

Close-to-nature transformation: enhancing the chemical stability of organic carbon in *Pinus massoniana* and *Cunninghamia lanceolata* forests

Weiwei Shu

Experimental Center of Tropical Forestry, Chinese Academy of Forestry

Angang Ming

mingangang0111@163.com

Experimental Center of Tropical Forestry, Chinese Academy of Forestry

Kun Yang

Experimental Center of Tropical Forestry, Chinese Academy of Forestry

Hua Li

Experimental Center of Tropical Forestry, Chinese Academy of Forestry

Huilin Min

Experimental Center of Tropical Forestry, Chinese Academy of Forestry

Yi Tao

Experimental Center of Tropical Forestry, Chinese Academy of Forestry

Ji Zeng

Experimental Center of Tropical Forestry, Chinese Academy of Forestry

Zhongguo Li

Experimental Center of Tropical Forestry, Chinese Academy of Forestry

Juling Wei

Experimental Center of Tropical Forestry, Chinese Academy of Forestry

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Abstract

The close-to-nature forest management affects the soil carbon cycle process by changing the vegetation composition of the stand. However, the effects of close-to-nature forest management on the chemical stability of soil organic carbon (SOC) have been poorly understood. We analyzed chemical composition and stability of organic carbon taken from three group (soil, litter and fine roots) of *Pinus massoniana* and *Cunninghamia lanceolata* close-to-nature forests (PCN and CCN) and their control pure forests (PCK and CCK). Compared with the control pure forests, the proportion of soil alkyl C, the alkyl C/O-alkyl C (A/O-A) ratio, and the evenness of distribution of the four main SOC chemical compositions were all higher in the close-to-nature transformation forests, especially in the PCN. The microbial biomass carbon (MBC) was significantly higher than that of control forests (CCK, PCK). However, the close-to-nature transformation did not significantly affect the soil bacterial richness and diversity. The Alkyl C and O-alkyl C in litter and fine roots were significantly correlated with the SOC chemical components, and there was also a positive correlation between the soil A/O-A ratio and the litter A/O-A ratio as well as the fine root A/O-A ratio. The RDA analysis showed that fine root alkyl C and fine root O-alkyl C were the two most critical factors affecting the chemical composition of SOC. Our findings highlight that the close-to-nature transformation exhibits a relatively high resistance to decomposition. Converting the pine and fir plantations into uneven-aged mixed forests through close-to-nature transformation is capable of enhancing the chemical stability of organic carbon in coniferous plantations.

1. Introduction

Forests constitute the main part of the terrestrial ecosystem, possessing a carbon storage of 861 Pg (calculated in terms of C) ($1 \text{ Pg} = 10^{15} \text{ g} = 1 \text{ billion t}$), which accounts for 40% of the carbon pool in the terrestrial ecosystem and represents the largest carbon reservoir on land apart from permafrost (Dixon et al. 1994). The augmentation of carbon storage mainly hinges on the biomass of planted forests and the storage of soil organic carbon (SOC). The capacity of planted forests to alleviate the pressure of logging secondary forests for timber has been gradually acknowledged, while simultaneously promoting CO_2 fixation and the restoration of degraded soil (Kelty 2006). However, the *P. massoniana* and *Cunninghamia lanceolata* coniferous forests, which are most extensively distributed in the subtropical region of China, are confronting issues like soil fertility degradation, productivity decline, and the frequent occurrence of pests and diseases resulting from continuous clear-cutting and rotation (Liu et al. 2014). To heighten the stability of forests and the resilience to climate change, it is essential to implement strategies that combine forestry mitigation and adaptation. (Li et al. 1996). Optimizing the management of planted forests and implementing multi-objective adaptive management of planted forests, such as afforestation with high-carbon-sequestration tree species, tree species mixing, the introduction of nitrogen-fixing tree species, and the adjustment of forest stand structure, can not only maintain high soil fertility, long-term productivity, and enhance the resilience to climate change, but also contribute to forest carbon sequestration and sink enhancement, especially increasing the storage and stability of forest soil organic carbon (Liu et al. 2024).

Soil organic carbon is a compound composed of a variety of complex organic carbon functional groups (Margenot et al. 2015). These organic molecules have different degrees of chemical resistance and anti-aging (Lorenz and Lal 2007) in the ecosystem, and therefore contribute differently to the long-term stable retention ability of SOC. Alkyl C mainly derived from cutin, suberin and wax can be preferentially and stably retained in the soil for a long time and is the main contributor to soil organic carbon (Chang et al. 2018). Alkyl C/O-alkyl (A/O-A) values are commonly used as indicators of SOC quality to characterize SOC as a metabolic substrate for microorganisms and the extent to which it can be decomposed (Baldock et al. 1992). Aromatic C, which represents substances with benzene rings such as lignin, softwood, polypeptides or black carbon. Traditional views maintain that aromatic C is a relatively stable carbon component in soil. However, research in recent years have proposed that aromatic C is easily lost and cannot be stably preserved in the soil for a long time (Zhu et al. 2019). Therefore, an increase in the proportion of alkyl carbon in soil helps stabilize the soil organic carbon pool and enhances the sustainable carbon sequestration capacity of forests. With the progress of research methods and the rapid development of analytical techniques, it is not only the early "soil continuum theory" (Lehmann and Kleber 2015), the step-by-step decomposition theory of plant residues, but also the "ecosystem property model" proposed recently (Schmidt et al. 2011). It is widely believed that any kind of organic carbon component, despite its natural resistance, will eventually decompose when the right environmental and biological conditions arise. Therefore, Wang et al. (2019) proposed a new view on the diversity of SOC components and took the uniformity of organic carbon component distribution as a predictor of SOC relative stability. That is, the more uniform and diverse the SOC chemical composition, the lower the risk of C being broken down by environmental changes.

As an important indicator of potential changes in C release, SOC stability is closely related to tree species diversity (Luan et al. 2018). Higher tree species diversity can significantly improve soil carbon sequestration by increasing biomass and adjusting forest composition (Augusto and Boča 2022). In addition, increased tree diversity can provide a greater variety of organic compounds, favoring a more diverse community of decomposers and more diverse extracellular enzymes (Ei Moujahid et al. 2017), resulting in a more uniform and stable organic carbon component, which in turn decreases the sensitivity of unstable carbon components to environmental changes and minimizes the risk of SOC decomposition (Wang et al. 2019). Compared with *Pinus massoniana* plantations, the soil soluble carbon content of the three broad-leaved forests was higher, with lower alkyl C, higher O-alkyl C and lower A/O-A (Wang et al. 2010). The introduction of nitrogen-fixing species *Acacia mangium* plantation in *Eucalyptus* plantations for close-to-nature forest management is conducive to the accumulation of relatively stubborn soil carbon components (alkyl C) and contributes to the improvement of SOC stability in subtropical plantations (Huang et al. 2016). However, it is not solely tree species diversity that determines the functions of forest ecosystems (including the stability of the soil carbon pool), but rather the functional traits and composition of tree species (Liu et al. 2024). It also implies that there is uncertainty about whether the potential stability of the SOC pool can be maintained only by increasing tree species richness (Wang et al. 2023). Although increasing tree species richness through close-to-nature transformation is increasingly recognized as a key driver of C dynamics, whether there is higher

stability of SOC in the process of converting coniferous plantation pure forests to mixed forests through close-to-nature management in tropical regions remains to be further investigation.

