the subject identifier. Structural equation modeling (SEM) was used to explore hypothesized statistical associations among biochar addition, soil physicochemical properties, plant variables, and soil CO₂ efflux, based on the biochar-soil-plant conceptual framework, previous studies, and preliminary relationships among measured variables. SEM paths were interpreted as model-based statistical associations rather than causal mechanisms. All analyses were conducted in R 4.4.3 using the “stats,” “lavaan,” and “car” packages.

3 Results

3.1 Direct effects of biochar addition on soil carbon components and soil CO₂ efflux

Statistical analysis revealed comparable annual cumulative soil CO₂ efflux for *Cinnamomum subavenium*, *Phoebe chekiangensis* and *Pseudolarix amabilis* under all biochar regimes during the short-term observation period ($p > 0.05$; Table 1). The incorporation of biochar

TABLE 1 Annual cumulative soil CO₂ efflux from plantation plots of *Cinnamomum subavenium*, *Phoebe chekiangensis*, and *Pseudolarix amabilis* under biochar amendment across experimental periods.

Tree species	Biochar addition rate (t/ha)	Short-term annual cumulative soil CO ₂ efflux (g C·m ⁻² ·a ⁻¹)	Long-term annual cumulative soil CO ₂ efflux (g C·m ⁻² ·a ⁻¹)
<i>Cinnamomum subavenium</i>	0	800.48 ± 41.59Aa	733.67 ± 98.01Aa
	5	849.34 ± 99.15Aa	641.17 ± 22.00Aa
	10	827.70 ± 70.80Aa	902.75 ± 115.70Aa
<i>Phoebe chekiangensis</i>	0	949.67 ± 57.56Aa	543.39 ± 85.71Aa
	5	755.44 ± 52.55Aa	834.33 ± 169.73Aa
	10	790.02 ± 79.13Aa	711.02 ± 85.16Aa
<i>Pseudolarix amabilis</i>	0	761.44 ± 61.66Aa	812.84 ± 60.82Aa
	5	768.12 ± 144.45Aa	651.72 ± 107.67Aa
	10	765.12 ± 127.40Aa	701.21 ± 34.16Aa
Mixed tree species	0	837.20 ± 39.49A	696.63 ± 57.61A
	5	790.97 ± 54.81A	709.07 ± 66.26A
	10	794.28 ± 48.73A	771.66 ± 53.79A

Uppercase letters represent differences between different biochar treatments, while lowercase letters represent differences between species under the same biochar treatment. Dissimilar letters denote significant differences, whereas identical letters denote no statistical significance.

(5 and 10 t ha⁻¹) significantly increased the measured soil carbon fractions, effectively bolstered the soil carbon fractions (SOC and MBC), showing a significant upward trend over the SCK control ($p < 0.01$; Table 2). Specifically, SOC content increased by 4.63% and 12.45%, and MBC content increased by 8.06% and 12.39%, respectively ($p < 0.01$; Table 2). However, DOC content remained statistically unchanged across the different biochar application rates ($p > 0.05$; Table 2).

During the long-term observation period, different biochar treatments had no significant effect on annual cumulative soil CO₂ efflux for any of the three tree species ($p > 0.05$; Table 1). Compared to the long-term control treatment (LCK), biochar additions at 5 t·ha⁻¹ and 10 t·ha⁻¹ (L5BC and L10BC) significantly increased SOC and MBC contents ($p < 0.001$; Table 2). Specifically, SOC content increased by 39.45% and 65.14%, and MBC content increased by 19.67% and 30.84%, respectively ($p < 0.001$). However, these two treatments did not significantly affect DOC content ($p > 0.05$). Relative to the short-term effects, long-term biochar addition exerted a significantly greater enhancing effect on SOC and MBC accumulation ($p < 0.01$; Supplementary Table S4).

Soil CO₂ efflux rates exhibited significant seasonal fluctuations in both the short- and long- duration observation periods ($p < 0.001$), but were not significantly influenced by biochar addition treatments. No significant interaction among biochar addition, season, and tree species was detected for soil CO₂ efflux rates. However, the interaction between season and tree species significantly affected soil CO₂ efflux rates during the short-term observation period ($p < 0.05$), whereas this interactive effect was no longer significant during the long-term observation period ($p > 0.05$; Supplementary Table S5).

