Soil microbial community and physicochemical properties together drive soil organic carbon in *Cunninghamia lanceolata* plantations of different stand ages

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Carbon sequestration in forest soil is critical for reducing atmospheric greenhouse gas concentrations and slowing down global warming. However, little is known about the difference in soil organic carbon (SOC) among different stand ages and the relative importance of biotic and abiotic variations such as soil microbial community and soil physicochemical properties in the regulation of SOC in forests. In the present study, we measured the SOC of the topsoil (0-10 cm) in Chinese subtropical *Cunninghamia lanceolata* plantations of three different stand ages (young plantation of 6 years, middle-aged plantation of 12 years, and mature plantation of 25 years). We further measured microbial community composition by phospholipid fatty acid (PLFA) analysis and soil organic carbon physical fractions by wet sieving and density floating as well as other physicochemical properties. The effects of the main impact factors on SOC were investigated. The results showed that: The middle-aged plantation had significantly higher SOC (10.63 g kg^{-1}) than the young plantation (5.33 g kg^{-1}), and that of the mature plantation (7.83 g kg^{-1}) was in between. Besides, the soil total PLFAs and all the functional groups (i.e. bacteria, fungi, actinomycetes, Gram-positive bacteria, and Gram-negative bacteria) of PLFAs were significantly higher in the middle-aged plantation than in the young plantation and the mature plantation. Soil physicochemical properties, including physical fractions, differed among plantations of the three stand ages. Notably, the proportion of organic carbon protected within microaggregates was significantly higher in the middle-aged plantation (40.4%) than those in the young plantation (29.2%) and the mature plantation (27.8%), indicating that the middle-aged *Cunninghamia lanceolata* plantation had stronger soil organic carbon stability. Both soil microbial community and physicochemical properties exerted dominant effects on SOC and jointly explained 82.7% of the variance of SOC among different stand ages. Among them, total and all the functional groups of PLFAs, nitrate nitrogen, total nitrogen, and organic carbon protected

within microaggregates had a significant positive correlation with SOC. These results highlight the important role of soil biotic and abiotic factors in shaping the contents of SOC in forests of different stand ages. This study provides a theoretical basis for forestry management and forest carbon cycling models.

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Abstract

Carbon sequestration in forest soil is critical for reducing atmospheric greenhouse gas concentrations and slowing down global warming. However, little is known about the difference in soil organic carbon (SOC) among different stand ages and the relative importance of biotic and abiotic variations such as soil microbial community and soil physicochemical properties in the regulation of SOC in forests. In the present study, we measured the SOC of the topsoil (0-10 cm) in Chinese subtropical *Cunninghamia lanceolata* plantations of three different stand ages (young plantation of 6 years, middle-aged plantation of 12 years, and mature plantation of 25 years). We further measured microbial community composition by phospholipid fatty acid (PLFA) analysis and soil organic carbon physical fractions by wet sieving and density floating as well as other physicochemical properties. The effects of the main impact factors on SOC were investigated. The results showed that: The middle-aged plantation had significantly higher SOC (10.63 g kg^{-1}) than the young plantation (5.33 g kg^{-1}), and that of the mature plantation (7.83 g kg^{-1}) was in between. Besides, the soil total PLFAs and all the functional groups (i.e. bacteria, fungi, actinomycetes, Gram-positive bacteria, and Gram-negative bacteria) of PLFAs were significantly higher in the middle-aged plantation than in the young plantation and the mature plantation. Soil physicochemical properties, including physical fractions, differed among plantations of the three stand ages. Notably, the proportion of organic carbon protected within microaggregates was significantly higher in the middle-aged plantation (40.4%) than those in the young plantation (29.2%) and the mature plantation (27.8%), indicating that the middle-aged

Cunninghamia lanceolata plantation had stronger soil organic carbon stability. Both soil microbial community and physicochemical properties exerted dominant effects on SOC and jointly explained 82.7% of the variance of SOC among different stand ages. Among them, total and all the functional groups of PLFAs, nitrate nitrogen, total nitrogen, and organic carbon protected within microaggregates had a significant positive correlation with SOC. These results highlight the important role of soil biotic and abiotic factors in shaping the contents of SOC in forests of different stand ages. This study provides a theoretical basis for forestry management and forest carbon cycling models.

Keywords *Cunninghamia lanceolata* plantation; stand age; SOC; microbial community; physicochemical properties； soil organic matter fractions

1 Introduction

Global warming caused by greenhouse gas enrichment is changing the structure, functions and some key ecological processes of the terrestrial ecosystem (*Jones et al., 2009*), thus has become the focus of global change research (*Peters et al., 2013*). The global forest, with a coverage area of 4.0×10^9 hectares, is the main carbon pool of terrestrial ecosystems (*Pan et al., 2011*), and plays an important role in regulating the global carbon cycle and climate change (*He, 2012*). Afforestation and plantation management are the main ways to increase forest cover, thereby fixing more CO_2 from the atmosphere and slowing down global warming. Plantation management, such as harvesting and forest fires, could create plantations of different stand ages. Stand age and corresponding biomass were found to be the most important factors in determining

the net primary production (NPP) of forest plantations (*Michaletz et al., 2014*). What's more, soil organic carbon (SOC) content (*Hou et al., 2019*) and its decomposition (*Wu et al., 2020b*) were also found to vary with stand age. How does soil organic carbon in subtropical plantations varies with stand age and what are the main drivers need more research.