To address these questions, SOC chemical composition, microbial diversity were examined in close-to-nature transformation of *P. massoniana* and *C. lanceolata* artificial forests (PCN, CCN) and their control forests (PCK, CCK) in the southern subtropical region. With reference to the community species diversity index, Pielou's evenness index was used to estimate the evenness of organic carbon composition distribution in soil, litter and fine root total organic carbon. The objective of this study was to investigate the impact close-to-nature transformation of *P. massoniana* and *C. lanceolata* will change the chemical composition of soil organic carbon and its controlling factors. Specifically, we hypothesize that: a) Based on previous case studies in the same region (Huang et al. 2016; Wang et al. 2019; Wang et al. 2022), after implementing near-natural transformation, the proportion of stable organic carbon components will increase, and the homogeneity of the chemical composition of soil organic carbon will also be higher. And b: The changes in the chemical composition of soil organic carbon and the homogeneity of its distribution may be related to the characteristics of the carbon chemical components derived from the inputs of litter and fine roots.

2 Methods

2.1 Site description

The study area is located at the Guangxi Youyiguan Forest Ecosystem Research Station of the Experimental Center of the Tropical Forestry Chinese Academy of Forestry (22°10'N, 106°50'E). The region belongs to the south subtropical monsoon climate zone with a humid to semi-humid climate. The area has sufficient sunlight and abundant precipitation, with an average annual precipitation of 1200 ~ 1500 mm mainly occurring from April to September, and an annual evaporation of 1200 ~ 1400 mm, with a relative humidity of 80%~84%. Mean annual temperature ranges from 20.5 °C to 21.7 °C. The main landform type is low mountains and hills, and the main soil types are lateritic red soil and red soil, with a soil layer thickness of over 80cm. The natural vegetation in this region is the south subtropical evergreen broad-leaved forest.

2.2 Experimental design

The experimental forest was established in 1993 on a *C. lanceolata* felling site with a pure forest of *P. massoniana* and *C. lanceolata*, with an initial planting density of 2500 trees /ha. In 2004, the first growth harvesting was carried out with a thinning intensity of around 30%. In 2007, after completing the baseline investigation of vegetation and soil, close-to-nature transformation operations were carried out mainly by thinning the *P. massoniana* and *C. lanceolata* pure forest (retaining a density of 600 trees / ha) while protecting natural regeneration, in combination with the second growth harvesting. In early 2008, indigenous species such as *Castanopsis fissa* and *Erythrophleum fordii* were planted under the thinned *P. massoniana* and *Cunninghamia lanceolata* forest, with a planting density of 600 trees /ha (300 trees /ha for both *E. fordii* and *C. fissa*), forming a mixed forest of needle-leaf and broad-leaved trees of

different ages, with a total density of 1200 trees /ha. Meanwhile, the *P. massoniana* and *C. lanceolata* pure forest that had not undergone close-to-nature transformation with the same total density (1200 trees/ ha) were retained as controls. Four replicates were set up for each of the four forests. The tree species layout of the unmodified pure pine forest and the close-to-nature transformation forest is shown in Fig. S1.

This experiment was a two-factor experiment, one was the species factor, two typical conifer species of South tropical *P. massoniana* and *C. lanceolata* were selected, the other was the treatment factor, and two treatments were set for modification and non-modification. There were 4 block groups with 4 stand types in each block group, close-to-nature transformation forest of *P. massoniana* (PCN), close-to-nature transformation forest of *C. lanceolata* (CCN), *P. massoniana* pure forest (PCK) without transformation, and *C. lanceolata* pure forest (CCK) without transformation, respectively. There were 16 experimental plots in 4 stand types and 4 replicates, each with an area of 1hm².

2.3 Soil, litter and fine root samplings

In July 2022, soil samples were collected to determine the carbon chemical components, physical and chemical properties of soil. Five 20 m×20 m plots were randomly set in each experimental plot (i.e. each stand). Three random soil collection points were selected at the top, middle, and bottom of each stand. Soil surface coverings were removed, and soil from 0-10cm was collected using an auger with an inside diameter of 5 cm. The soil samples from 9 collection points were mixed evenly, passed through a 2 mm sieve, and sorted to remove stones, roots, and other animal remains. The soil samples were air-dried and kept for later use.

Three small sample plots (1m×1m) were randomly selected from each sample plot, and fresh litter of different tree species was collected for chemical analysis. The litter samples of three plots in pure forests of *P. massoniana* and *C. lanceolata* were mixed separately. The litter samples of different tree species were separated in modified forests of *P. massoniana* and *C. lanceolata*, and mixed three litter samples in each plot according to tree species.

Three plants of *P. massoniana* and *C. lanceolata* were randomly selected in each control plot, and three plants of *P. massoniana*, *C. lanceolata*, *C. fissa* and *E. fordii* were randomly selected in each modified plot, and collecting their fine roots (diameter ≤ 2 mm) for chemical analysis. Fine roots of the same tree species in the same plot were mixed together.

2.4 Soil, litter and fine root chemical properties

SOC and total nitrogen (TN) content were determined by dichromate oxidation method and Kjeldahl method, respectively. Total phosphorus (P) content was determined by alkaline fusion molybdenum antimony colorimetric method, while available phosphorus (AP) content was determined by spectrophotometric method following extraction with hydrochloric acid and sulfuric acid solution. The content of NH₄⁺ was determined by indophenol blue colorimetric method after extraction using 2 mol L⁻¹

¹ KCl, and the content of NO₃⁻ was determined by ultraviolet spectrophotometric method. The soil pH value was determined using a FE20 pH meter, with a soil to water ratio of 1:2.5.