3.2 Plant photosynthetic performance and growth responses under biochar addition

During the short-term observation period, for the two species with available Pn data, different biochar addition gradients significantly increased plant net photosynthesis rate (Pn) ($p < 0.001$). However, long-term observations showed that the enhancement in Pn was approximately 20% lower compared to the short-term effect ($p < 0.05$). The variation in Pn was significantly influenced by the interaction between season and tree species (Supplementary Table S6). Specifically, in the short term observation period, Pn values in the *Cinnamomum subavenium* forest and *Phoebe chekiangensis* forest peaked during summer and reached their minimum in winter. In the long term observation period, the average Pn values for these two forest types declined to 6–8 μmol·m⁻²·s⁻¹ and 2–4 μmol·m⁻²·s⁻¹, respectively (Figure 3).

During the short-term observation period, biochar addition generally increased annual basal diameter growth in all three forest types ($p < 0.05$), whereas tree height and crown width increments remained statistically unchanged ($p > 0.05$). *Pseudolarix amabilis* showed relatively high absolute basal diameter growth, especially under the S10BC treatment, but the magnitude of biochar-associated responses varied among species (Table 3). Over the 10-year observation period, biochar application mainly enhanced mean annual basal diameter growth, while tree height and crown area responses varied among species and treatments ($p < 0.01$; Supplementary Table S7). Among these forest types, the *Cinnamomum subavenium* forest under the L10BC treatment exhibited the greatest annual cumulative basal diameter growth, which was significantly greater than that in the *Phoebe*

TABLE 2 Soil carbon pool responses (SOC, MBC, and DOC) to biochar treatments during different observation periods.

Group	Soil organic carbon content (g/kg)	Soil microbial biomass carbon content (mg/kg)	Soil dissolved organic carbon content (mg/kg)
SCK	18.56 ± 0.33B	200.83 ± 3.59B	47.42 ± 1.82A
LCK	25.93 ± 0.81A	214.49 ± 6.70A	47.62 ± 1.41A
S5BC	19.42 ± 0.36B	217.00 ± 3.48B	48.64 ± 2.11A
L5BC	36.16 ± 0.97A	256.69 ± 8.23A	49.01 ± 1.55A
S10BC	20.87 ± 0.35B	225.72 ± 3.48B	51.44 ± 2.22A
L10BC	42.84 ± 1.35A	280.64 ± 7.95A	48.84 ± 1.56A

Statistical differences among experimental groups are indicated by different uppercase letters ($p < 0.05$). SCK and LCK refer to control groups; S5BC/S10BC and L5BC/L10BC refer to short-term and long-term observation periods with 5 and 10 t/ha of biochar, respectively.

chekiangensis and *Pseudolarix amabilis* forests (Figure 4). Compared with the corresponding LCK treatment, tree height and crown area of *Cinnamomum subavenium* under L10BC increased from 26.90 ± 1.81 cm to 40.98 ± 3.45 cm and from 0.53 ± 0.07 m² to 0.71 ± 0.07 m², respectively (Table 3).

3.3 Indirect pathways through which biochar addition affects soil CO₂ efflux

In the short-term observation period, the SEM fitted the observed data well (Supplementary Table S8), accounting for 74% of the variance in soil CO₂ efflux (Figure 5a). Statistical analysis revealed that the direct path of biochar addition on soil CO₂ efflux was not statistically significant ($p > 0.05$), while the total effect was weakly negative (total effect = -0.04). This indirect pathway through soil physicochemical properties was also weakly negative (-0.05), with the significant negative impact of soil pH being the main contributing factor (path coefficient = -0.18) soil CO₂ efflux. In addition, net photosynthetic rate (Pn) and basal diameter growth (BDG) showed path coefficients of 0.04 and -0.02 , respectively, indicating minimal effects on soil CO₂ efflux (Supplementary Table S9).

The long-term observation period, SEM achieved an acceptable fit to the observed data (Supplementary Table S8), accounting for 69% of the variance in soil CO₂ efflux (Figure 5b). Biochar addition showed a weak positive total effect on soil CO₂ efflux (0.09). The indirect positive effect mediated by soil physicochemical properties was 0.37, primarily associated with soil moisture content (0.32) and total nitrogen (0.16). Soil physicochemical properties mediated an indirect positive effect of 0.37, primarily associated with soil moisture content (0.32) and total nitrogen (0.16). Meanwhile, net photosynthesis rate (Pn) and basal diameter growth showed statistical associations of 0.19 and -0.18 on soil CO₂ efflux, respectively, with both effects significantly enhanced compared to short-term results (Supplementary Table S10).