It is worth noting that SOC could be partly protected from decomposition by being encapsulated in aggregates or adsorbed by minerals (*Krull et al., 2003*). Therefore, according to the stability and turnover time, SOC could be divided into different components, including mineral-associated organic carbon (SC), organic carbon protected within microaggregates (SA), and particulate organic matter (POM) (*Mitchell et al., 2018*). However, most studies about SOC in forests focused on its total content (*Guan et al., 2019; Thom et al., 2019*) and there is still a lack of studies about SOC fractions. We assume that the ratio of different components of SOC as well as other soil physicochemical properties will affect SOC storage. Soil microbes are an important part of the soil ecosystem, affecting soil fertility, nutrients, and carbon cycling processes (*Soong et al., 2020*). Plantations of different stand ages have diverse NPP (*Anderson-Teixeira et al., 2016*), litter, and root exudates (*Wu et al., 2020a*), which could directly lead to microbial differences. Previous studies have found that microbial community composition plays an important role in the formation and persistence of soil organic matter (*Condron et al., 2010*). Therefore, we assume that microbial community is one of the most important drivers of SOC among plantations of different stand ages.

There is a large area of artificial forests in China. *Cunninghamia lanceolata* is an excellent fast-growing native species, which is not susceptible to pests and diseases and is widely used in

commercial wood. *Cunninghamia lanceolata* has the largest planting area (8.5×106 hm^2) among China's artificial forests and it accounts for 21.4% of the total forest area in China based on the seventh national forest inventory statistics. In this study, we compared SOC in *Cunninghamia lanceolata* plantations of three different stand ages (young plantation of 6 years, middle-aged plantation of 12 years, and mature plantation of 25 years) in subtropical China. To explore the key determinant of SOC, we further detected microbial community composition, SOC physical fractions, and other physicochemical properties. We then conducted variation partitioning analysis (VPA) to quantify the relative contribution of microbial community and physicochemical properties to the variations of SOC among different stand ages. The results will reveal patterns and drivers of SOC in different developmental stages of *Cunninghamia lanceolata* plantations and provide a theoretical basis for the scientific management of the artificial forest.

2 Materials & Methods

2.1. Study site and soil sample collection

Our study was conducted at Gaojingmiao Forest Farm (31°00′59″N, 119°12′08″E) in Langxi County, Xuancheng City, Anhui Province, China. Gaojingmiao Forest Farm is a state-owned forest farm, covering an area of 10.37 km^2, with large areas of *Pinus massoniana*, *Pinus elliottii*, and *Cunninghamia lanceolata*. This forest farm is located in the southeast of Anhui Province and has a humid subtropical monsoon climate with four distinct seasons and sufficient

sunlight. The mean annual temperature in this area is 15.9℃, and the coldest and warmest months are January (with a mean temperature of 2.7°C) and July (with a mean temperature of 28.1°C), respectively. The region receives an average total annual precipitation of 1294.4 mm mostly occurring in summer. The area has 1784 h of sunshine and 228 days of Frost-free period per year. The soil is acidic yellow-red soil.

In December 2018, *Cunninghamia lanceolata* plantations of three stand ages (young plantation of 6 years, middle-aged plantation of 12 years, and mature plantation of 25 years) with basically the same site conditions (30-50 m above sea level, slope less than 30°) were selected as the sample blocks. Three plots (10 m×10 m) were randomly set in each of the stand ages of *Cunninghamia lanceolata* plantation. Five soil cores were randomly collected from the 0-10 cm soil layer in each plot using a corer 6 cm in diameter. These five soil cores were then combined and mixed thoroughly as one sample for each plot. The individual soil samples of each plot were collected in plastic bags and placed in a cooler in the field, then transported to the laboratory for further analysis.

After visible plant residues and stones were removed, each soil sample was divided into two subsamples. One subsample was used to determine pH, soil organic carbon (SOC), and total nitrogen (TN) content after air-dried; one subsample was stored at -20°C and used to determine microbial community composition, ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), and dissolved organic carbon (DOC) (*Yuan et al., 2020*). Cylinders were used to obtain 4 undisturbed soil cores from the 0-10 cm profile of each plot. One of these soil cores was used to determine soil bulk density (BD) and soil water content (SWC), while the other three of them

were used to determine SOC physical fractions. The three soil cores used to determine SOC fractions were gently broken apart along the natural breakpoints and passed through a 2 mm sieve to remove visible organic debris and stones, and then air-dried for further analysis (*Mitchell et al., 2018*).

