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The multi-pathway mechanism of soil organic carbon accumulation in *Pinus massoniana* plantations under nitrogen and phosphorus addition: from soil biota to carbon stability

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Abstract

Background The decline in soil organic carbon accumulation caused by intensified nitrogen deposition is concerning. Although phosphorus input may alleviate the negative impacts, there is still a research gap regarding the mechanisms, particularly those involving the soil biota, that drive the stability of soil organic carbon.

Methods We conducted a 2-year nitrogen (0, 30 and 90 kg N ha⁻¹ yr⁻¹) and phosphorus (0, 30 kg P ha⁻¹ yr⁻¹) addition experiment with six treatments in a 25-year-old *Pinus massoniana* plantation in subtropical China.

Results The addition of external nutrients improved soil nutrient availability but led to a decrease in pH. Low nitrogen input promoted the particulate organic carbon (POC) and total organic carbon, whereas high nitrogen input had the opposite effect. Phosphorus addition alleviated these negative impacts to some extent. Nitrogen and phosphorus addition significantly affected the dissimilarity of soil biological communities. Nitrogen treatments generally reduced the alpha diversity index of soil bacteria, while the trend for fungi was the opposite. Arthropods showed a rise followed by a decline, with phosphorus addition weakening these effects. Soil respiration decreased with increasing nitrogen addition, and phosphorus addition didn't alter this trend. The POC was primarily influenced by the soil environment-microorganism-respiration and environment-microorganism pathways, whereas the mineral-associated organic carbon (MAOC) was mainly influenced by the soil environment-arthropod pathway. POC (Path coefficient, pc=0.524) and MAOC (pc=0.237) directly determine the accumulation of organic carbon. This conceptual model explained 59.4% of the variation in total organic carbon (Goodness-of-fit, GOF=0.594), thereby delineating the integrated mechanisms underlying SOC accumulation.

Conclusions Excessive nitrogen input was unfavorable for organic carbon accumulation, while phosphorus addition partially mitigated the negative effects of nitrogen excess. Under this context, active organic carbon was significantly influenced by soil microorganisms and soil respiration, whereas stable organic carbon was primarily affected by soil arthropods.

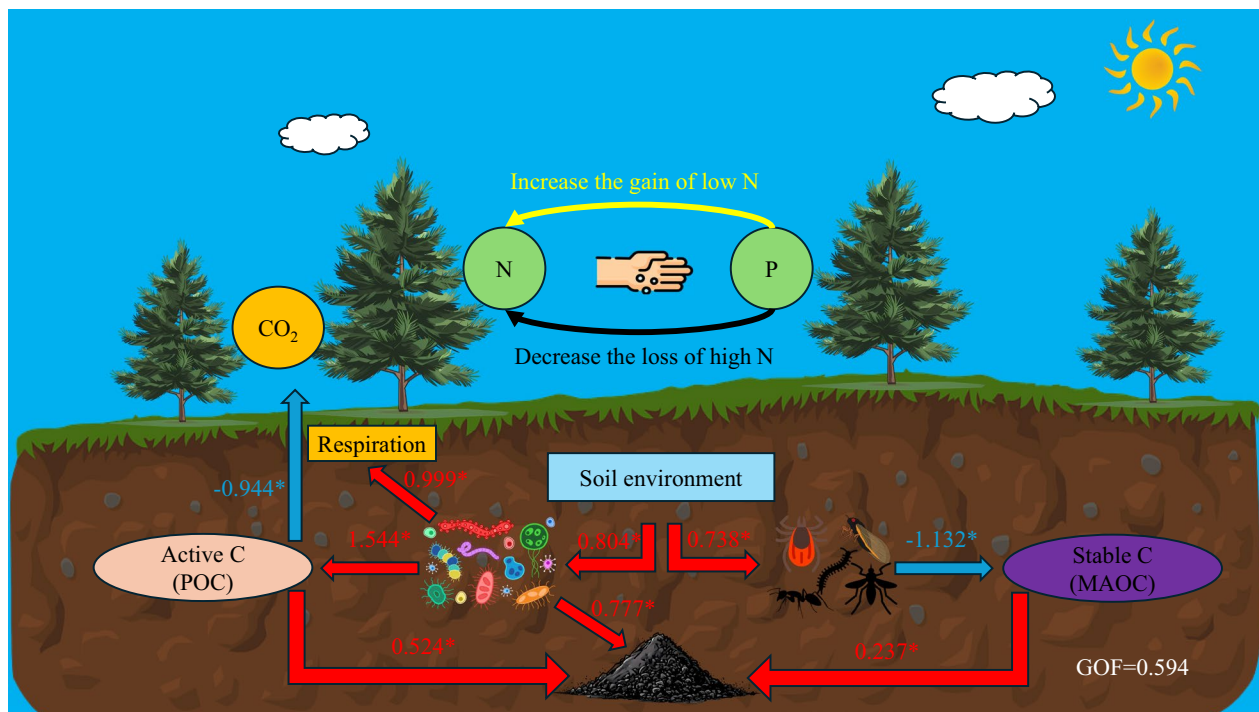
Keywords Nitrogen and phosphorus addition, Soil biota, Soil respiration, Soil organic carbon component

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Graphical Abstract



Introduction

Soil organic carbon is a crucial component of the global carbon cycle, and its fixation patterns have profound implications for strategies aimed at mitigating the current global greenhouse effect (Lima et al. 2025; Xu et al. 2024a). With the global push for ecological restoration, plantation forests, as an important part of forest resources, have become significant carbon reservoirs in terrestrial ecosystems (Yang et al. 2021). However, as global warming accelerates, the release of soil carbon in plantation forests is increasing, raising concerns about the risk of these forests transitioning from carbon “sinks” to carbon “sources” (Wu et al. 2014). In response to this potential issue, a substantial body of research has been conducted. Although the choice of tree species for afforestation is a direct factor influencing soil organic carbon, affecting it through litter decomposition and root activity (Hassan et al. 2019; Florio et al. 2021), substantial evidence suggests that climate change and human activities—especially the input of exogenous nutrients—play a key role in determining the impact on SOC in plantation forests (Liu et al. 2025a, b; Ling et al. 2025).

Nitrogen, as a critical limiting nutrient in soils, plays a major role in terrestrial soil carbon cycling (Xu et al. 2024b). Forest soil carbon fixation is significantly

impacted by nitrogen deposition, and the pathways of this effect are not singular (Lu et al. 2021a). The traditional view holds that nitrogen input promotes SOC accumulation (Song et al. 2017). However, in recent years, with the intensification of nitrogen deposition, long-term high nitrogen input has led to a decrease in SOC accumulation (Kangi et al. 2025). This process is co-regulated by multiple interacting factors. For example, nitrogen deposition affects nutrient return by influencing litter decomposition, which in turn impacts soil organic carbon (Dong et al. 2020). Similarly, soil microorganisms respond rapidly to nitrogen inputs, and changes in their community structure and biological activity alter the rate of nutrient mineralization, thereby affecting organic carbon accumulation (Wang et al. 2023). Additionally, soil arthropods, as key components of soil biota, play a significant role in SOC dynamics through processes such as litter decomposition and their own mortality. Fluctuations in nitrogen availability can substantially affect their diversity, thereby influencing SOC accumulation (Siepel et al. 2018). Moreover, soil respiration, a major pathway for soil organic carbon release, is significantly influenced by nitrogen deposition (Zhang et al. 2021). For instance, nitrogen input affects different respiratory components (autotrophic respiration and heterotrophic respiration)