Biochar addition showed contrasting statistical associations with soil CO₂ efflux between the first-year and tenth-year observation periods. The total SEM effect was weakly negative during the first-year observation period (total effect: -0.04), whereas it was weakly positive during the tenth-year observation period (total effect: 0.09). Similarly, the indirect association mediated by soil physicochemical properties was negative during the first-year observation period (-0.05) but

positive during the tenth-year observation period (0.37). Indirect associations related to plant variables, including net photosynthetic rate and basal diameter growth, were stronger during the tenth-year observation period than during the first-year observation period. In addition, SOC showed a negative indirect association with soil CO₂ efflux in both observation periods.

4 Discussion

4.1 Contrasting first-year and tenth-year plant photosynthetic and growth responses to biochar amendment

Biochar-induced changes in plant photosynthesis and growth differed between the first-year and tenth-year observation periods, with clear seasonal variation and species-specific responses. In the first-year observation period, biochar significantly increased net photosynthesis rate and promoted basal diameter growth, but had no significant effect on tree height or crown width. Similar results were reported by [Palviainen et al. \(2018\)](#), who found that biochar initially enhanced photosynthetic performance and basal diameter growth potentially through changes in soil nutrient-related conditions and rhizosphere environments, whereas whole-tree growth responses may appear only after a certain time delay. However, root development and detailed rhizosphere processes were not directly measured in the present study, and therefore these explanations should be interpreted cautiously. Moreover, different tree species showed marked differences in their responses to biochar, particularly in growth indicators: *Pseudolarix amabilis* showed relatively high absolute basal diameter growth compared with *Cinnamomum subavenium* and *Phoebe chekiangensis*, suggesting that coniferous species may be more responsive to changes in soil nutrient availability, whereas broadleaf species may adopt a relatively more conservative resource utilization strategy ([Nguyen et al., 2017](#)).

In the tenth-year observation period, biochar was associated with sustained impetus for radial stem expansion and concurrently accelerating the mean annual increments of tree height and canopy spread, suggesting that measured soil and plant variables related to growth differed between the two observation periods rather than indicating a confirmed shift in biochar mechanisms. Biochar may facilitate organo-mineral association, thereby potentially affecting soil hydraulic conductivity and aeration. These processes may influence the bioavailability of mineral nitrogen (N) and phosphorus (P), while concurrently mitigating soil toxicity and fostering a stable microenvironment conducive to root system expansion. In summary, these effects may partly contribute to coordinated plant growth in terms of stem thickening, height increment, and crown expansion ([Joseph et al., 2010](#); [Glaser et al., 2015](#); [Thomas and Gale, 2015](#)). Nevertheless, organo-mineral association, hydraulic conductivity, root biomass, nutrient bioavailability, and root system expansion were not directly measured in this study; therefore, these processes should be considered plausible explanations rather than mechanisms directly demonstrated by our data.

Tenth-year observations revealed that the enhancement of Pn induced by biochar was weaker than that observed during the first-year observation period, which may be attributed to the gradual aging of biochar in soil, declining surface reactivity, and diminished