2.2. Soil physicochemical properties analysis

Both SOC and TN were measured using a C/N analyzer (Elementar, Vario Max CN, Germany) with a combustion temperature of 900 °C. Soil samples collected with cylinders were oven-dried to calculate BD and SWC according to the volume of the cylinder and the soil weight before and after drying. Soil pH was measured at a soil: water ratio of 1:2.5 using a pH meter (Mettler Toledo, Greifensee, Switzerland). Soil NH_4^+-N and NO_3^--N were extracted from 20 g of fresh soil with 1 mol L^{-1} KCl (soil: extract, 1:5) and analyzed using a flow-injection autoanalyzer ((CFA)-AA3, SEAL, Germany). Soil DOC was extracted using deionized water (soil: extract = 1:5) and analyzed using a TOC analyzer (Elementar, Vario TOC cube, Hanau, Germany).

Wet sieving and density flotation methods were used to detect SOC fractions (*Mitchell et al., 2018*). First, soil samples were dispersed by low-energy sonication. Briefly, add 30 g of air-dried soil into 150 mL water and then put them into an ultrasonic treatment (KQ5200DE, Kunshan Ultrasound Instrument Co., Ltd., China) with the output energy of 22 J ml^{-1}. The macroaggregates were disrupted in this step and only the more stable microaggregates were left. Secondly, the dispersed suspension was then wet sieved over a 53 μm aperture sieve several

143 times until the rinsing water was clear. Soil samples were separated into two parts in this step:
144 the fraction > 53 μm which was left on the sieve and the fraction < 53 μm which passed through
145 the sieve and remained in suspension. The fraction > 53 μm was rinsed repeatedly with deionized
146 water, dried in the oven at 40 °C, and weighed. Thirdly, density flotation was conducted to
147 separate the fraction > 53 μm from the second step into organic carbon protected within
148 microaggregates (SA) and particulate organic matter (POM). The fraction > 53 μm from the
149 second step was stirred with sodium polytungstate (SPT) at a density of 1.8 g cm^{-3} and then
150 centrifuged at 1000g for 15 min. The light part was POM, which could be decanted and then
151 washed with deionized water to remove all SPT, dried at 40°C, and weighed. The heavy part was
152 also dried at 40 °C and weighed as SA. At last, the fraction < 53 μm from the second step was
153 filtered through a 0.45 μm aperture nylon mesh, and the material > 0.45 μm was dried at 40°C
154 and weighed as mineral-associated organic carbon (SC).

155 **2.3. Phospholipid fatty acid (PLFA) analysis**

156 The PLFA contents of the samples were analyzed using the method described by *Bååth and*
157 *Anderson (2003)*. Briefly, lipids were extracted from freeze-dried soil (8 g) in a single-phase
158 mixture of chloroform:methanol:phosphate buffer (1:2:0.5). After extraction, the lipids were
159 separated into neutral lipids, glycolipids, and polar lipids (phospholipids) on a silicic acid
160 column. The samples were analyzed using a Thermo ISQ gas chromatography-mass
161 spectroscopy system (TRACE GC Ultra ISQ). The concentrations of the individual compounds
162 were obtained by comparing the peaks with an internal standard (nonadecanoic acid methyl ester

19:0). The fatty-acid signatures 15:0, i15:0, a15:0, i16:0, 16:1ω7c, 17:0, i17:0, cy17:0 and cy19:0 were used as bacterial biomarkers (B). The fatty acids 18:2ω6 and 18:1ω9c were used as fungal indicators (F) and 10Me16:0 and 10Me18:0 were used as indicators for the actinomycetes (ACT). The fatty acids i15:0, a15:0, i16:0, and i17:0 were used to represent Gram-positive bacteria (G^+), while 16:1ω7c, cy17:0, and cy19:0 were used to represent Gram-negative bacteria (G^-). The total PLFAs (TPLFA) were the sum of bacterial, fungal, and actinomyces PLFA biomarkers.

2.4. Statistical analysis

A one-way ANOVA was performed to identify significant differences in SOC, soil physicochemical properties, and PLFAs among the three stand ages. A principal components analysis (PCA) was used to analyze soil microbial community structure. The Pearson correlation method was used to analyze the correlation between SOC and some important physicochemical properties and PLFAs. One-way ANOVA and Pearson correlation analysis was performed using SPSS 19.0 (SPSS Inc., Chicago, USA). PCA was conducted with CANOCO for Windows 4.5. Other figures were generated using the Origin 9.5 package (Origin Lab Corporation, Northampton, USA). $P < 0.05$ was considered to be statistically significant. Mean±standard deviation was shown in tables and figures.

Variation partitioning analysis (VPA) was conducted to quantify the relative importance of soil microbial community and soil physicochemical properties to the variations of SOC among *Cunninghamia lanceolata* plantations of different stand ages. To avoid collinearity, variables

with a large variance inflation factor (VIF) were removed in order until the VIF of the retained variables does not exceed 10. Next, principal component analysis was performed on microbial variables and physicochemical variables respectively (the first 2 principal components were retained). Then VPA was employed to quantify their explanation for the variations of SOC among different stand ages. VPA was conducted in R 4.0.2 (*R Core Team, 2020*) and the vegan package (*Oksanen, et al. 2020*).