The solid-state ¹³C CPMAS NMR spectroscopy (Germany Bruker AVANCE 400) was used to analyze the different chemical shift ranges of ¹³C atoms, the corresponding functional group composition, spectral information, etc., in litter, roots, and soil solid samples to determine their organic carbon chemical structures. Before analyzing soil samples by the ¹³C nuclear magnetic resonance spectroscopy, they were pre-treated with a 10% (v/v) hydrofluoric acid solution (Schmidt et al. 2010). The ¹³C nuclear magnetic resonance spectrum was divided into the following common chemical shift regions: alkyl C (0–45 ppm), O-alkyl C (45–110 ppm), aromatic C (110–165 ppm), carbonyl C (165–210 ppm), and the relative intensity of each region was determined by integration (Solomon et al. 2007).

Species evenness refers to the degree of similarity in the number of individuals of each species in given environment. The evenness of a community can be represented by the Pielou's index (J'): $J' = H'/H_{max}$ (Cph, et al.), where H' is the Shannon's diversity index, H_{max} is the maximum value of H' , and $H_{max} = \ln S$, where S is the total number of species. The Shannon's diversity index (H') = $-\sum p_i \log_2 p_i$, where $p_i = n_i/N$, n_i is the abundance of the first species, and N is the total abundance (Shannon and Weaver 1963). In this study, the Pielou's evenness index was used to estimate the evenness of the proportion of various carbon chemical compositions in the total organic carbon in soil, litter and fine roots. If the distribution of SOC chemical components is uneven or the proportion of a specific carbon component is too high, the risk of carbon emission posed by any one SOM constituent having a huge impact due to climate change and other disturbances could rise. Therefore, a better understanding of the distribution uniformity of various organic carbon components in forest soil is crucial for improving the predictive ability of ecosystems to resist environmental changes (Wang et al. 2019). In this formula, S equals to 4 (four types of organic carbon chemistry compositions, namely Alkyl C, O-alkyl C, Aromatic C, and Carbonyl C); n_i is the proportion of the i_{th} organic C chemical composition in total organic C calculated from the relative intensity in each carbon component region of ¹³C NMR spectra, and N is the total proportion of various organic carbon chemical compositions (Wang et al. 2019).

2.5 Soil microbial community composition

DNA was extracted from soil samples using MO BIO Power Soil DNA Extraction kit (MO BIO Laboratories, Carlsbad, USA), and quality was detected using 1% agarose gel electrophoresis. DNA concentration and purity were determined using NanoDrop2000. The V3-V4 variable region of bacterial 16S rRNA gene was amplified by PCR using 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). After that, the samples were fully mixed with equal mass according to the concentration of PCR products, and then purified by agarose Gel electrophoresis (concentration 2%) and AxyPrep DNA Gel extraction kit (Axygen Biosciences, Union City, CA, USA). The NEXTFLEX® Rapid DNA-Seq Kit was used for library construction, and Illumina's Miseq PE300/NovaSeq PE250 platform was used for sequencing (Shanghai Majorbio Bio-pharm Technology Co., Ltd.).

2.6 Statistical analysis

Adopting one-way ANOVA, the least significant difference (LSD) method, and multiple comparisons, the study analyzed soil properties, the distribution of carbon chemical compositions of soil, litter, and fine roots, MBC, and soil bacteria richness and diversity index in different treated artificial forests. Pearson correlation analysis was used to examine the relationship of A/O-A, Pielou's evenness index, and MBC among different components (soil, litter fine roots). The Canoco 5 software (from micro Power, Inc., Ithaca, NY, USA) was used to conduct redundancy analysis (RDA). The changes in SOC chemical composition (alkyl C, O-alkyl C, aromatic C, and carbonyl C) were divided into parts explained by plant, soil, and microbial community parameters. The explanatory variables considered included litter and fine root carbon chemical composition, SOC, TN, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, TP, AP, MBC, MBN, soil bacterial richness and diversity. Random forest analysis was carried out by using the "rfPermute" package to identify the soil characteristics that have a significant impact on the chemical components of organic carbon. Subsequently, regression analysis was conducted using the lm function to examine the relationship between the chemical composition of soil organic carbon and the chemical compositions of litter carbon and fine root carbon. All variables follow a normal distribution and meet the homogeneity of variance test. Data analysis and plotting were completed using R 4.1.3.

3 Results

3.1 Soil physicochemical properties

Compared with the control forest, the SOC, AN, TP and AP of the CCN increased by 9.14%, 30.42%, 8.70% and 4.50%, respectively, while the $\text{NH}_4^+\text{-N}$ and MBC increased significantly by 103.46% and 33.32% ($P < 0.05$). The SOC, TN, AN and TP of the PCN slightly increased compared with the control forest, but none of them reached a significant level of difference, while the AP content was significantly lower than that of the PCK ($P < 0.05$), and the $\text{NO}_3^-\text{-N}$, $\text{NH}_4^+\text{-N}$ MBC and MBN significantly increased by 121.54%, 118.91%, 28.90% and 36.42% respectively ($P < 0.05$) (Table 1).

Table 1

Effects of close-to-nature transformation on soil physical and chemical properties of *pinus-Cunninghamia* forests. Different small letters meant significant difference among different same stands at 0.05 level. Mean $\pm$ se

Soil physical and chemical properties	CCK	CCN	PCK	PCN
Organic carbon (g/kg)	21.10 $\pm$ 4.08a	23.03 $\pm$ 0.42a	30.35 $\pm$ 2.84a	30.38 $\pm$ 2.82a
Total nitrogen (g/kg)	1.50 $\pm$ 0.25a	1.37 $\pm$ 0.03a	1.82 $\pm$ 0.01a	1.93 $\pm$ 0.04a
Total phosphorus (g/kg)	0.23 $\pm$ 0.01b	0.25 $\pm$ 0.02ab	0.28 $\pm$ 0.01ab	0.29 $\pm$ 0.01a
Available phosphorus (mg/kg)	1.11 $\pm$ 0.03b	1.16 $\pm$ 0.03b	1.55 $\pm$ 0.02a	1.28 $\pm$ 0.12b
NO ₃ ⁻ N(mg/kg)	5.38 $\pm$ 0.88b	4.37 $\pm$ 1.24b	5.71 $\pm$ 0.24b	12.65 $\pm$ 1.56a
NH ₄ ⁺ -N(mg/kg)	7.81 $\pm$ 0.10b	15.89 $\pm$ 4.38a	4.23 $\pm$ 1.31c	9.26 $\pm$ 0.23b
Microbial biomass carbon (mg/kg)	234.44 $\pm$ 14.75c	312.50 $\pm$ 16.26b	301.12 $\pm$ 12.27b	388.13 $\pm$ 5.88a
Microbial biomass nitrogen (mg/kg)	36.41 $\pm$ 3.22b	46.51 $\pm$ 2.11ab	39.07 $\pm$ 3.29b	53.30 $\pm$ 4.06a
Fine root biomass (g m ⁻²)	401.57 $\pm$ 53.71a	675.26 $\pm$ 21.50a	536.92 $\pm$ 65.16a	862.86 $\pm$ 202.43a