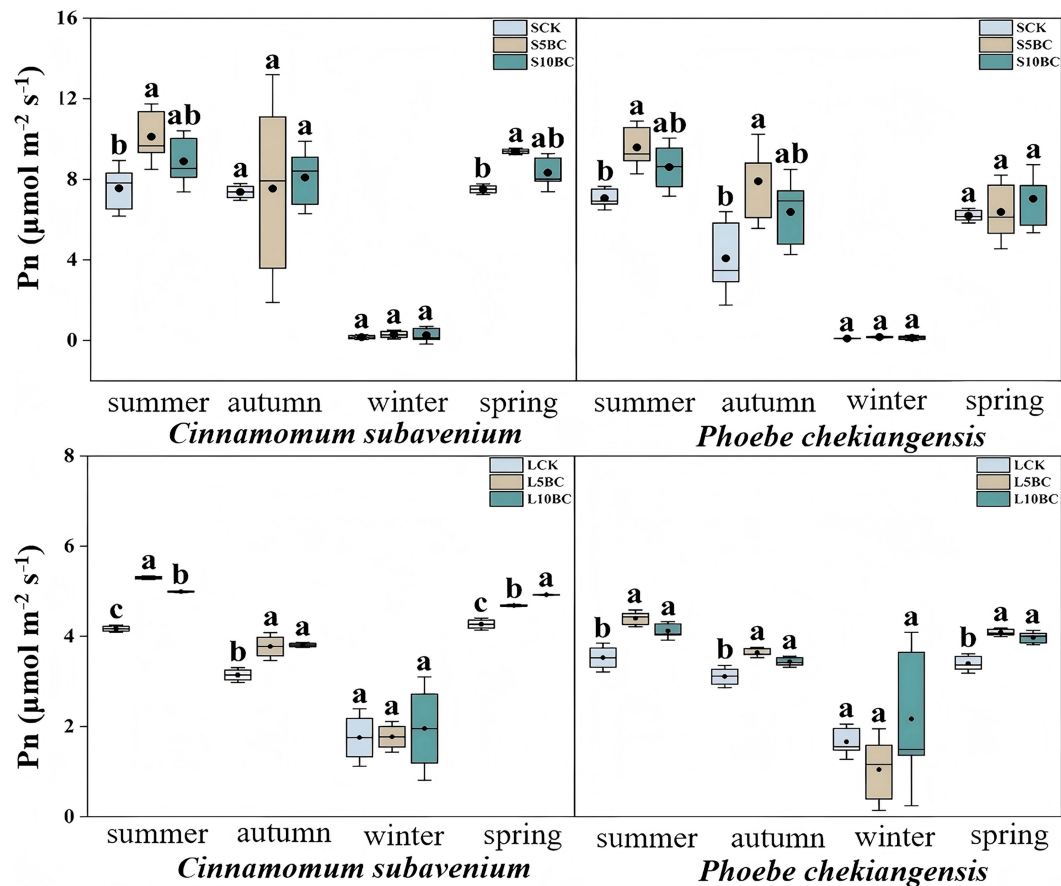


FIGURE 3

Seasonal variations in net photosynthetic rates of *Cinnamomum subavenium* and *Phoebe chekiangensis* across growth periods. Values followed by different lowercase letters indicate a significant disparity between treatments ($p < 0.05$). Boxes display 25th–75th percentiles; whiskers represent standard deviation; median values are indicated by horizontal lines, while mean values are denoted by black dots. Pn data are shown only for the two species with complete seasonal photosynthetic measurements. SCK, S5BC, and S10BC refer to the first-year phases with 0, 5, and 10 t/ha biochar; LCK, L5BC, and L10BC refer to the decadal-term observation phases with corresponding application rates. The different lowercase letters indicate significant differences among treatments within the same species and observation period at $p < 0.05$.

nutrient release capacity, potentially limiting sustained positive regulation of the photosynthetic processes (Nguyen et al., 2010; Lehmann and Joseph, 2015). However, several biochar properties, such as ash content, specific surface area, volatile matter, H/C and O/C ratios, and aromaticity indices, were not measured in our study. This limitation may constrain detailed interpretation of biochar aging, persistence, and soil–biochar interaction mechanisms. Nevertheless, biochar-treated plots maintained relatively high levels of Pn, which may be related to improved leaf nutritional status and photosynthetic physiological processes, including enhanced carboxylation efficiency and photochemical efficiency of photosystem II (Liu et al., 2013; Ding et al., 2016). However, leaf nutritional status, carboxylation efficiency, and photochemical efficiency of photosystem II were not directly measured in this study, so these physiological processes should be interpreted as possible explanations based on previous studies rather than direct evidence from the present data. Furthermore, seasonal fluctuations in Pn were evident, with peak values occurring in summer and minimum values occurring in winter. Similar seasonal patterns were also reported by Jeffery et al. (2011) and Li Y. et al. (2018), emphasizing the important role of seasonal environmental variation in regulating plant photosynthetic responses.