3 Results

3.1 Soil organic carbon content

Soil organic carbon content exhibited large variability among different stand ages (Fig. 1). The middle-aged plantation had the highest SOC (10.63 g kg^{-1}) followed by the mature plantation (7.83 g kg^{-1}), and the young plantation had the lowest SOC (5.33 g kg^{-1}). Soil organic carbon content of the middle-aged plantation was 99.4% and 35.8% higher than those of the young and the mature plantation, respectively. The difference in SOC between the middle-aged and the young plantations was significant.

3.2 Soil physicochemical properties

Soil physicochemical properties of the *Cunninghamia lanceolata* plantations varied among different stand ages (Table 1). The middle-aged plantation had the highest pH, SWC and TN. The mature plantation had a significantly higher NH_4^+-N content than the other stand ages. Soil

BD, C/N, NO_3^--N, and DOC showed no significant difference among the three stand ages.

Soil organic carbon fractions were different among *Cunninghamia lanceolata* plantations of different stand ages (Fig. 2). The proportion of SA was significantly higher in the soil of the middle-aged plantation (40.4%) than those of the young (29.2%) and the mature (27.8%) plantation. The other two fractions, SC and POM, showed no significant difference among the three stand ages.

3.3 Soil microbial community

Soil PLFAs showed significant differences among different stand ages (Fig. 3). The middle-aged plantation had significantly higher soil total PLFAs, bacterial PLFAs, fungal PLFAs, actinobacterial PLFAs, Gram-positive and Gram-negative bacterial PLFAs than the young plantation and the mature plantation (Fig. 3a). Soil total PLFAs of the middle-aged plantation were 30.23 nmol g^{-1}, while those of the young plantation and the mature plantation were 72.5% and 60.3% lower than the middle-aged plantation. The ratio of F/B and the ratio of G^+/G^- showed no significant difference among stand ages(Fig. 3b). Principal components analysis (PCA) also confirmed that soil microbial community composition was different among the three stand ages and 94% of the variance was explained by the first component of the PCA (Fig. 4). The soil PLFAs of the middle-aged plantation were separated from those of the other two stand ages along with the first principal component.

3.4 Associations of SOC with the microbial community and physicochemical properties

The results of VPA showed that the soil microbial community and physicochemical properties explained 82.7% of the variance in SOC of different stand ages (Fig. 5). Pearson correlation analysis also showed that some of the microbial and physicochemical factors were significantly correlated with SOC (Table 2). Among the microbial factors, TPLFA, B, F, ACT, G^+, and G^-were positively correlated with SOC while F/B was negatively correlated with SOC. Among the physicochemical properties, TN, NO_3^--N, and SA were positively correlated with SOC.

4 Discussion

The SOC concentrations in this study (ranged from 5.33 to 10.63 g kg^{-1}, Fig. 1) were lower than those measured in some other subtropical *Cunninghamia lanceolata* plantations, which ranged from 18 to 25 g kg^{-1} (*Chen et al., 2013; Song et al., 2017*). The possible reason for the lower SOC in our study is that the plantations here were converted from farmland decades ago, and the SOC of farmlands is generally lower than that of forest plantations (*Xie et al., 2007*). This implies that the forest plantations in this study area still have large carbon sequestration potential with proper management.

The middle-aged *Cunninghamia lanceolata* plantation had the highest SOC (Fig. 1) and the highest fraction of SA (Fig. 2). Compared with the POM fraction, the SOC occluded within microaggregates is more stable because microaggregates excluded microbes and enzymes from

238 pores (*Yu et al., 2012*), indicating that the middle-aged plantation has more stable SOC. Forest
239 SOC mainly comes from above-ground litter, fine root decomposition, and root exudates
240 (*Berhongaray et al., 2019*). Young plantations, which are in the fast-growing stage, produce little
241 litter. With the growth of the plantation age, the above-ground litterfall increased (*Zheng et al.,*
242 *2019*). What's more, the increasing canopy closure will also accelerate the withering of
243 understory vegetation (*Verburg et al., 2001*), and further increase the amount of surface litter.
244 Most of the previous studies found that the mature forests had the highest SOC due to higher
245 levels of litter accumulation and lower soil respiration (*Nath et al., 2022, Wang et al, 2019*).
246 However, our study found that the SOC of mature plantations was lower than that of middle-
247 aged plantations. This may be due to inactive root activities in the mature plantations. The
248 decomposition process of litter takes a longer time, while the carbon input from root exudates to
249 soil is more rapid. A previous study conducted in subtropical China showed that middle-aged
250 *Cunninghamia lanceolata* plantations had higher underground carbon allocations than young and
251 mature plantations (*Chen et al., 2008*). Because mature forests grow slowly and require fewer
252 nutrients, they do not need as many roots for nutrients. The higher underground carbon
253 allocations in middle-aged forests provided more organic carbon to the soil in the form of
254 exudates and dead roots. While delivering large amounts of carbon directly to the soil, root
255 exudate contains macromolecular viscose and promotes the formation of microaggregates
256 (*Traoré et al., 2000*).