by altering soil active materials, leading to increased CO₂ release (Ma et al. 2020). Importantly, intensified nitrogen deposition also exacerbates phosphorus limitation in soils, further impacting soil carbon turnover processes (Chen et al. 2025). Regarding phosphorus, its input alleviates nitrogen-induced phosphorus limitation, thereby mitigating some of the negative effects of nitrogen excess, which has become widely accepted (Liu et al. 2025c; Hui et al. 2020). For example, studies have confirmed that the addition of exogenous phosphorus alleviates phosphorus limitation caused by nitrogen deposition and influences SOC accumulation through multiple pathways. On one hand, the addition of exogenous phosphorus alters the composition of soil biological communities, which may alter the production of carbon-degrading enzymes and affect carbon input (Mori et al. 2018). On the other hand, phosphorus addition can change the competition intensity between plant roots and soil biota, modulating soil respiration and affecting carbon output (Wang et al. 2017). However, the combined effects of exogenous phosphorus and nitrogen on SOC accumulation mechanisms remain underexplored.

The fixation pattern of SOC accumulation mainly depends on its input and output, and its stability is the key determinant of the final accumulation effect (Dong et al. 2022). SOC can be broadly divided into active and stable organic carbon based on its stability. Different organic carbon fractions have varying turnover rates and sensitivities to environmental changes (Liu et al. 2025a; Schmidt et al. 2011; Gulde et al. 2008). Therefore, the accumulation patterns of different organic carbon fractions under nitrogen and phosphorus addition may differ significantly. Some studies have explored the effects of nutrient additions on various SOC fractions, such as evidence showing that nitrogen and phosphorus addition significantly increases the content of active SOC in temperate and tropical forests (Chen et al. 2025; Wang et al. 2024a), or that nitrogen and phosphorus addition alters soil microbial community structure, influencing the accumulation of stable SOC in tropical forests (Huang et al. 2025), these studies have provided valuable insights into the responses of different SOC fractions to exogenous nutrient additions. However, in subtropical plantations of China, existing studies have predominantly focused on the addition of a single nutrient (e.g., nitrogen or phosphorus), while the effects of combined nitrogen and phosphorus additions remain largely unexplored. Moreover, research on the responses of SOC components to nutrient enrichment—particularly regarding the biological mechanisms driving SOC stability—has typically considered only one group of soil biota (e.g., microbes or fauna). This gap limits our ability to accurately assess SOC accumulation and may lead to

an underestimation of the role of soil biotic communities in this process. Therefore, this study aims to analyze the community structure and diversity of soil microorganisms and arthropods, and to evaluate their relative contributions to the accumulation of soil organic carbon.

Pinus massoniana, as a widely distributed conifer species in southern China, is renowned for its strong nutrient retention ability and resilience, exhibiting high environmental adaptability and rapid growth (Jian et al. 2022). As a pioneer afforestation species in subtropical China, it has a broad distribution and plays a crucial role in controlling soil erosion, making it an ideal subject for this study (Wu et al. 2024a). We made different experimental treatments of nitrogen and phosphorus addition in Taizi Mountains in Hubei Province, China, conducting sampling and measurement of relevant indicators 2 years after the treatments began. These indicators included soil environmental factors (pH, total nitrogen, total phosphorus, available nitrogen, available phosphorus), soil microbial characteristics (bacterial and fungal diversity, microbial biomass carbon, microbial biomass nitrogen and phosphorus), soil arthropod characteristics (diversity, biomass), soil respiration (autotrophic respiration, heterotrophic respiration, total respiration), and SOC fractions (active: particulate organic carbon (POC); stable: mineral-associated organic carbon (MAOC)). To explore the ecological regulatory mechanisms behind the accumulation of different SOC fractions, we hypothesized that: (1) Low nitrogen addition promotes SOC accumulation, whereas excessive nitrogen input has the opposite effect. (2) Phosphorus input enhances the positive effects of low nitrogen and mitigates the negative impacts of high nitrogen addition. (3) The accumulation of different SOC components is regulated by distinct biological pathways: active carbon (POC) is primarily governed by a pathway involving soil environment, microorganisms, and soil respiration, whereas stable carbon (MAOC) is mainly controlled by a pathway linking soil environment and arthropods.

Materials and methods

Study region

The study area is located in Taizi Mountain, Jingmen City, Hubei Province, China (112°48′–113°03′ E, 30°48′–31°02′ N), with a forest coverage rate of 85%. The plantation was established in 1999 on a site with a prescribed burning following the harvesting of *Pinus elliotii* plantation. After reforestation it was managed through regular tending operations, and beginning in 2021 selective felling was implemented to remove trees affected by pine wood nematode (*Bursaphelenchus xylophilus*). This region has a subtropical monsoon climate, with an average annual precipitation of 1094.6 mm and a frost-free

period of 240 days. The average annual temperature is 16.4 °C, the maximum temperature can reach 39.2 °C, while the minimum temperature can drop as low as −19.6 °C. According to *Classification and codes for Chinese soil* (Tian et al. 2009), the soil type in the study area is leaching soil-yellow silica sand soil. The dominant tree species mainly include *Quercus acutissima*, *Cupressus funebris*, *Pinus massoniana*, etc. (Li et al. 2025a).

Methodology

Plot design

In July 2022, a 25-year-old *Pinus massoniana* plantation was selected within the study area and established as a set of 6 nitrogen and phosphorus treatments. These included: control (CK), phosphorus (P, 30 kg P ha^{−1} yr^{−1}), low nitrogen (LN, 30 kg N ha^{−1} yr^{−1}), low nitrogen with phosphorus (LN+P, 30 kg N ha^{−1} yr^{−1}, 30 kg P ha^{−1} yr^{−1}), high nitrogen (HN, 90 kg N ha^{−1} yr^{−1}), and high nitrogen with phosphorus (HN+P, 90 kg N ha^{−1} yr^{−1}, 30 kg P ha^{−1} yr^{−1}). Each treatment group consisted of three replicate plots, each measuring 20 m×20 m, for a total of 18 plots, and the buffer zone between plots was 20 m. Starting in July 2022, nitrogen and phosphorus treatments were applied at the beginning of each month (between the 1st and 5th) during the growing season (April to October), based on the local phenology of *Pinus massoniana*. The fertilizers (HN₄NO₃, NaH₂PO₄) were dissolved in 10 L of distilled water and then evenly sprayed onto the surface of each plot (control plots were sprayed with an equal amount of distilled water). The treatments were continuously applied for 24 months. The minimum nitrogen input amount is based on the annual regional atmospheric nitrogen deposition; to control variables and reduce resource waste, the phosphorus addition amount is consistent with the minimum nitrogen input amount of this study, as determined by similar research (Chen et al. 2025; Fang et al. 2019). The nitrogen level treatments were designed to simulate the effects of progressive nitrogen deposition, while the phosphorus concentration treatments aimed to mitigate potential phosphorus limitation and other possible constraints in the plantation soils.