4.2 Contrasting first-year and tenth-year responses of soil carbon fractions and microbial biomass

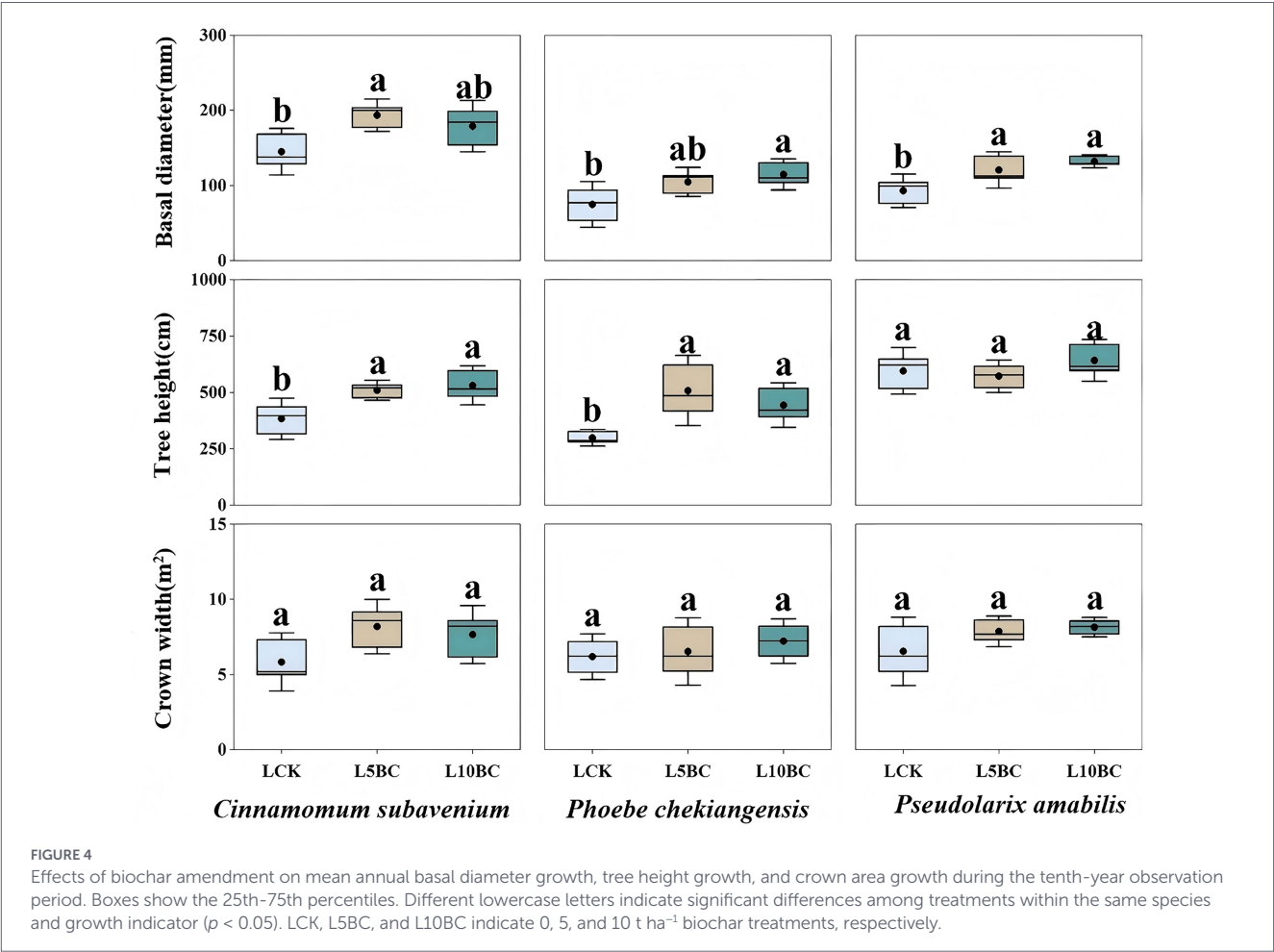
Biochar addition affected soil carbon fractions differently between the first-year and tenth-year observation periods, whereas annual soil CO₂ efflux showed no significant treatment response. In the short term observation period, biochar treatments significantly increased SOC and MBC. This increase may be partly related to the relatively stable aromatic structure of biochar, as well as its well-developed porous structure that can enhance physical protection of SOC by adsorbing organic carbon, reducing microbial access to organic substrates, and promoting microaggregate formation (Lehmann et al., 2011). Concurrently, the porous structure of biochar may provide microbial microhabitats and improve soil aeration and water-holding capacity, thereby facilitating MBC accumulation (Zimmerman et al., 2011). DOC concentration remained largely unchanged after biochar addition, possibly because stable biochar contributed little to the labile C pool, while the limited DOC released from biochar may have been rapidly consumed by microorganisms during the first-year observation period (Gao et al., 2025).

In the tenth-year observation period, the positive effects of biochar addition on SOC and MBC became even more pronounced. The

TABLE 3 Mean annual growth rates of basal diameter, tree height, and crown area across different biochar treatments during both short-term and long-term observation periods.