257 Soil total PLFAs and all microbial groups were highest in the middle-aged plantation (Fig.
258 3a). The possible reason is that the middle-aged plantations, had higher litter accumulation than

the young plantations and more root allocation than the mature plantations and this provided more carbon sources for the growth and reproduction of microbes. Higher microbial biomass generally corresponds to better soil structure and nutrient status (*Kang et al., 2021*). The ratio of F/B could reflect ecosystem stability and soil nutritional status (*Liu et al., 2019*). The ratios of F/B were all smaller than 1 (Fig. 3b), indicating the absolute dominance of bacteria. And in the present study, there was no significant difference in the ratio of F/B among plantations of different stand ages. The ratios of G^+/G^- were all bigger than 1 (Fig. 3b). This may be due to the different water requirements of G^+ and G^- bacteria, the subtropical humid soil environment is more suitable for the growth and reproduction of G^+ bacteria (*Zhou et al., 2017*).

Our results illustrated the roles of soil microbial community and physicochemical properties in regulating SOC across different stand ages (Fig. 5). We found that all microbial groups (PLFAs) were positively correlated with SOC (Table 2). While SOC provides food sources for microorganisms, soil microorganisms and their debris are important components of SOC (*Guo et al., 2021*). The higher root exudates of middle-aged plantations provided more carbon sources for the growth and reproduction of microorganisms and produced more microbial necromass C to SOC. Besides, soil physicochemical properties, especially TN, NO_3^--N, and SA, were also found to regulate SOC significantly (Table 2). The interactions between soil C and N have been intensively studied as N could regulate plant photosynthesis, allocation, rhizosphere priming effect, and greenhouse gas emissions (*Gärdenäs et al., 2011*). For example, *Tian et al*. (2019) found that high N increased SOC mainly by decreasing CO_2 efflux. Organic carbon protected within microaggregates is isolated from microorganisms and hard to be decomposed. As a result,

higher SA facilitates the storage of soil SOC. Unexpectedly, soil moisture, which is an important factor in regulating SOC decomposition (*Meyer et al., 2018*), was insignificantly related to SOC in our study (Table 2). We measured SWC only one time, and it could not be representative of long-term conditions of soil moisture, which has a lasting impact on SOC decomposition. Higher soil pH was found in the middle-aged plantation than in the young and mature plantation (Table 1). In acidic soils, lower pH suppresses the growth of microorganisms and elevated pH could promote microbial biomass (*Silva-Sánchez et al., 2019*). The higher pH and higher microbial biomass of middle-aged forests in this study are consistent. Although soil pH was not significantly related to SOC, pH can indirectly affect SOC through the regulation of microorganisms.

5 Conclusions

Our study detected the variation in SOC of different stand ages, which has important implications for forest carbon sink function. Overall, middle-aged plantations had higher total SOC and organic carbon protected within microaggregates, which indicated that the SOC stability of middle-aged forests was stronger. Moreover, our study clarified that the physicochemical properties and microbial communities are the two main driving factors of SOC. The middle-aged plantation had significantly higher soil total PLFAs and all the functional groups of PLFAs than those of the young and mature plantations, indicating that the middle-aged plantation had better soil structure and nutrient status. In general, these findings jointly highlight the important role of stand age and soil biotic and abiotic factors in shaping the contents of SOC

in subtropical *Cunninghamia lanceolata* plantations. These results will greatly improve our understanding and prediction of soil carbon dynamics in forests with their development.

Acknowledgments

We thank Shan Gao for his assistance in field and laboratory work. We also thank the academic editor and anonymous reviewers for their constructive comments, which helped in improving the manuscript.

References

Anderson-Teixeira, K.J., Wang, M.M., McGarvey, J.C. and LeBauer, D.S., 2016. Carbon dynamics of mature and regrowth tropical forests derived from a pantropical database (T rop F or C-db). *Global Change Biology*, 22(5): 1690-1709.

Bååth E., Anderson T.H., 2003. Comparison of soil fungal/bacterial ratios in a pH gradient using physiological and PLFA-based techniques. *Soil Biology and Biochemistry*, 35(7): 955-963.

Berhongaray, G., Cotrufo, F.M., Janssens, I.A. and Ceulemans, R., 2019. Below-ground carbon inputs contribute more than above-ground inputs to soil carbon accrual in a bioenergy poplar plantation. *Plant and Soil*, 434(1): 363-378.

Chen, G.S. et al., 2008. Changes in belowground carbon allocation in a Chinese fir chronosequence in Fujian Province, China. *Chinese Journal of Plant Ecology*, 32(6): 1285.

Chen, G.S. et al., 2013. Carbon storage in a chronosequence of Chinese fir plantations in southern China. *Forest Ecology and Management*, 300: 68-76.

Condron, L., Stark, C., O'Callaghan, M., Clinton, P. and Huang, Z., 2010. The role of microbial communities in the formation and decomposition of soil organic matter, Soil microbiology and sustainable crop production. Springer, pp. 81-118.

Gärdenäs, A.I. et al., 2011. Knowledge gaps in soil carbon and nitrogen interactions–From molecular to global scale. *Soil Biology and Biochemistry*, 43(4): 702-717.