Soil sampling

In July 2024, after 24 months of continuous nutrient treatments, soil samples were collected from each plot 15 days after the final fertilization event to capture the treatment effects while avoiding the immediate pulse effect of fertilizer application. Soil samples were collected from each plot using a “S-shaped” sampling method (along the diagonal of each plot, soil was sampled every 5 m, resulting in a total of five subsamples per plot). PVC pipes (10 cm high, 5 cm diameter) were used to extract

soil samples from a depth of 10 cm, excluding surface litter. The soil samples from each plot were mixed together, and any fine roots or debris were removed. The samples were then sieved through a 2 mm mesh and divided into two portions for further analysis. One portion was air-dried for the measurement of soil factors and organic carbon components. Another portion was designated for soil microbial analyses. Specifically, samples for microbial biomass determination were stored at 4 °C and processed within 1 week, while those for high-throughput sequencing were preserved at −80 °C for subsequent DNA extraction. Soil factors were analyzed as follows: soil pH was measured with a BPH-7100 pH meter; soil organic matter (SOC) was determined using the potassium dichromate oxidation method with external heating; total nitrogen (STN) was measured by the Kjeldahl method; available nitrogen (SAN) was determined by alkali diffusion; total phosphorus (STP) and available phosphorus (SAP) were determined by the molybdenum-antimony colorimetric method, following H₄SO₄-HClO₄ digestion and NaHCO₃ extraction, respectively (Tie et al. 2021; Zhang et al. 2024b). Particulate organic carbon and mineral-associated organic carbon were determined using the sodium hexametaphosphate-potassium dichromate titration method (Blair et al. 1995; Luan et al. 2010; Garten 2002).

Soil microbial biomass carbon (MBC), nitrogen (MBN), and phosphorus (MBP) were measured using the chloroform fumigation-K₂SO₄ extraction method (Zhang et al. 2024a). For high-throughput sequencing of soil microbial communities, bacterial community sequencing was performed using 16S rRNA gene amplicon technology, with specific primers F (5'-ACTCCTACGGGAGGCAGC A-3') and R (5'-GGACTACHVGGGTWTCTAAT-3') targeting the V3-V4 regions of 16S rRNA for sequencing (Caporaso et al. 2011; Youssef et al. 2009). Fungal community sequencing was performed using primers ITS_5F (GGAAGTAAAAGTCGTAACAAGG) and ITS_2R (GCTGCGTTCTTCATCGATGC) for the amplification and sequencing of the ITS V1 region (Nilsson et al. 2006; Segata et al. 2011). Sequencing was carried out by Shanghai Personal Biotechnology Co., Ltd (Shanghai, China) (<http://www.personalbio.cn/>). Species information was identified based on sequencing annotations, and microbial diversity indices (Simpson and Shannon index) for both soil bacteria and fungi were calculated using R (Version 4.0.3).

Soil arthropod sampling

Soil arthropods were sampled from both the surface and subsurface layers to provide a more comprehensive representation of their distribution. For surface sampling, 5 small plots (1 m×1 m) were randomly selected within each plot, and sampling was conducted within these

small plots. Five trap cups with a diameter of 8 cm and a height of 10 cm were randomly placed within each small plot (avoiding clustering and edges), with the top of the cups flush with the soil surface. Each cup was filled with 55 ml of a mixture of glycerol and 75% ethanol (in a 1:10 volume ratio), with a total of 90 traps set across all plots. Traps were harvested every 3 days, with samples placed in 75% ethanol for transport back to the laboratory, and traps were reset each time. Sampling occurred four times over a 12-day period, starting 15 days after the nitrogen and phosphorus treatments ended in July 2024 (Masoomah and Esmail 2012). For subsurface sampling, four sampling points were set 30 cm from the trap cups in the east, south, west, and north directions within each plot. A soil auger (5 cm diameter, 20 cm length) was used to collect 0–20 cm soil samples from each sampling point, with four samples collected over the same 12-day period as the trap harvests. The soil samples were taken back to the laboratory and processed using the Berlese–Tullgren funnel method. The soil was placed on a 2 mm mesh sieve, and the collected arthropods were preserved in 75% ethanol in the collection container. After 24 h of dark incubation at room temperature, the samples were exposed to 40 W incandescent light for 48 h to induce arthropod extraction. Afterward, the arthropods were washed repeatedly with 75% ethanol and stored in clean 75% ethanol (Peguero et al. 2021). Following the treatments, the four samples from each plot were combined to form a single composite sample. The arthropods were identified and classified using a binocular microscope with a double-lens system, following the “China Pictorial Keys to Soil Animals” (Yin 2000) and by consulting relevant taxonomic references (Li et al. 2021). After identification, the specimens were placed in paper envelopes and dried in an oven at 65 °C to constant weight to measure their biomass. Finally, the species data were analyzed using R (Version 4.0.3), calculating the Simpson and Shannon index for arthropod diversity.

Soil respiration measurements

In July 2022, after the establishment of the plots, we randomly installed two PVC rings (respiration rings) with a diameter of 20 cm at each plot (avoiding the edges of the plot), spaced 2 m apart. The rings were inserted 7 cm into the soil with 5 cm exposed above the surface. One of the respiration rings was surrounded by a trench 50 cm wide and 80 cm deep, and root removal was carried out. During the 12 months that the respiration rings were in place, we periodically cleared fallen litter and live plants from both the rings and the trench. Starting in July 2023, soil respiration measurements were taken in the middle of each month (during the growing season, they were specifically conducted 15 days after the cessation of nitrogen

and phosphorus addition treatments), the CO₂ flux from each respiration ring was measured using the LI-8100 (Li-Cor Inc., USA). The measurement time was selected between 9:00 am and 11:00 am on clear days. If it rained, the measurement was delayed by 4 days. The respiration rings with normal installation were used to measure the total soil respiration, and the rings with root removal were used to measure the heterotrophic respiration of the soil. Soil autotrophic respiration was calculated using the following equation:

$$R_a = R_s - R_h$$

where R_a is the soil autotrophic respiration rate, R_s is the total soil respiration rate, R_h is the soil heterotrophic respiration rate.

Statistical analyses

One-way analysis of variance was used to compare soil environmental factors (pH, STN, STP, SAN, SAP); soil organic carbon components (SOC, POC, MAOC); soil microbial factors (MBC, MBN, MBP and the alpha diversity indices of bacteria and fungi: Simpson and Shannon index), soil arthropod factors (biomass and the alpha diversity indices of arthropod: Simpson and Shannon index); soil respiration components (total, heterotrophic and autotrophic). Prior to data analysis, Levene's test was used to assess the homogeneity of variances, and Duncan's HSD test was used for post-hoc comparisons to ensure the reliability of the analytical results ($P < 0.05$ was considered statistically significant).

Principal coordinate analysis (PCoA) was used to study the beta diversity of soil microbial communities (bacteria and fungi) and arthropod communities based on Bray–Curtis distance. PERMANOVA/ANOSIM tests were then conducted to explore the dissimilarities in community composition between different treatments. $P < 0.05$ was considered statistically significant.

Partial least squares path modeling (PLS-PM) was used to explore the mechanisms influencing soil organic carbon components under nitrogen and phosphorus treatments. The results of the PLS-PM are based on the construction of latent variables. During the analysis, items with loadings < 0.7 in each latent variable were eliminated from the analysis.