Tree species	Metric	SCK	LCK	S5BC	L5BC	S10BC	L10BC
<i>Cinnamomum subavenium</i>	Basal diameter (mm)	10.36 ± 0.79Bb	11.78 ± 1.25Ba	14.73 ± 0.36ABa	16.81 ± 0.79Aa	14.92 ± 2.07Aab	15.12 ± 1.41ABa
	Tree height (cm)	35.83 ± 0.48Ab	26.90 ± 1.81Bb	36.37 ± 0.02Ab	38.23 ± 2.17Aa	38.33 ± 4.48Ab	40.98 ± 3.45Ab
	Crown width (m²)	0.41 ± 0.09Ab	0.53 ± 0.07Aa	0.31 ± 0.03Ab	0.76 ± 0.07Aa	0.31 ± 0.03Ab	0.71 ± 0.07Aa
<i>Phoebe chekiangensis</i>	Basal diameter (mm)	10.40 ± 0.69Bb	5.60 ± 1.33Bb	15.79 ± 1.23Aa	8.19 ± 0.74ABb	12.81 ± 0.98ABb	9.52 ± 0.79Ab
	Tree height (cm)	28.83 ± 3.77Ab	19.24 ± 1.59Bb	24.50 ± 6.17Ab	38.18 ± 5.84Aa	25.72 ± 7.38Ab	32.93 ± 3.34ABb
	Crown width (m²)	0.40 ± 0.03Ab	0.56 ± 0.06Aa	0.48 ± 0.04Ab	0.58 ± 0.09Aa	0.47 ± 0.14Ab	0.66 ± 0.06Aa
<i>Pseudolarix amabilis</i>	Basal diameter(mm)	15.01 ± 0.28Ba	7.54 ± 0.76Bb	15.94 ± 0.82ABa	9.86 ± 0.79Ab	17.94 ± 0.75Aa	11.03 ± 0.31Ab
	Tree height (cm)	58.30 ± 8.17Aa	49.62 ± 4.30Aa	52.67 ± 0.87Aa	45.46 ± 2.60Aa	60.27 ± 5.29Aa	53.27 ± 3.19Aa
	Crown width (m²)	0.90 ± 0.07Aa	0.62 ± 0.08Aa	0.95 ± 0.11Aa	0.73 ± 0.02Aa	0.97 ± 0.09Aa	0.77 ± 0.02Aa
Mixed tree species	Basal diameter (mm)	11.92 ± 0.83B	8.31 ± 1.08B	15.48 ± 0.48A	11.62 ± 1.37AB	15.22 ± 1.01A	11.89 ± 0.96A
	Tree height (cm)	40.99 ± 5.15A	31.92 ± 4.78A	37.84 ± 4.46A	40.62 ± 2.29A	41.44 ± 5.83A	42.39 ± 3.39A
	Crown width (m²)	0.57 ± 0.09A	0.57 ± 0.04B	0.58 ± 0.10A	0.69 ± 0.04A	0.65 ± 0.11A	0.71 ± 0.03A

Statistical differences among experimental groups are indicated by different uppercase letters ($p < 0.05$). SCK and LCK refer to control groups; S5BC/S10BC and L5BC/L10BC refer to short-term and long-term observation periods with 5 and 10 t/ha of biochar, respectively.