Guan, J.H. et al., 2019. Soil organic carbon density and its driving factors in forest ecosystems across a northwestern province in China. *Geoderma*, 352: 1-12.

Guo, Z. et al., 2021. Contribution of soil microbial necromass to SOC stocks during vegetation recovery in a subtropical karst ecosystem. *Science of the Total Environment*, 761: 143945.

He, J., 2012. Carbon cycling of Chinese forests: From carbon storage, dynamics to models. *Science China*. *Life Sciences*, 55(2): 188.

Hou, G., Delang, C.O., Lu, X. and Gao, L., 2019. Soil organic carbon storage varies with stand ages and soil depths following afforestation. *Annals of Forest Research*, 62(2): 3-20.

Jari Oksanen, F. Guillaume Blanchet, Michael Friendly, Roeland Kindt, Pierre Legendre, Dan McGlinn, Peter R. Minchin, R. B. O'Hara, Gavin L. Simpson, Peter Solymos, M. Henry H. Stevens, Eduard Szoecs and Helene Wagner (2020). vegan: Community Ecology Package. R package version 2.5-7. https://CRAN.R-project.org/package=vegan

Jones, C., Lowe, J., Liddicoat, S. and Betts, R., 2009. Committed terrestrial ecosystem changes due to climate change. *Nature Geoscience*, 2(7): 484-487.

340 Kang, H., Yu, W., Dutta, S. and Gao, H., 2021. Soil microbial community composition and
341 function are closely associated with soil organic matter chemistry along a latitudinal
342 gradient. *Geoderma*, 383: 114744.
343 Krull, E.S., Baldock, J.A. and Skjemstad, J.O., 2003. Importance of mechanisms and processes
344 of the stabilisation of soil organic matter for modelling carbon turnover. *Functional Plant*
345 *Biology*, 30(2): 207-222.
346 Liu, M., Sui, X., Hu, Y. and Feng, F., 2019. Microbial community structure and the relationship
347 with soil carbon and nitrogen in an original Korean pine forest of Changbai Mountain,
348 China. *BMC Microbiology*, 19(1): 218.
349 Meyer, N., Welp, G. and Amelung, W., 2018. The temperature sensitivity (Q10) of soil
350 respiration: Controlling factors and spatial prediction at regional scale based on
351 environmental soil classes. *Global Biogeochemical Cycles*, 32(2): 306-323.
352 Michaletz, S.T., Cheng, D., Kerkhoff, A.J. and Enquist, B.J., 2014. Convergence of terrestrial
353 plant production across global climate gradients. *Nature*, 512(7512): 39-43.
354 Mitchell, E. et al., 2018. Amount and incorporation of plant residue inputs modify residue
355 stabilisation dynamics in soil organic matter fractions. *Agriculture, Ecosystems &*
356 *Environment*, 256: 82-91.
357 Nath, P.C., Sileshi, G.W., Ray, P., Das, A.K., and Nath, A.J., 2022. Variations in soil properties
358 and stoichiometric ratios with stand age under agarwood monoculture and polyculture on
359 smallholder farms. *Catena*, 213: 106174.

Pan, Y. et al., 2011. A large and persistent carbon sink in the world's forests. *Science*, 333(6045): 988-993.

Peters, G.P. et al., 2013. The challenge to keep global warming below 2 °C. *Nature Climate Change*, 3(1): 4-6.

R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Silva-Sánchez, A., Soares, M., and Rousk, J., 2019. Testing the dependence of microbial growth and carbon use efficiency on nitrogen availability, pH, and organic matter quality. *Soil Biology and Biochemistry*, 134: 25-35.

Song, X., Kimberley, M.O., Zhou, G. and Wang, H., 2017. Soil carbon dynamics in successional and plantation forests in subtropical China. *Journal of Soils and Sediments*, 17(9): 2250-2256.

Soong, J.L. et al., 2020. Microbial carbon limitation: the need for integrating microorganisms into our understanding of ecosystem carbon cycling. *Global Change Biology*, 26(4): 1953-1961.

Thom, D. et al., 2019. The climate sensitivity of carbon, timber, and species richness covaries with forest age in boreal–temperate North America. *Global Change Biology*, 25(7): 2446-2458.

Tian, J. et al., 2019. Long-term nitrogen addition modifies microbial composition and functions for slow carbon cycling and increased sequestration in tropical forest soil. *Global Change Biology*, 25(10): 3267-3281.

Traoré, O., Groleau-Renaud, V., Plantureux, S., Tubeileh, A. and Boeuf-Tremblay, V., 2000. Effect of root mucilage and modelled root exudates on soil structure. *European Journal of Soil Science*, 51(4): 575-581.

Verburg, P., Johnson, D. and Harrison, R., 2001. Long-term nutrient cycling patterns in Douglas-fir and red alder stands: a simulation study. *Forest Ecology and Management,* 145(3): 203-217.