3.2 Organic carbon chemical composition of fine roots, litter and soil

The fine root was composed of 14.72–19.01% alkyl C, 58.53–60.00% O-alkyl C, 14.81–17.55% aromatic C, and 7.23–7.67% carbonyl C in the total C across the PCK, PCN CCK and CCN forests (Fig. 1a). The litter revealed 23.82–28.78% alkyl C, 51.46–55.39% O-alkyl C, 11.75–14.46% aromatic C, and 7.14–8.34% carbonyl C in the total C across the four forests (Fig. 1b). Among them, the close-to-nature transformation of *P. massoniana* significantly increased the proportion of alkyl C in litter, fine roots and soil and the proportion of carbonyl C in litter. In addition, the close-to-nature transformation also significantly decreased the proportion of O-alkyl C in litter and soil and the proportion of aromatic C in fine roots and litter. However, the close-to-nature transformation has no significant impact on each organic carbon chemical component in the litter, fine roots and soil of *C. lanceolata*.

The soil, litter and fine roots A/O-A ratio was lower in the PCK forest than in the PCN forest ($p < 0.05$) (Fig. 2a). The PCN forest had the highest evenness of distribution of the various C chemical compositions (Pielou's index) in both litter and fine roots (Fig. 2c).

3.3 Microbial diversity and factors affecting SOC chemical composition

Close-to-nature transformation significantly increased soil MBC (Table 1), but there was no significant difference in chao1 index and Shannon-Wiener index (Fig. S2).

Litter and fine root organic carbon chemical components had a significant impact on soil organic carbon chemical components (Fig. 3). Soil alkyl C was significantly positively correlated with litter alkyl C, litter A/OA, fine root alkyl C, fine root A/OA, and fine root Pielou index, and was significantly negatively correlated with litter O-alkyl C and fine root O-alkyl C. Soil O-alkyl C was significantly positively correlated with litter O-alkyl C, fine root aromatic C, and fine root Arom/OA, and was significantly negatively correlated with litter carbonyl C, litter Pielou, fine root alkyl C, and fine root A/OA. Soil carbonyl C was significantly negatively correlated with fine root aromatic C and fine root Arom/OA (Fig. 3). Linear regression results revealed that the A/O-A ratio of litter and fine roots in the four forests were positively correlated with the soil A/O-A ratios (Fig. 4a and b).

Random forest analysis indicated that soil NO_3^- -N, AP and TN were the strongest predictors of alkyl C, while soil TP was the strongest predictor of O-alkyl C. Carbonyl C was significantly predicted by soil AP (Fig. S3).

The results showed that the first RDA axis explained 61.7% of the variation, while the second RDA axis explained 21.4% of the variation. Permutation tests showed that soil SOC composition was significantly correlated with F_alkyl C ($F = 9.2$, $P = 0.002$), and F_O-alkyl C ($F = 2.9$, $P = 0.036$). F_alkyl C was significantly positively correlated with soil carbonyl C, and F_O-alkyl C was significantly positively correlated with soil aromatic C (Fig. 5).

4. Discussion

A large amount of research has found that close-to-nature transformation through silvicultural measures such as thinning and replanting can alter stand structure, species composition and diversity, leading to rapid increases in stand productivity and carbon storage (Liu et al. 2011; Andreas et al. 2019). Forest SOC also increases with increasing tree species diversity (Liu et al. 2018). Previous studies have found that 8 years after close-to-nature transformation of artificial *P. massoniana* and *C. lanceolata* forests, SOC significantly increased in the 0-100 cm layer (Angang et al. 2019). In this study, our further demonstrated the significant changes in the chemical composition of SOC after the close-to-nature transformation of coniferous forests. The proportion of soil alkyl C was higher in the close-to-nature transformation forests (CCN, PCN), while the proportions of aromatic C and O-alkyl C were lower than those in the control pure forests (CCK, PCK, Fig. 1). These results proved that the close-to-nature transformation of coniferous forests, especially that of *P. massoniana* forests, could increase the proportion of recalcitrant alkyl C in the plantations and reduce the aromaticity of SOC. Our results are supported by previous research findings. After the mixture of *P. massoniana* and *Magnolia sumatrana*, and the combination of *Eucalyptus* and *Acacia mangium*, the proportion of soil alkyl C increased significantly (Huang et al. 2016). Moreover, through close-to-nature management, as the proportion of coniferous tree species declined, the residual quantity of lignin-rich litter reduced (Krishna and Mohan

2017), and it might also potentially accelerate the decomposition of aromatic C functional groups in conifers (Wang et al. 2018). These results also provided possible reasons for the decline of aromatic C in the soil after the close-to-nature transformation of coniferous forests.

Furthermore, the A/O-A ratios of fine roots, litter, and soil in the close-to-nature transformation forests as well as the SOC evenness (Pielou's index) were all higher than those in the control forests (Fig. 2), indicating that the distribution of potential substrates (including unstable or recalcitrant components) of the decomposition community in the transformation forests was more uniform. Recently, Lehmann et al. (2020) successively put forward the concept that the diversity and functional complexity of organic carbon components determine the decomposability of soil carbon. Wang et al. (2019) proposed that the high evenness of organic carbon functional groups can minimize the carbon decomposition risk caused by the significant impact of any SOC component during interference. This also demonstrated that the close-to-nature transformation forests (CCN and PCN) in this study had a greater potential ability to resist the decomposition of SOC.

Close-to-nature transformation significantly increased soil MBC (Table 1). Previous studies have shown that the soil MBC in broad-leaved forests or mixed forests with a higher proportion of broad-leaved tree is higher than that in coniferous forests with a higher proportion of coniferous trees (Marcin and Niklińska 2010; Wang, et al. 2016). However, our research results found that the close-to-nature transformation did not significantly affect the soil bacterial richness and diversity (Fig. S2), indicating that the composition of the plant community may have no significant relationship with the soil bacterial diversity. These findings well supported the "single hypothesis" that the composition and diversity of soil microbial communities were more dependent on the presence of a certain specific plant rather than the plant diversity itself (Johnson et al. 2010) (Porazinska et al. 2003). A single aboveground habitat might strengthened the role of deterministic processes in the construction of the microbial community and reduced biodiversity within a local scope (Chase 2010). In addition, our results also found that the changes in bacterial diversity indices had opposite trends in *P. massoniana* and *C. lanceolata* forests, with *C. lanceolata* transformation forests being less than control forests and *P. massoniana* the other way around. Research on tea plantations under long-term fertilization conditions showed that the content of $\text{NO}_3^- - \text{N}$ was a key factor affecting microbial diversity and community structure, and there was a significant positive correlation between the content of $\text{NO}_3^- - \text{N}$ and the diversity index (Gu and Research 2019). Therefore, under the background of the same soil type, climatic conditions, and forest management measures, the soil bacterial diversity index in the transformed Masson pine forest was higher than that in the control forest, probably because the soil nitrate nitrogen content in the transformed forest was significantly higher than that in its control forest, while the difference in Chinese fir forest was not significant and was lower than that in the control forest, which further became one of the important factors for the opposite results of soil bacterial diversity in the two transformed forests. In addition, $\text{NO}_3^- - \text{N}$ was also one of the soil properties that significantly affected SOC alkyl C (Fig. S3). In conclusion, the significantly increased $\text{NO}_3^- - \text{N}$ content after the close-to-nature transformation of *P.*

massoniana increased the diversity of decomposers and might accelerate the decomposition of unstable organic carbon components (O-alkyl C).