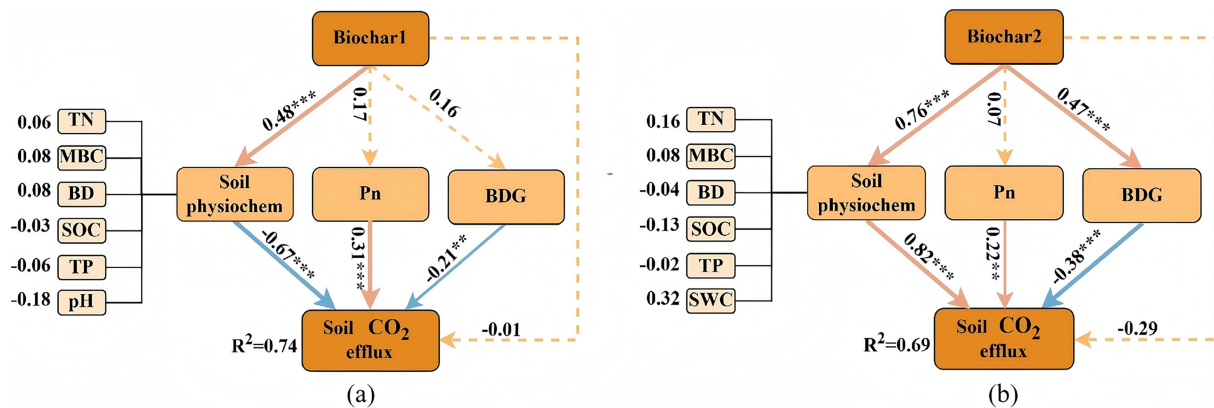


FIGURE 5

Model-based statistical pathways linking biochar amendment to soil CO₂ efflux during the first-year [(a), Biochar 1] and tenth-year [(b), Biochar 2] periods, as modeled by structural equation modeling. The models quantify how biochar affects soil CO₂ efflux through its effects on soil physicochemical properties, Pn, and basal diameter growth (BDG). The solid and dashed lines represent statistically significant and non-significant pathways, respectively, with the standardized coefficients labeled next to the corresponding arrows, and R² statistics indicate the degree to which the model accounts for variability in soil CO₂ efflux. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

sustained accumulation of SOC and MBC may reflect the persistence of relatively stable biochar-derived C and its potential contribution to soil carbon retention over time (Jones et al., 2011). In contrast, DOC showed no significant change during the tenth-year observation period, possibly because most unstable carbon fractions in biochar were largely lost through prolonged microbial decomposition or leaching. In addition, as a carbon source directly available to microorganisms, DOC often fluctuates with seasonal microbial activity and may exhibit a trade-off relationship with MBC, particularly under high summer temperatures when it is rapidly consumed (Kuzayakov et al., 2014).

Regarding soil CO₂ efflux, cumulative annual emissions did not differ significantly between biochar and control treatments in either the short- or long-term observation period ($p > 0.05$; Table 1). This null net change may reflect the combined influence of statistically opposite pathways rather than the absence of biochar effects. Path analysis (Section 4.3) suggested a dominant negative indirect association related to elevated pH during the first-year observation period, whereas positive indirect associations related to soil moisture, total nitrogen and photosynthetic rate were observed during the tenth-year observation period. Meanwhile, SEM identified a negative direct statistical pathway between biochar addition and soil CO₂ efflux, which was opposite in direction to the positive indirect pathways mediated by soil physicochemical properties and plant-related variables. Biochar's aromatic structure and high chemical stability may partly contribute to soil carbon retention by slowing the decomposition of biochar-derived C and potentially influencing native SOC mineralization (Major et al., 2010b; Spokas et al., 2012). However, this process should be regarded as a plausible explanation based on previous studies rather than direct evidence for the SEM-derived direct pathway in the present study. Soil CO₂ efflux exhibited seasonal summer peaks, but these peaks were not significantly intensified by biochar amendment. One possible explanation is that biochar may have reduced the accessibility of organic matter to microbial decomposition through physical protection or adsorption processes (Zimmerman et al., 2011). However, aggregate formation, adsorption processes, and related physical protection mechanisms were not directly measured in this study. Therefore, the absence of a significant increase in annual soil CO₂ efflux should be interpreted in the context of heterogeneous

responses reported in previous biochar studies, and the present results should be considered site- and system-specific rather than universally applicable. Although biochar increased SOC and MBC without increasing annual soil CO₂ efflux, these results reflect changes in measured soil carbon storage indicators, while a full ecosystem carbon-budget assessment is still needed to evaluate net carbon sequestration. It should be noted that SOC was reported as concentration rather than stock in this study. Although soil bulk density was measured as part of the initial soil characterization, SOC stock was not calculated because equivalent-soil-mass or depth-specific stock estimates were not available for all observation periods. Therefore, the observed SOC increase should be interpreted as a change in soil carbon concentration or soil carbon storage indicators rather than direct evidence of SOC stock accumulation.