All the analyses were done in the R (Version 4.0.3) statistical environment (R Core Team 2019).

Results

Response patterns of soil environmental factors and organic carbon components

For soil environmental factors (Fig. 1), nitrogen input significantly increased STN and SAN while decreasing soil pH value ($P < 0.05$). As nitrogen concentration

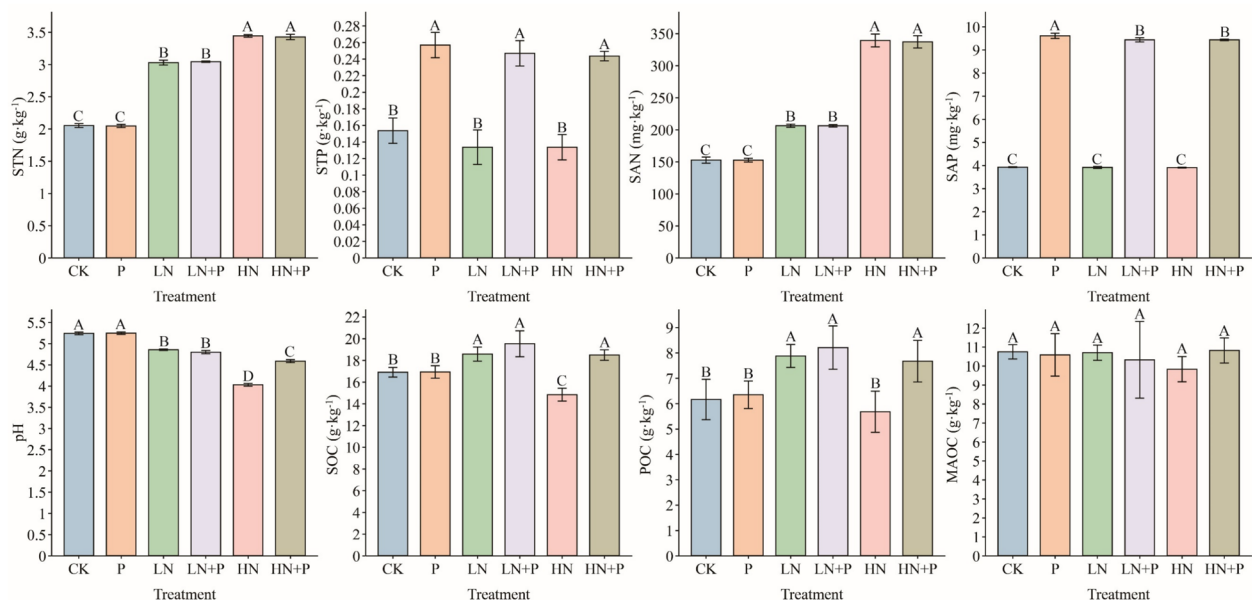


Fig. 1 Differences in soil environmental factors and organic carbon components among different treatments. STN represents soil total nitrogen, STP represents soil total phosphorus, SAN represents soil available nitrogen, SAP represents soil available phosphorus, SOC represents soil organic carbon, POC represents soil particulate organic carbon, MAOC represents soil mineral-associated organic carbon. The error bars in the bar charts represent standard error. Different capital letters indicate significant differences among different treatments at the 0.05 significance level

increased, this effect became more significant ($P < 0.05$). Phosphorus input significantly increased STP and SAP, and to some extent alleviated the decline in soil pH under high nitrogen input. Low nitrogen input significantly increased SOC content, especially when combined with additional phosphorus input, which amplified this effect ($P < 0.05$). In contrast, high nitrogen treatment alone significantly reduced SOC, and phosphorus input alleviated this negative impact ($P < 0.05$). Low nitrogen input also significantly increased POC, while high nitrogen input weakened this effect ($P < 0.05$). Additional phosphorus input alleviated the negative effects caused by high nitrogen input. None of the nutrient addition treatments had a significant impact on MAOC ($P \geq 0.05$).

Response patterns of soil microbial factors

We analyzed the high-throughput sequencing of soil microbes (bacteria and fungi) from all sampling sites (Fig. 2). For bacterial communities, the top 10 phyla by relative abundance were identified, with Acidobacteriota, Proteobacteria, Actinobacteriota, Verrucomicrobiota, and Chloroflexi being the dominant phyla (Average relative abundance $> 5\%$). In the principal coordinate analysis, the variance contribution of axis 1 was 72.73%, and axis 2 contributed 11.03%, with a cumulative contribution of 83.76%. All treatments differed significantly from the control (CK) in soil bacterial communities ($P < 0.05$). The HN+P treatment significantly increased the soil

bacterial Simpson index, while the LN, LN+P, and HN treatments significantly decreased the soil bacterial Shannon index ($P < 0.05$). Regarding fungal communities, the top 8 phyla by relative abundance were identified, with Basidiomycota and Ascomycota being the dominant phyla (Average relative abundance $> 5\%$). In the principal coordinate analysis, the variance contribution of axis 1 was 68.01%, and axis 2 contributed 29.97%, with a cumulative contribution of 97.98%. All treatments differed significantly from the control (CK) in soil fungal communities ($P < 0.05$). The P treatment significantly decreased the soil fungal Simpson index. Nitrogen inputs in the treatments significantly increased the Simpson and Shannon index ($P < 0.05$).

For soil microbial biomass, low nitrogen input significantly increased the contents of MBC and MBN, whereas high nitrogen input significantly reduced both MBC and MBN ($P < 0.05$). The additional phosphorus input partially alleviated the negative effects caused by high nitrogen input. Moreover, the content of MBP was significantly positively affected only by treatments involving phosphorus addition ($P < 0.05$).

Response pattern of soil arthropod factors

A total of 54,373 soil arthropods were captured in the study sites, comprising 62 taxa. Figure 3 shows the top 10 families by relative abundance of soil arthropods, with Parasitidae, Entomobryidae, Formicidae,