Litter contains a variety of organic compounds, such as lignin, cellulose, wax, cutin, etc. (Crow et al. 2010). The impact of vegetation on the chemical composition of SOC might have been the result of the diversity of C chemical compositions in plant residues (Hannam et al. 2004). Actually, we also found in this study that the Alkyl C and O-alkyl C in litter and fine roots were significantly correlated with the SOC chemical components (Fig. 3), there was also an interesting positive correlation between the soil A/O-A ratio and the litter A/O-A ratio as well as the fine root A/O-A ratio (Fig. 4). This indicates that after the close-to-nature transformation, the changes in the chemical compositions of various organic carbons in the plantation soil may be related to the characteristics of the carbon chemical compositions in the input of litter and fine root residues. However, our further research found that F_alkyl C and F_O-alkyl C were the two most critical factors affecting the chemical composition of SOC (Fig. 5), suggesting that the impact of fine roots on the SOC chemical components may be stronger than that of litter. Recent research on the contribution of roots to SOC found that SOC $\delta^{13}\text{C}$ was consistent with root $\delta^{13}\text{C}$, indicating that roots were the main source of SOC (Yang et al. 2023). This was mainly due to: (1) The continuous input of rhizosphere deposits into the soil during the root growth process (Henneron et al. 2019). (2) The "high-quality" C rhizosphere deposition during root development could be more effectively converted into microbial products (Bradford et al. 2013). It is worth noting that the relative contributions (or impacts) of litter and roots to soil organic carbon components were not consistent among different forest types. This difference might be the combined effect of complex interactions among microbial activities, the quality and quantity of litter and roots, and the input carbon with soil minerals and soil organisms (Sun et al. 2024). Besides, since global climate change might alter the carbon allocation in the aboveground and underground parts of plants, the relative contributions of litter and fine roots to the chemical composition of SOC might also change under different climate zones and forest types. Therefore, the impact of litter treatment combined with global change driving factors on soil organic carbon sequestration requires further research.

Moreover, the fine root alkyl C vector pointed to the main SOC chemical components of CCN and PCN, and the fine root O-alkyl C vector pointed to PCK. These results indicated that alkyl C contributed more to the formation of SOC components through the plantation of broad-leaved trees, while O-alkyl C contributed more to SOC in the soil of coniferous forests.

5. Conclusions

In this study, we found that the distribution of various SOC chemical components in the close-to-nature transformed plantation forest was more uniform than that in the untransformed forest. The increase in the proportion of stable SOC in the transformed forest and the uniformity of the distribution of SOC chemical components were likely related to the characteristics of the carbon chemical components input from litter and fine root residues. Compared with litter and microorganisms, the chemical components of fine root organic carbon were the key factors that led to the differences in the chemical

components of SOC between the transformed forest (CCN, PCN) and the untransformed forest (CCK, PCK). These results indicated that the close-to-nature transformation of the pine and fir plantation forest would increase the chemical stability of SOC and might reduce the potential risk of carbon decomposition. Therefore, in forest management practices, the impact of tree species configuration on the stable components of soil carbon should be considered.

Declarations

Competing Interests

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

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Author Contribution

Author Contributions Conceptualization and Methodology by AM and JZ, Material preparation, investigation by KY, HL, HM and ZL, data collection and analysis were performed by YT and JW. The first draft of the manuscript was written by WS, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability

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

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Figures

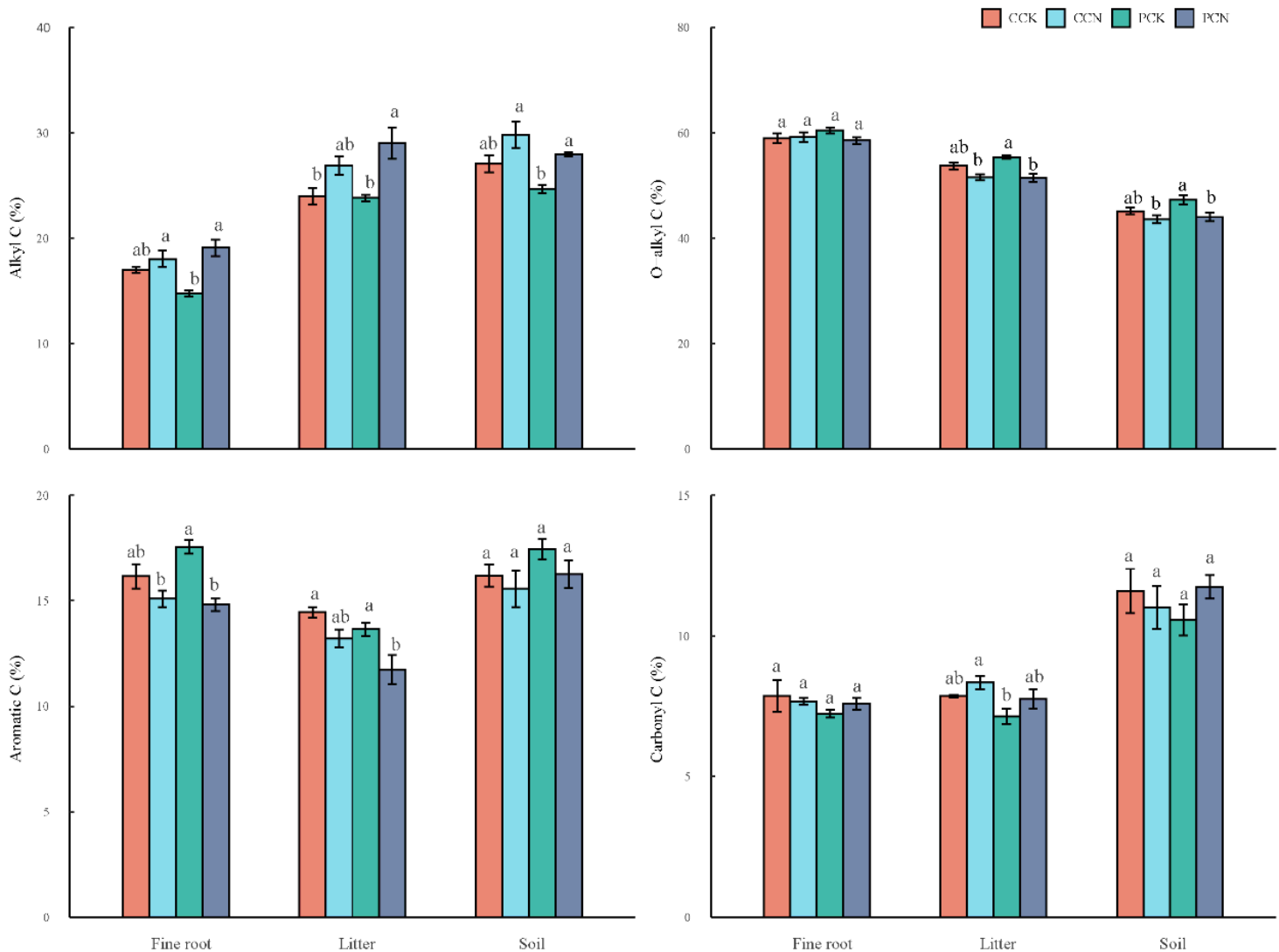


Figure 1

Differences in the mean values of the proportion of litter carbon (a), fine root carbon (b), soil organic carbon (c), and functional species (alkyl C, O-alkyl C, aromatic C, carbonyl C) in the total organic carbon across the four planted forest types (*Pinus massoniana* and *Cunninghamia lanceolata* close-to-nature forests (PCN, CCN) and their control pure forests (PCK, CCK)). Error bars indicate the standard error. Different lowercase letters of the same category represent statistically significant differences ($p < 0.05$).

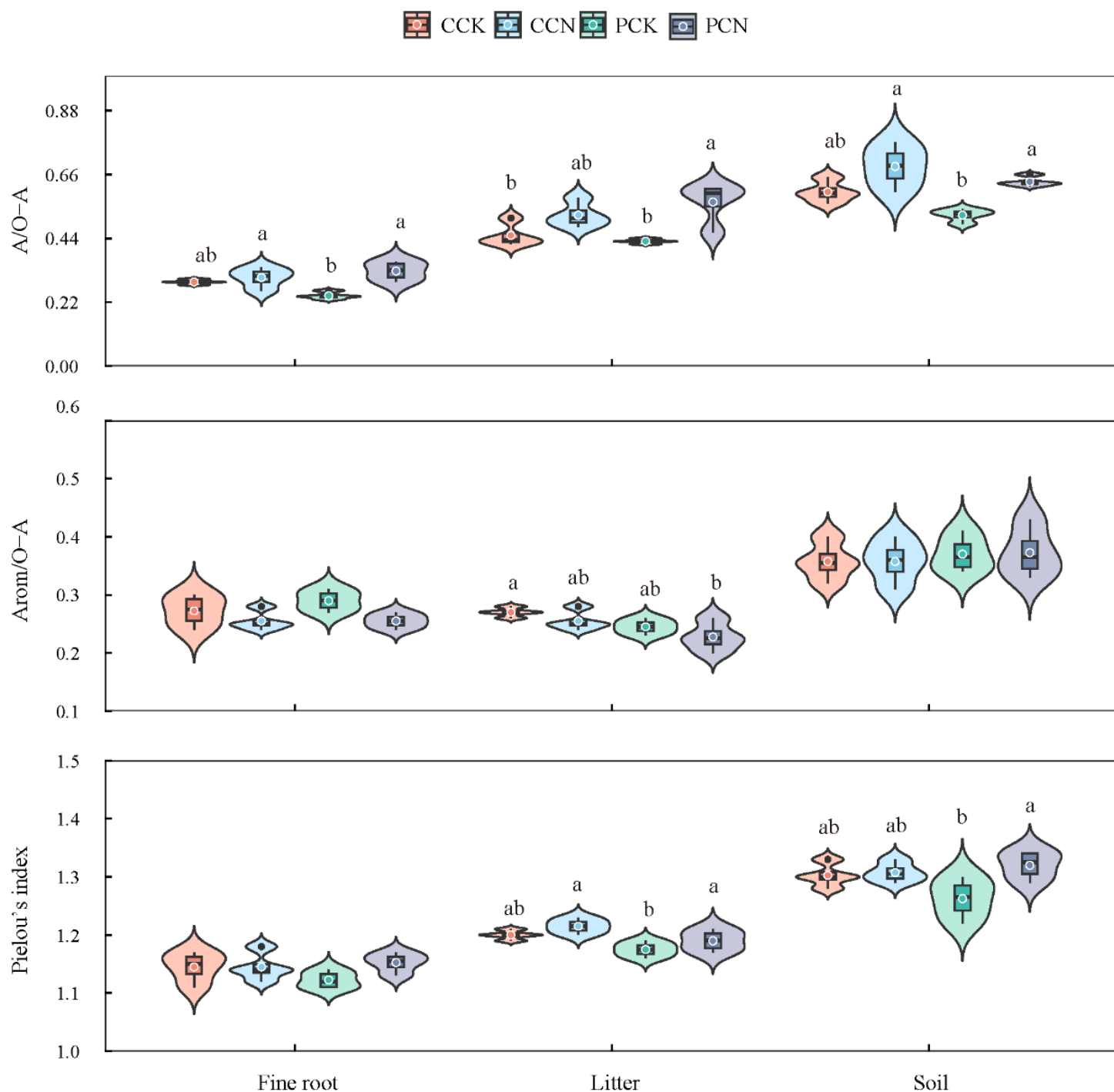


Figure 2

Differences in the mean values of alkyl C/O-alkyl C (A/O-A) ratios, Aromatic C/O-alkyl C (Arom/OA) ratios and of the evenness of distribution of various soil organic carbon functional compositions (Pielou's index) across the four planted forest types (*Pinus massoniana* and *Cunninghamia lanceolate* close-to-nature forests (PCN, CCN) and their control pure forests (PCK, CCK)). Error bars indicate standard errors. Different lowercase letters of the same category represent statistically significant differences ($p < 0.05$).