Wang, Y., Liu, L., Yue, F., and Li, D., 2019. Dynamics of carbon and nitrogen storage in two typical plantation ecosystems of different stand ages on the Loess Plateau of China. *PeerJ*, 7: e7708.

Wu, H. et al., 2020a. Soil phosphorus bioavailability and recycling increased with stand age in Chinese fir plantations. *Ecosystems*, 23(5): 973-988.

Wu, X. et al., 2020b. Land use change and stand age regulate soil respiration by influencing soil substrate supply and microbial community. *Geoderma*, 359: 113991.

Xie, Z. et al., 2007. Soil organic carbon stocks in China and changes from 1980s to 2000s. *Global Change Biology*, 13(9): 1989-2007.

Yu, H. et al., 2012. Effects of long-term compost and fertilizer application on stability of aggregate-associated organic carbon in an intensively cultivated sandy loam soil. *Biology and Fertility of Soils*, 48(3): 325-336.

Yuan, Y., Dai, X., Fu, X., Kou, L., Luo, Y., Jiang, L., and Wang, H., 2020. Differences in the rhizosphere effects among trees, shrubs and herbs in three subtropical plantations and their seasonal variations. *European Journal of Soil Biology*, 100: 103218.

Zheng, L.T., Chen, H.Y. and Yan, E.R., 2019. Tree species diversity promotes litterfall productivity through crown complementarity in subtropical forests. *Journal of Ecology*, 107(4): 1852-1861.

Zhou, W., Shen, W., Li, Y. and Hui, D., 2017. Interactive effects of temperature and moisture on composition of the soil microbial community. *European Journal of Soil Science*, 68(6): 909-918.

Figure 1

Soil organic carbon (SOC) content in Cunninghamia lanceolata plantations of three different stand ages

Different lower case letters indicated a significant difference among plantations of different stand ages at 0.05 level

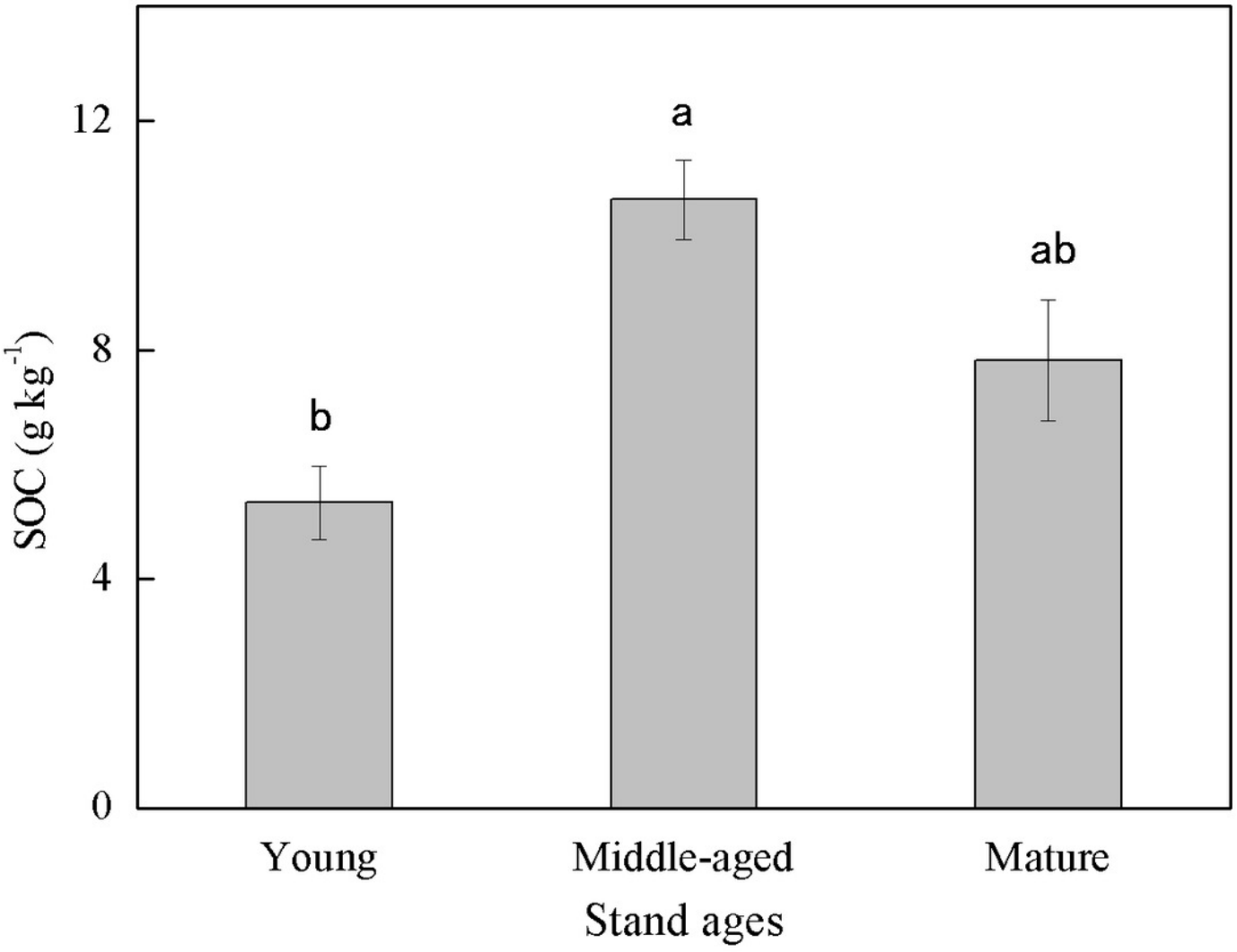

Figure 2

Soil organic carbon physical fractions in *Cunninghamia lanceolata* plantations of three different stand ages