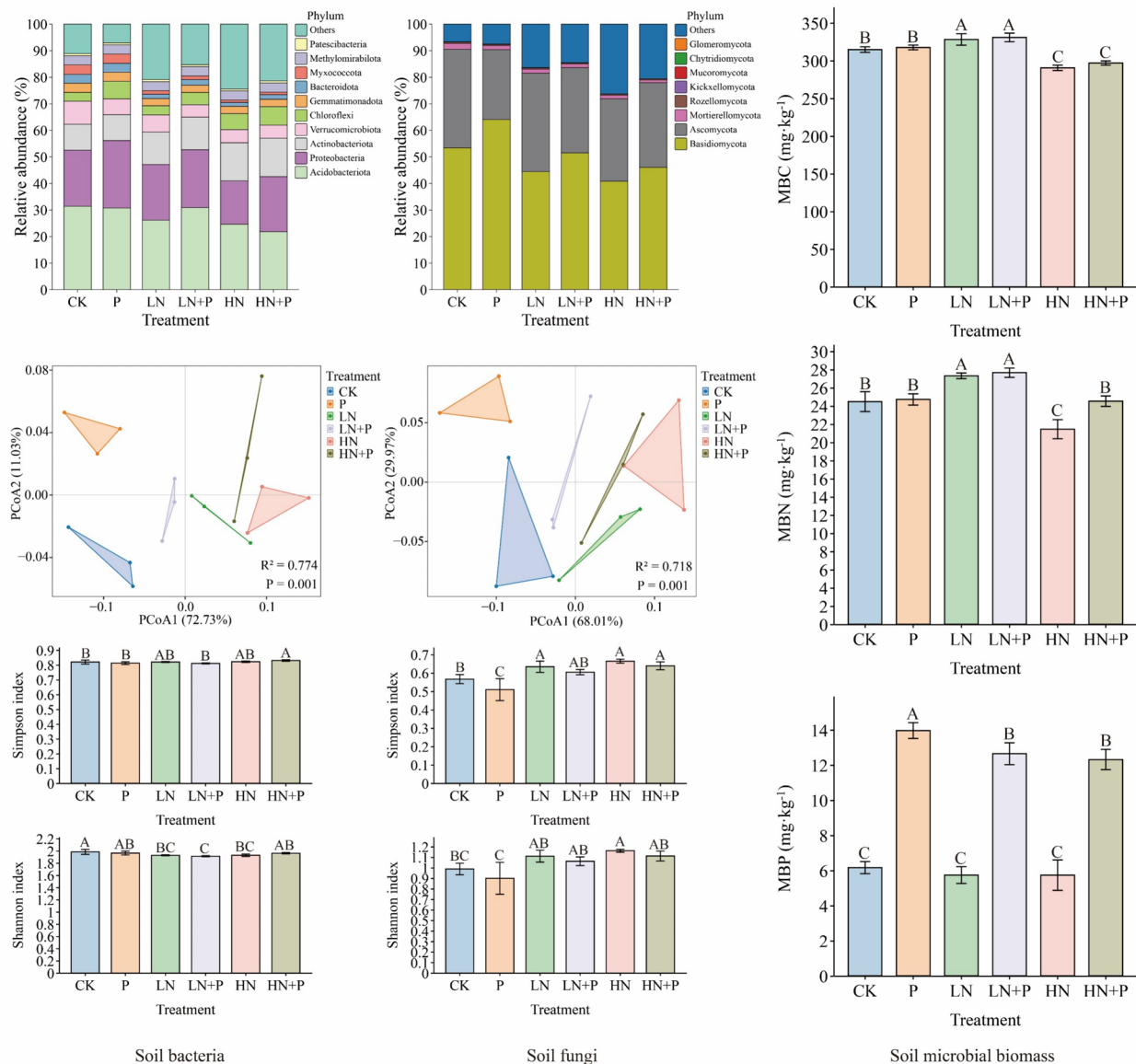


Fig. 2 Soil microbial community characteristics and biomass among different treatments. MBC represents soil microbial biomass carbon, MBN represents soil microbial biomass nitrogen, MBP represents soil microbial biomass phosphorus. The stacked column chart shows the relative abundance of soil microbial communities. Different triangles represent the community differences of soil microorganisms between different treatments. The error bars in the bar charts represents standard error. Different capital letters indicate significant differences among different treatments at the 0.05 significance level

and Paradoxosomatidae being the dominant families (Average relative abundance >5%). The results of the principal coordinate analysis showed that axis 1 explained 77.45% of the variance and axis 2 explained 13.03%, with a cumulative contribution of 90.48%. With the exception of all HN+P treatments, the effects of each treatment on the soil arthropod community were significantly different from CK ($P < 0.05$).

Phosphorus treatment and low nitrogen with phosphorus treatment significantly increased arthropod biomass ($P < 0.05$). Low nitrogen treatment significantly increased the Simpson index and Shannon index, and the additional phosphorus input enhanced this effect ($P < 0.05$). High nitrogen treatment significantly decreased the Simpson and Shannon index, while the additional phosphorus input alleviated this effect ($P < 0.05$).

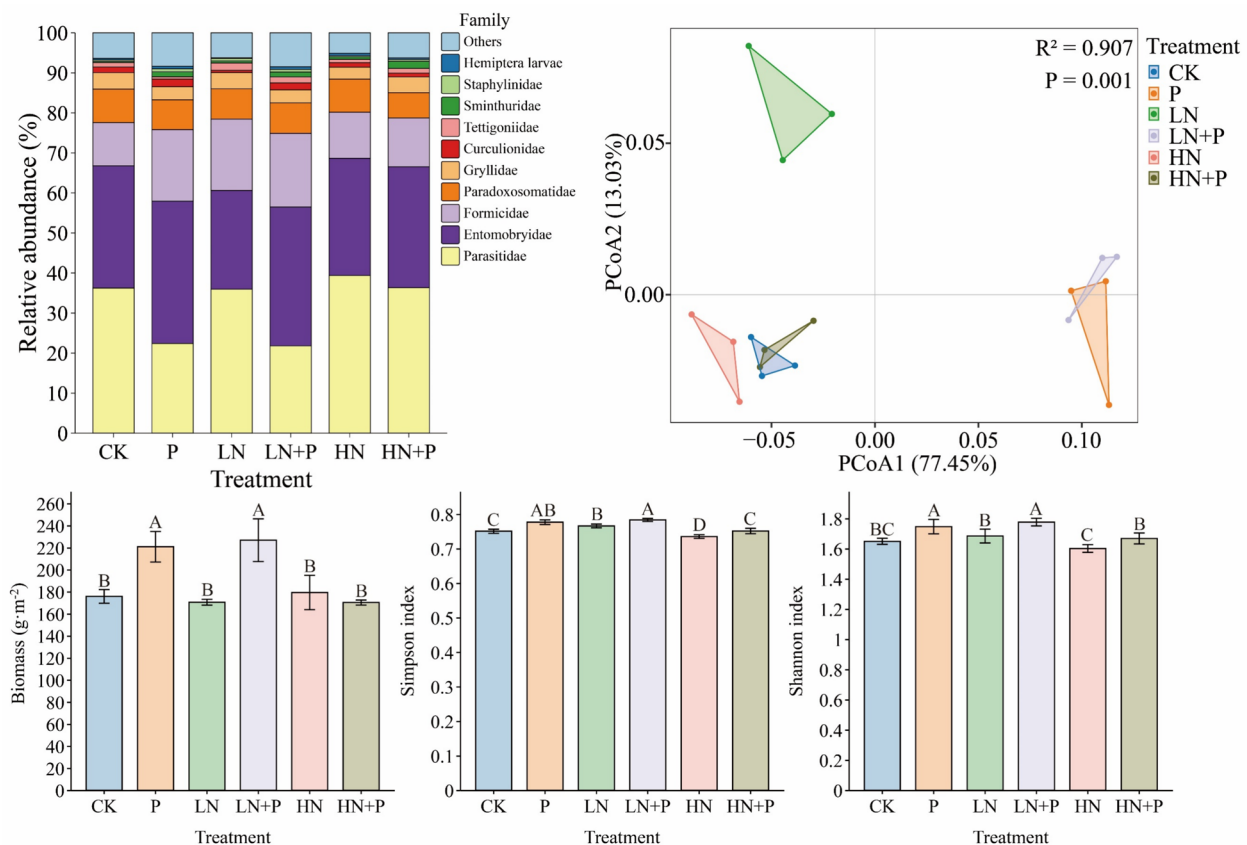


Fig. 3 Soil arthropod community characteristics and biomass among different treatments. The stacked column chart shows the relative abundance of soil arthropods. The error bars in the bar charts represents standard error. Different triangles represent the community differences of soil arthropods between different treatments. Different capital letters indicate significant differences among different treatments at the 0.05 significance level

Response pattern of soil respiration

Figure 4 shows the monthly dynamic changes of soil respiration components from July 2023 to July 2024. Overall, different soil respiration components increased in summer and decreased in winter. After 2 years of nitrogen and phosphorus treatments, significant differences in the soil respiration components were observed among the treatments ($P < 0.05$). In general, increased nitrogen input significantly reduced total soil respiration, heterotrophic respiration, and autotrophic respiration (especially with high nitrogen input, $P < 0.05$). However, additional phosphorus input did not alleviate this effect.