4.3 Contrasting soil and plant associations with soil CO₂ efflux between the first and tenth years

Building on the two-period comparison described above, the factors statistically associated with soil CO₂ efflux differed between the first and tenth years after biochar application. In the first-year observation period, SEM results suggested that biochar addition was weakly and negatively associated with soil CO₂ efflux, mainly through the pathway related to soil pH. During the early stages of biochar application, the increase in soil pH showed a negative indirect association with soil CO₂ efflux. Lehmann et al. (2011) also reported that biochar amendment can rapidly raise the pH of acidic soils because of its alkaline nature. However, the effect of pH increase on soil CO₂ efflux in acidic soils is not necessarily unidirectional. Moderate pH increases may relieve acidity stress and stimulate microbial activity in some acidic soils, whereas context-specific pH shifts may also alter microbial community structure, substrate availability, or enzyme-mediated organic matter decomposition (Rousk et al., 2010). Therefore, the negative pH-related pathway observed in this study should be interpreted as a context-specific statistical association rather than a universal inhibitory mechanism. However, microbial community composition and extracellular enzyme activity were not directly measured in this study; therefore,

this explanation should be interpreted as a plausible interpretation rather than direct evidence from the present data. Although total soil nitrogen and microbial biomass carbon showed positive associations with soil CO₂ efflux, these associations did not offset the negative pH-related association in the SEM. This pattern was consistent with previous studies in acidic red soils and subtropical forest soils (Zheng et al., 2013). Therefore, during the first-year observation period, biochar showed a weak negative association with soil CO₂ efflux, primarily through the pH-related statistical pathway, although the interpretation of this pathway should remain context-specific.

In the tenth-year observation period, biochar showed positive indirect associations with soil CO₂ efflux through changes in soil physicochemical properties, particularly soil moisture and total nitrogen. This pattern may be partly related to the gradual stabilization of biochar structure over time, which could improve water retention and soil moisture conditions, thereby influencing microbial and root-related metabolic processes. Elevated nitrogen levels may also be associated with greater root activity and nitrogen cycling, potentially contributing to higher soil CO₂ efflux. This interpretation is consistent with previous studies suggesting that the longer-term effects of biochar may arise from sustained improvements in soil structure and nutrient status, thereby strengthening soil-plant interactions rather than relying solely on initial pH changes (Lehmann and Joseph, 2015; Yoshitake et al., 2025). However, because this study measured total soil CO₂ efflux without separating autotrophic and heterotrophic respiration, and did not directly measure available nitrogen, root respiration, microbial metabolic activity, or nitrogen cycling processes, these explanations should be regarded as plausible interpretations rather than mechanisms directly supported by the present data. Importantly, the difference in statistical associations between the first-year and tenth-year observation periods did not lead to a net increase in total soil CO₂ efflux, because the negative direct SEM pathway counterbalanced the positive indirect pathways. Collectively, the two-period comparison suggests that biochar-soil CO₂ efflux relationships differed between the first- and tenth-year observation periods. Biochar showed a weak negative association with soil CO₂ efflux in the first-year observation period, whereas a weak positive association was observed in the tenth-year observation period. The tenth-year response may be related to higher plant growth and changes in soil physicochemical conditions, but these relationships should be interpreted as statistical associations rather than direct evidence of a continuous mechanistic transition.

In addition, climatic variability across the decade, including inter-annual precipitation differences, possible extreme weather events, and stand development independent of biochar addition, was not explicitly separated in this study; therefore, the differences between the first-year and tenth-year observation periods should be interpreted cautiously as two-period contrasts rather than as changes attributable solely to biochar addition.

5 Conclusion

This study compared the responses of soil CO₂ efflux, soil carbon fractions, and plant growth to a single biochar application

during the first-year and tenth-year observation periods in subtropical evergreen plantations. Biochar significantly increased SOC and MBC, with greater accumulation observed during the tenth-year observation period, while annual soil CO₂ efflux did not differ significantly among treatments. These findings indicate that biochar boosts soil carbon storage via elevated SOC and MBC, yet they do not provide direct proof of whole-ecosystem net carbon sequestration. Overall, biochar may enhance SOC and MBC accumulation in the studied plantations, but its broader implications for net carbon sequestration require further verification across different sites, biochar types, soil conditions, and forest systems, as well as through complete carbon-budget assessments.

Data availability statement

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Author contributions

YY: Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft. XL: Conceptualization, Funding acquisition, Investigation, Methodology, Supervision, Writing – review & editing. NS: Investigation, Software, Writing – review & editing. XY: Conceptualization, Funding acquisition, Writing – review & editing. TG: Conceptualization, Funding acquisition, Writing – review & editing.

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Conflict of interest

NS was employed by Ningbo Chaosheng Ocean Technology Co., Ltd.

The remaining author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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