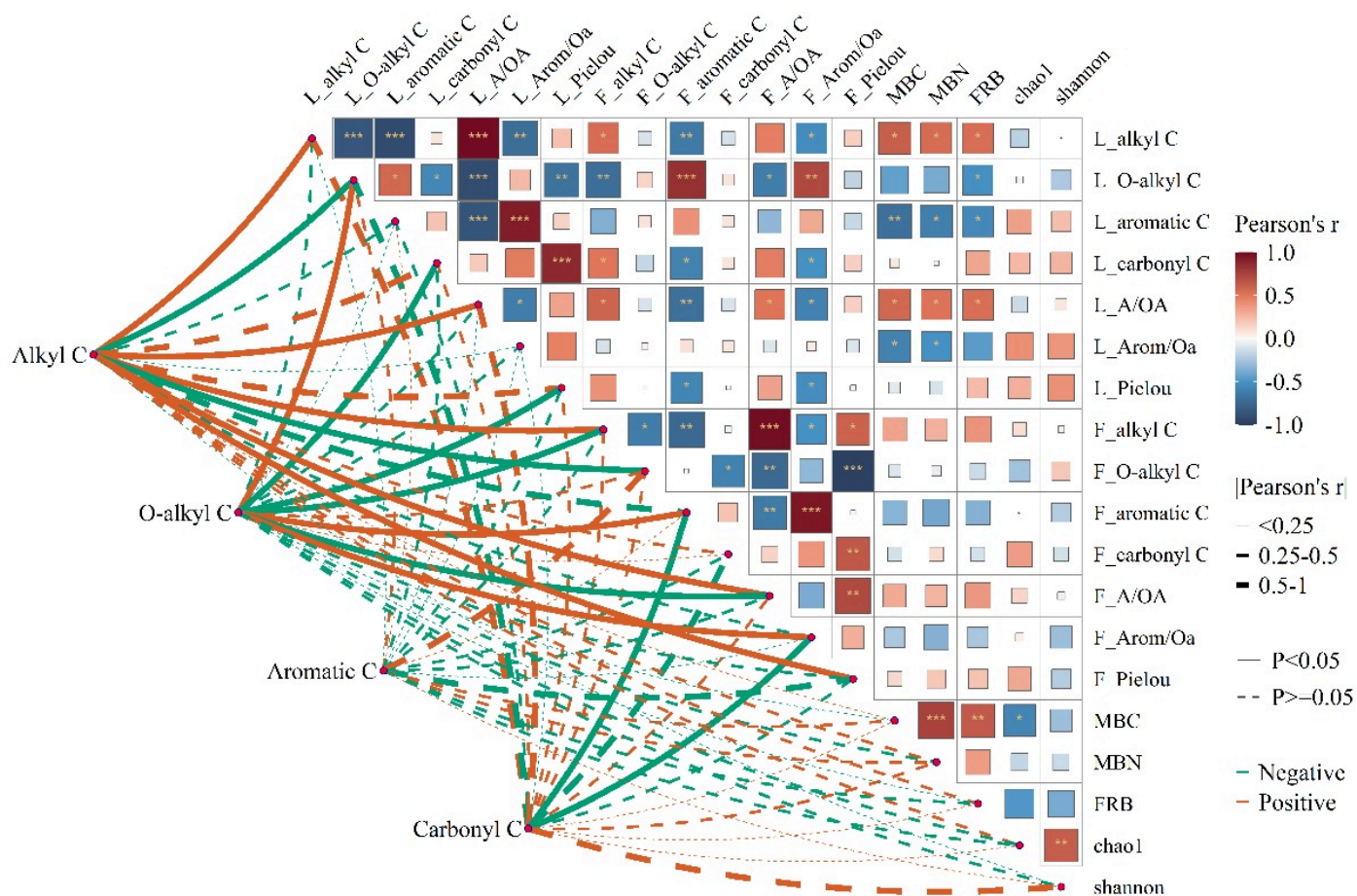


Figure 3

Correlation of chemical components of soil organic carbon with plant, soil and microbial indices by Mantel tests. Edge width corresponds to the Mantel's r statistic, and edge colour denotes the statistical significance. The size of the square indicates correlation strength, with a higher proportion representing higher correlation strength. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. L_alkyl C, Litter O-alkyl C; L_O-alkyl C, Litter O-alkyl C; L_aromatic C, Litter aromatic C; L_carbonyl C, Litter carbonyl C; F_alkyl C, Fine root O-alkyl C; F_O-alkyl C, Fine root O-alkyl C; F_aromatic C, Fine root aromatic C; F_carbonyl C, Fine root carbonyl C; L_A/OA, Litter alkyl C/O-alkyl C; F_A/OA, Fine root alkyl C/O-alkyl C; L_Arom/Oa, Litter Aromatic C/O-alkyl C; F_Arom/Oa, Fine root Aromatic C/O-alkyl C, L_Pielou, Evenness of litter C distribution (Pielou index); F_Pielou, Evenness of fine root C distribution (Pielou index); MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; chao1, soil bacterial chao1 index; Shannon, soil bacterial Shannon- wiener index.