Drivers of soil organic carbon component accumulation

Given that N and P additions differentially altered soil biota, respiration, and carbon components, we employed PLS-PM to disentangle the direct and indirect pathways governing SOC accumulation. Based on the PLS-PM (Fig. 5), soil organic carbon components

were driven by different pathways (Goodness-of-fit, $GOF = 0.594$). For the active organic carbon component (POC), under nitrogen and phosphorus addition, soil environmental factors significantly influenced microbial factors ($P < 0.05$). The increased microbial biomass and diversity substantially enhanced the contribution to POC (Path coefficient, $pc = 1.544$), but also significantly promoted soil respiration ($pc = 0.999$, $P < 0.05$), thereby accelerating the consumption of active carbon components ($pc = -0.944$). For the stable organic carbon component (MAOC), soil environmental factors significantly affected arthropod factors ($pc = 0.738$, $P < 0.05$), and higher arthropod biomass and diversity markedly reduced MAOC content ($pc = -1.132$). Regarding soil organic carbon (SOC) accumulation, increases in both POC ($pc = 0.524$) and MAOC ($pc = 0.237$) significantly promoted SOC accumulation. Additionally, microbial factors directly contributed to SOC accumulation ($pc = 0.777$, $P < 0.05$).

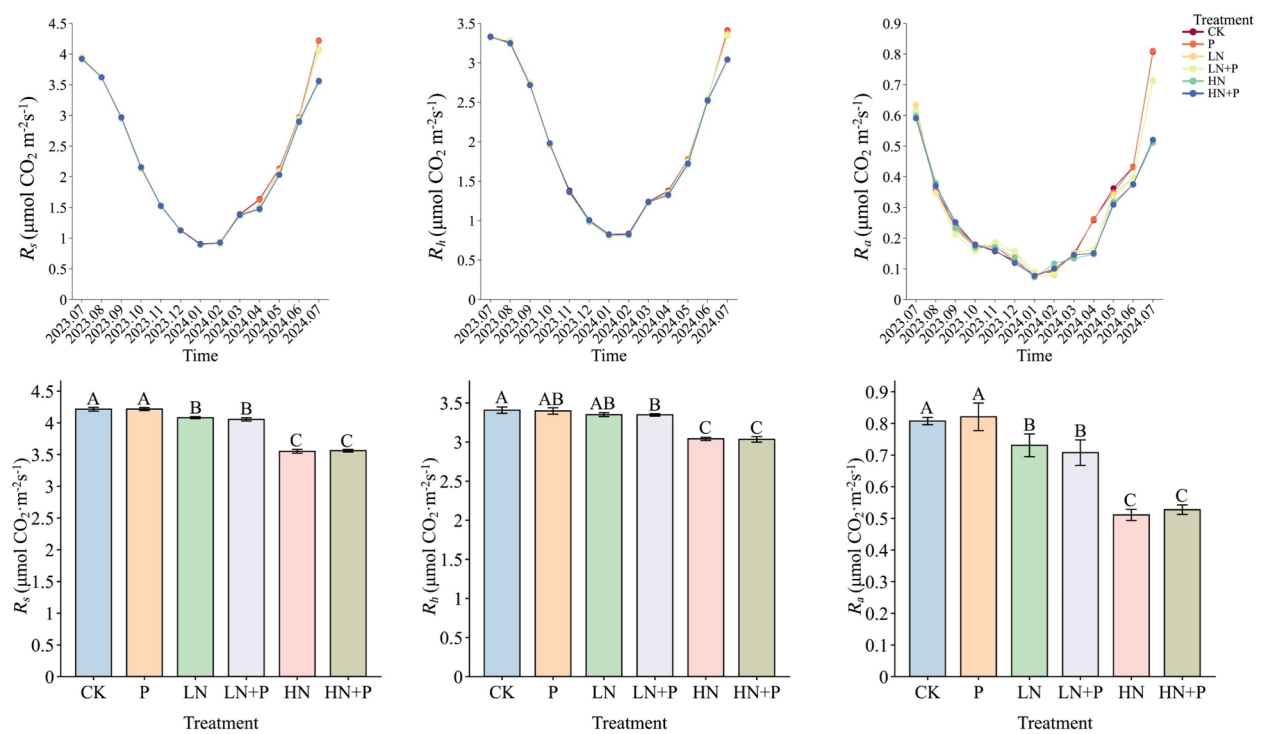


Fig. 4 Dynamic characteristics of soil respiration components among different treatments. The line graph represents the monthly variation of soil respiration components under different treatments. R_s represents total soil respiration, R_h represents soil heterotrophic respiration, R_a represents soil autotrophic respiration. The error bars in the line and bar charts represent standard error. Different capital letters indicate significant differences among different treatments at the 0.05 significance level

Discussion

Soil biological responses to nitrogen and phosphorus addition

Soil organisms, including soil microorganisms and soil arthropods, play crucial roles in the cycling of elements within forest soils (Tian et al. 2019; Tie et al. 2021). In terms of soil microbial biomass, MBC and MBN increased significantly with nitrogen addition at first, followed by a significant decrease. Although nutrients provided essential elements for microbial growth, excessive nitrogen led to a decrease in pH, potentially causing soil acidification and weakening carbon and nitrogen mineralization, which in turn suppressed microbial accumulation (Li et al. 2025b; Lu et al. 2021b; Zhu et al. 2020). The response of MBP, on the other hand, was significantly influenced by phosphorus addition rather than nitrogen, likely because the addition of phosphorus enriched soil phosphorus and total phospholipid fatty acids (PLFAs), leading to an increase in MBP (Wu et al. 2022; Xia et al. 2024). In microbial high-throughput sequencing results, significant differences in Beta diversity, based on the Bray–Curtis distance, were observed between different treatments, indicating that soil microbial communities are highly sensitive to changes in soil environments (Bian et al. 2025). Among soil bacteria, Acidobacteriota,

Proteobacteria, Actinobacteriota, Verrucomicrobiota, and Chloroflexi were dominant phyla. These bacteria not only represent a significant proportion of the soil's bacterial biomass but also exhibit strong environmental adaptability, making them dominant species across different treatments (Kielak et al. 2016; Sichert et al. 2020). Nitrogen input significantly decreased soil bacterial Shannon index, which may be due to the prolonged nitrogen addition, leading to a decrease in soil pH (Chen et al. 2024; Zhang et al. 2025). Phosphorus input alleviated the effects of high nitrogen addition, leading to a significant increase in the Simpson index, indicating that phosphorus input mitigated nitrogen-induced phosphorus limitation and enriched eutrophic microbial populations (Wang et al. 2024b; Sailike et al. 2024). For soil fungi, Basidiomycota and Ascomycota were the dominant phyla, with strong environmental adaptability due to their role as saprophytic fungi with excellent ability to decompose complex plant materials (Bastida et al. 2016). Nitrogen addition significantly increased fungal Simpson and Shannon indices. In contrast, phosphorus addition reduced the soil fungal Simpson index. This is likely because fungi, typically considered k-strategists, exhibit greater tolerance to acidified conditions, thereby the nitrogen input created an opportunity for fungal expansion (Yang et al.

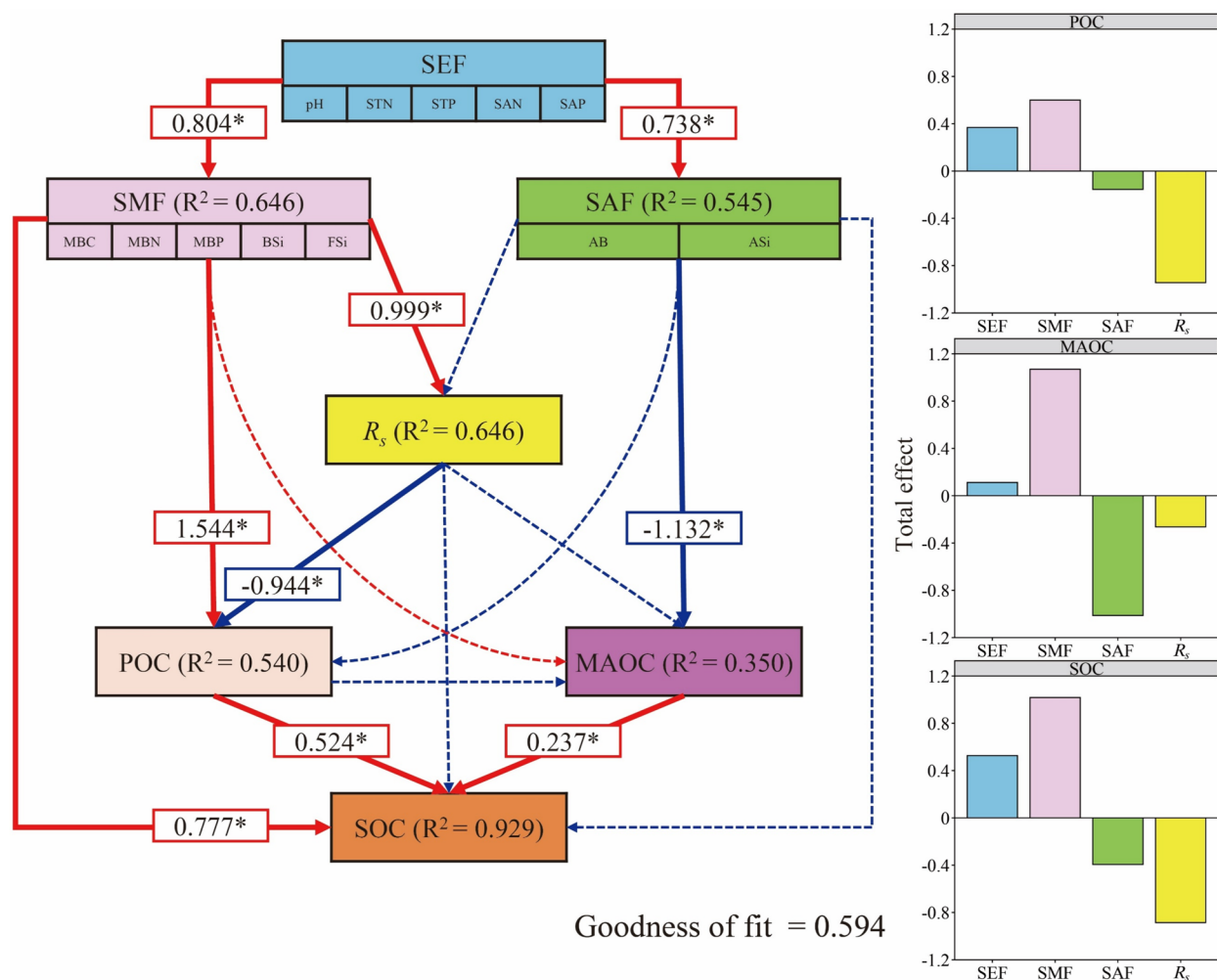


Fig. 5 Driving mechanisms of soil organic carbon accumulation. The partial least squares path modeling (PLS-PM) clarifies the accumulation mechanisms of soil organic carbon components with different stability under nitrogen and phosphorus treatments. SEF represents soil environmental factors, include pH, soil total nitrogen (STN), soil total phosphorus (STP), soil available nitrogen (SAN) and soil available phosphorus (SAP). SMF represents soil microbial factors, including soil microbial biomass carbon (MBC), soil microbial biomass nitrogen (MBN), soil microbial biomass phosphorus (MBP), bacterial Simpson index (BSi) and fungal Simpson index. SAF represents soil arthropod factors, include arthropod biomass (AB) and arthropod Simpson index (ASi). R_h represents soil heterotrophic respiration, POC represents soil particulate organic carbon, MAOC represents soil mineral-associated organic carbon, SOC represents soil organic carbon. Solid line represents $P < 0.05$, dashed line represents $P \geq 0.05$. The numbers on the lines indicate the standardized path coefficient. The bar chart represents the total effects of different driving factors on soil organic carbon components

2024; Lian et al. 2025). However, phosphorus input weakened the dominance of some fungal species. This is likely because phosphorus input alleviated soil acidification, thereby enhancing the competitiveness of r-strategist competitors (e.g., bacteria), which in turn curtailed fungal expansion (Liu et al. 2023; Pantigoso et al. 2018).

In soil arthropod results, significant differences in Beta diversity were observed among treatments, reflecting the adaptability of arthropods to environmental changes (Zhu and Zhu 2015). Dominant families included Parasitidae, Entomobryidae, Formicidae, and

Paradoxosomatidae. Entomobryidae and Formicidae, key species in organic matter decomposition and soil structure improvement, exhibited strong adaptability to environmental changes (Gaigher et al. 2025). Parasitidae, being parasitic, is influenced by its host species Formicidae (Alice 2021). Paradoxosomatidae, recognized as dominant species in subtropical forests due to their strong decomposition abilities and relatively large size, were also predominant in this study (Sierwald and Bond 2007). Low nitrogen input significantly increased the Shannon and Simpson indices for arthropods, and

phosphorus input further enhanced this effect. This may be because nitrogen input increased nutrient supply, and phosphorus balanced the nutrients, reducing resource competition (Li et al. 2024). High nitrogen input significantly decreased the Simpson and Shannon index for arthropods, but phosphorus input mitigated this effect. Such results are similar to the response of soil microorganisms, where high nitrogen usually leads to soil acidification, and disrupts the soil microenvironment, suppressing soil organism activity, but phosphorus addition alleviates the negative effects of soil acidification and weakens toxicity (Hu et al. 2024; Zhang et al. 2024c; Liu et al. 2018a). Additionally, food shortages caused by high nitrogen likely intensified competition among arthropods (Wang et al. 2022). This resulted in increased arthropod biomass and species richness, primarily driven by phosphorus input, although high nitrogen conditions offset the positive effects of phosphorus, highlighting the negative impact of nutrient accumulation (Tian and Niu 2015). These findings confirm our hypotheses (1) and (2).