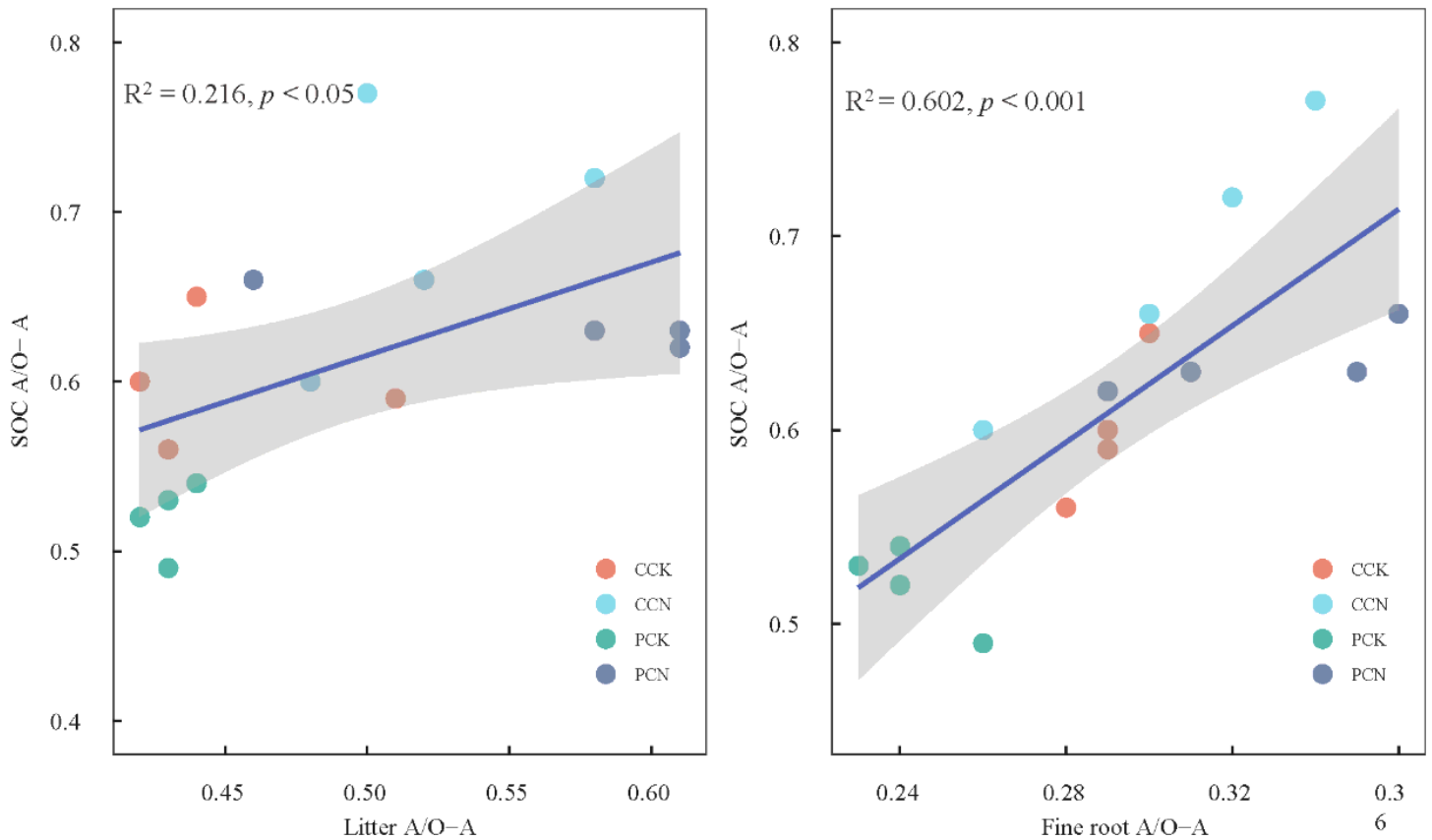


Figure 4

Relationships between soil organic carbon chemical composition and litter carbon, fine root carbon chemical composition across the four planted forest types (*Pinus massoniana* and *Cunninghamia lanceolata* close-to-nature forests (PCN, CCN) and their control pure forests (PCK, CCK)). The line and shaded area account for the fitted regression and the 95% confidence interval, respectively.

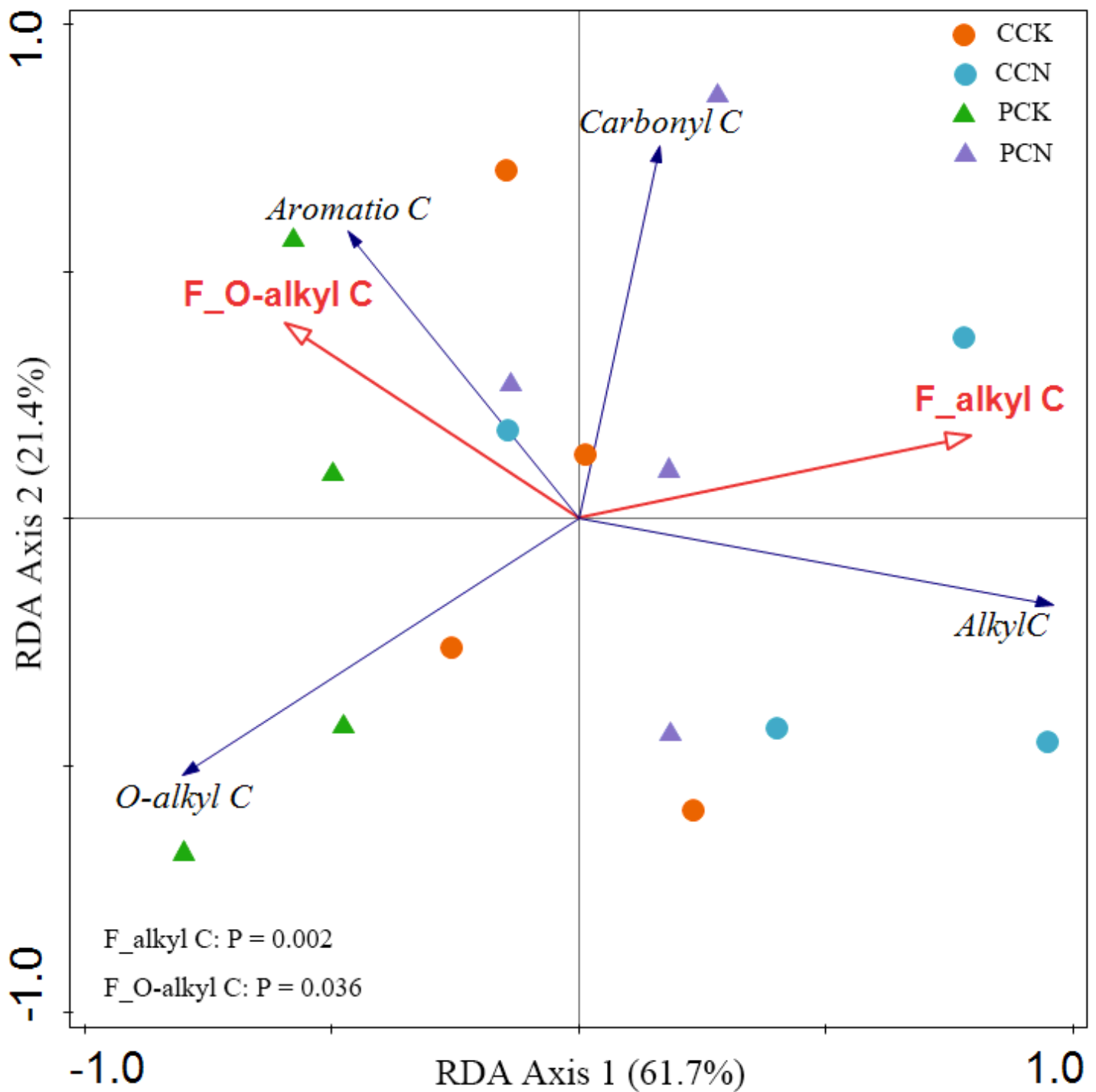


Figure 5

Soil organic carbon chemical composition distribution and the variation partition ordination plot of the redundancy analysis (RDA) results across the four planted forest types. SOC chemical composition (alkyl C, O-alkyl C, aromatic C, and carbonyl C) are the response variables, and F_alkyl C, F_O-alkyl C are the explanatory variables. Based on the permutation test ($p < 0.05$), the plot only includes plant parameters (alkyl C and O-alkyl C in fine roots) that have a significant impact on the distribution of SOC chemical composition

Supplementary Files

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