Mechanisms driving organic carbon accumulation under phosphorus and nitrogen addition

The stability of soil organic carbon in plantation forests determines whether they function as “carbon sinks” or “carbon sources” in the future (Wu et al. 2014). For the accumulation of organic carbon, our results show that soil organic carbon accumulation initially increased with nitrogen input but then decreased, with phosphorus addition protecting the carbon accumulation. This may be due to nitrogen input enriches soil nutrients, excessive nitrogen causes toxicity and reduces soil organic carbon content, while phosphorus input can mitigate this negative impact by increasing plant carbon input (Rezapour et al. 2023; Jiang et al. 2019). For the consumption of organic carbon, soil respiration, a major pathway for organic carbon release, is highly sensitive to changes in external nutrient inputs. In our study, soil respiration decreased significantly with increased nitrogen input, and additional phosphorus input did not alleviate this effect. External nutrient input typically exerts a negative impact on soil respiration, as it may induce soil acidification and nutrient limitation, which lowers soil microbial activity and inhibits respiration (Mann et al. 2024; Knorr et al. 2024).

In this study, we classified active and stable carbon components for further analysis. For active carbon components (POC), low nitrogen input generally increased their content. This may be because POC itself is highly sensitive to soil management, and nitrogen input stimulated the nutrient input from litter, thereby promoting POC accumulation (Jiang et al. 2021). Phosphorus addition amplified this effect. This is because phosphorus

addition enhances the effect of low nitrogen input by altering enzyme activity and soil microbial community structure (Ling et al. 2025; Zhang et al. 2024d). However, excessive nitrogen may exacerbate soil acidification, inhibits enzyme activity, delays plant-derived carbon input, and suppresses soil microbial activity, which in turn reduces litter decomposition, leading to no significant change in POC (Zhang et al. 2022; Lu et al. 2021b). Fortunately, the addition of phosphorus alleviated this negative impact. For stable carbon components, no significant responses of MAOC to different treatments were observed, likely due to their inherent stability (e.g., stable chemical bonds formed with minerals to ensure retention). However, the content of MAOC showed a declining trend under high nitrogen treatments. Previous studies have confirmed that excessive nitrogen-induced soil acidification may promote the release of Ca^{2+} , disrupting C-mineral cation bridges and causing a reduction in stable carbon components (Liu et al. 2025c; Ye et al. 2018). This also raises concerns about the potential decline in stable soil organic carbon components under future scenarios of excessive nitrogen input.

Furthermore, using a Partial Least Squares Path Modeling (PLS-PM), we explored the possible driving mechanisms of organic carbon accumulation in soils with different stabilities. Our model indicates that for active soil organic carbon (POC), under nitrogen and phosphorus addition, soil environmental factors significantly influenced microbial variables, which directly contributed to the accumulation of POC, but also enhanced soil respiration, thereby accelerating the consumption of POC. Previous evidence has shown that active carbon turnover typically occurs through the microbial-respiration pathway (Wu et al. 2024b, c; De Jesús et al. 2021). Microbial communities are highly responsive to changes in soil pH and nutrient environments, and their structure and biological activity quickly adjust to soil environmental changes (Liu et al. 2018b). These changes lead to fluctuations in soil respiration (especially heterotrophic respiration), which affects the consumption of active organic carbon (Tian et al. 2019). For stable organic carbon (MAOC), soil environmental factors primarily influence the activity of soil arthropods, which in turn regulate the accumulation of stable organic carbon. Existing studies have demonstrated that soil arthropod communities and biological activity show significant responses to external nutrient inputs, which can influence the accumulation of stable organic carbon components (Wang et al. 2022; Kaspari et al. 2017). Our results indicate that the abundance of soil arthropods has a significant negative effect on the accumulation of MAOC, which may be attributed to arthropod activity partially disrupting the physical structure of aggregate-mineral

associated organic carbon, thereby reducing its accumulation rate (Bai et al. 2025). On the other hand, soil arthropods may regulate the microbial community through food web predation, consequently decreasing microbial contributions to carbon cycling (Neher and Barbercheck 2019; Wang et al. 2005). Ultimately, the content of various organic carbon fractions determines the overall SOC content and stability (Angst et al. 2021). These findings confirm our hypothesis (3). This study addresses the common limitations of previous research, which often focused on single-nutrient additions or individual biological groups. It systematically reveals a novel mechanism through which different soil functional groups, under the interactive effects of nitrogen and phosphorus, drive the accumulation and stabilization of distinct soil organic carbon fractions via multiple pathways. However, this study is limited by its 2-year temporal scale. Longer-term monitoring is crucial to validate the persistence of the observed mechanisms and to assess the long-term trajectory of the MAOC pool under continuous nutrient addition. Future studies should also incorporate isotopic tracing techniques to directly quantify the flow of carbon through the microbial and faunal pathways identified here.

Conclusion

Our study demonstrates that nitrogen and phosphorus inputs affect soil organic carbon (SOC) accumulation through distinct pathways governed by different soil biota. The active carbon fraction (POC) was primarily regulated by a soil environment-microorganism-respiration pathway, while the stable carbon fraction (MAOC) was mainly controlled by a soil environment-arthropod pathway. Excessive nitrogen input poses a threat to SOC accumulation, particularly by destabilizing the MAOC pool, raising concerns about a potential carbon “sink-to-source” transition. In this context, appropriate phosphorus supplementation emerges as a key management strategy to mitigate nitrogen-induced negative effects and enhance carbon sequestration in subtropical plantation forests.

Abbreviations

SOC	Soil organic carbon
POC	Particulate organic carbon
MAOC	Mineral-associated organic carbon
pc	Path coefficient
GOF	Goodness-of-fit
CO ₂	Carbon dioxide
STN	Total nitrogen
SAN	Available nitrogen
STP	Total phosphorus
SAP	Available phosphorus
MBC	Soil microbial biomass carbon
MBN	Soil microbial biomass nitrogen
MBP	Soil microbial biomass phosphorus
CK	Control

P	Phosphorus
LN	Low nitrogen
LN + P	Low nitrogen with phosphorus
HN	High nitrogen
HN + P	High nitrogen with phosphorus
R_s	Total soil respiration rate
R_h	Soil heterotrophic respiration rate
R_a	Soil autotrophic respiration rate
PCoA	Principal coordinate analysis
PLS-PM	Partial least squares path modeling
SEF	Soil environmental factors
SMF	Soil microbial factors
SAF	Soil arthropod factors
BSi	Bacterial Simpson index
FSi	Fungal Simpson index
AB	Arthropod biomass
ASi	Arthropod Simpson index
PLFAs	Total phospholipid fatty acids

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Author contributions

Writing—original draft preparation: [J.L.], Resources: [R.C.], Formal analysis and investigation: [Y.Z.], Methodology: [Y.S.], Funding acquisition: [L.Z.], Writing—review and editing: [W.X.].

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Availability of data and materials

The datasets used in the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors have read and agreed to the published version of the manuscript.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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