SC, Mineral-associated organic carbon; SA, Organic carbon protected within microaggregates; POM, Particulate organic matter

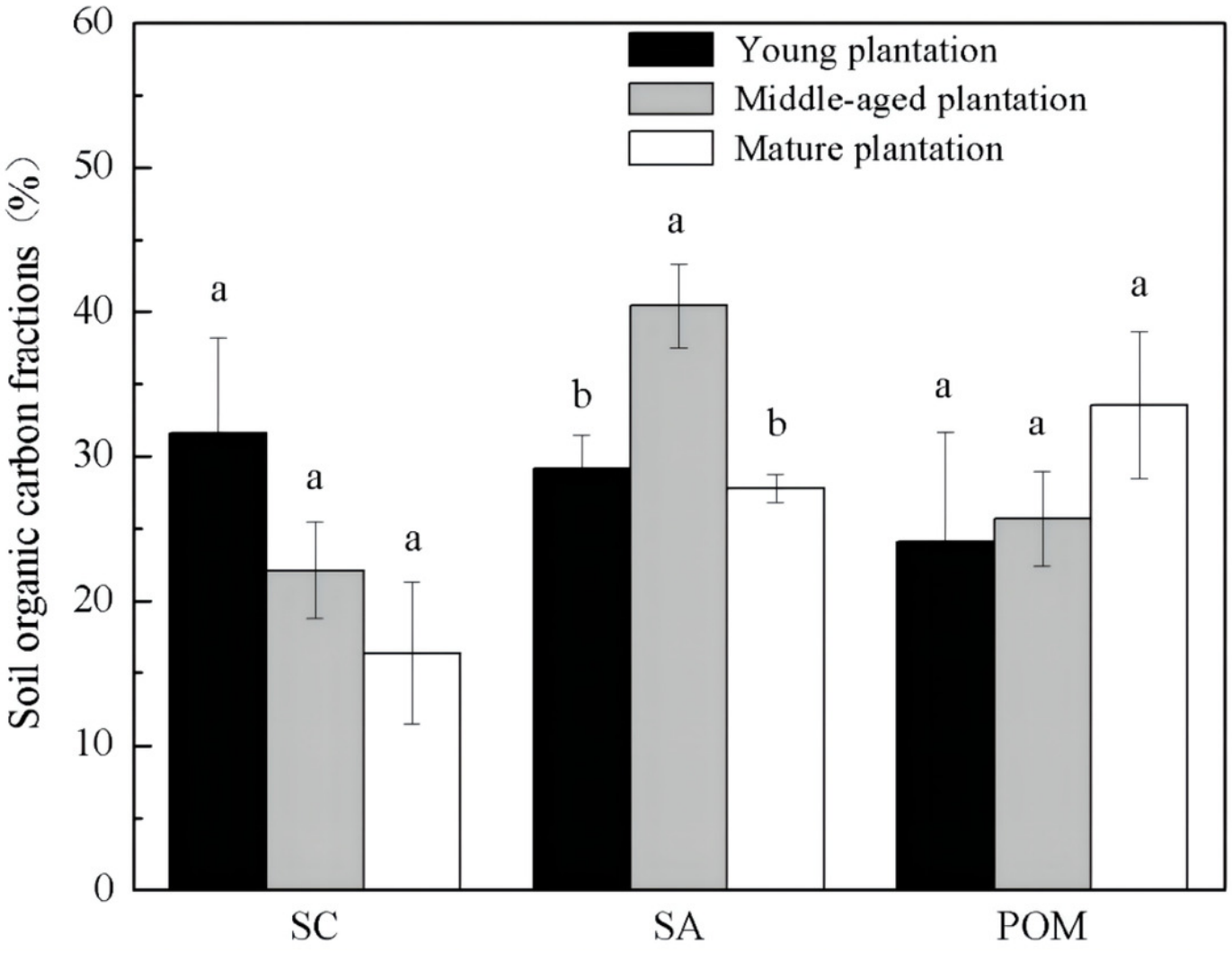

Figure 3

Soil microbial community structure in *Cunninghamia lanceolata* plantations of three different stand ages

TPLFA, Total phospholipid fatty acids; B, Bacteria; F, Fungi; ACT, Actinobacteria; G^+, Gram-positive bacteria; G^-, Gram-negative bacteria; F/B, Ratio of fungi to bacteria; G^+/G^-, Ratio of gram-positive bacteria to gram-negative bacteria

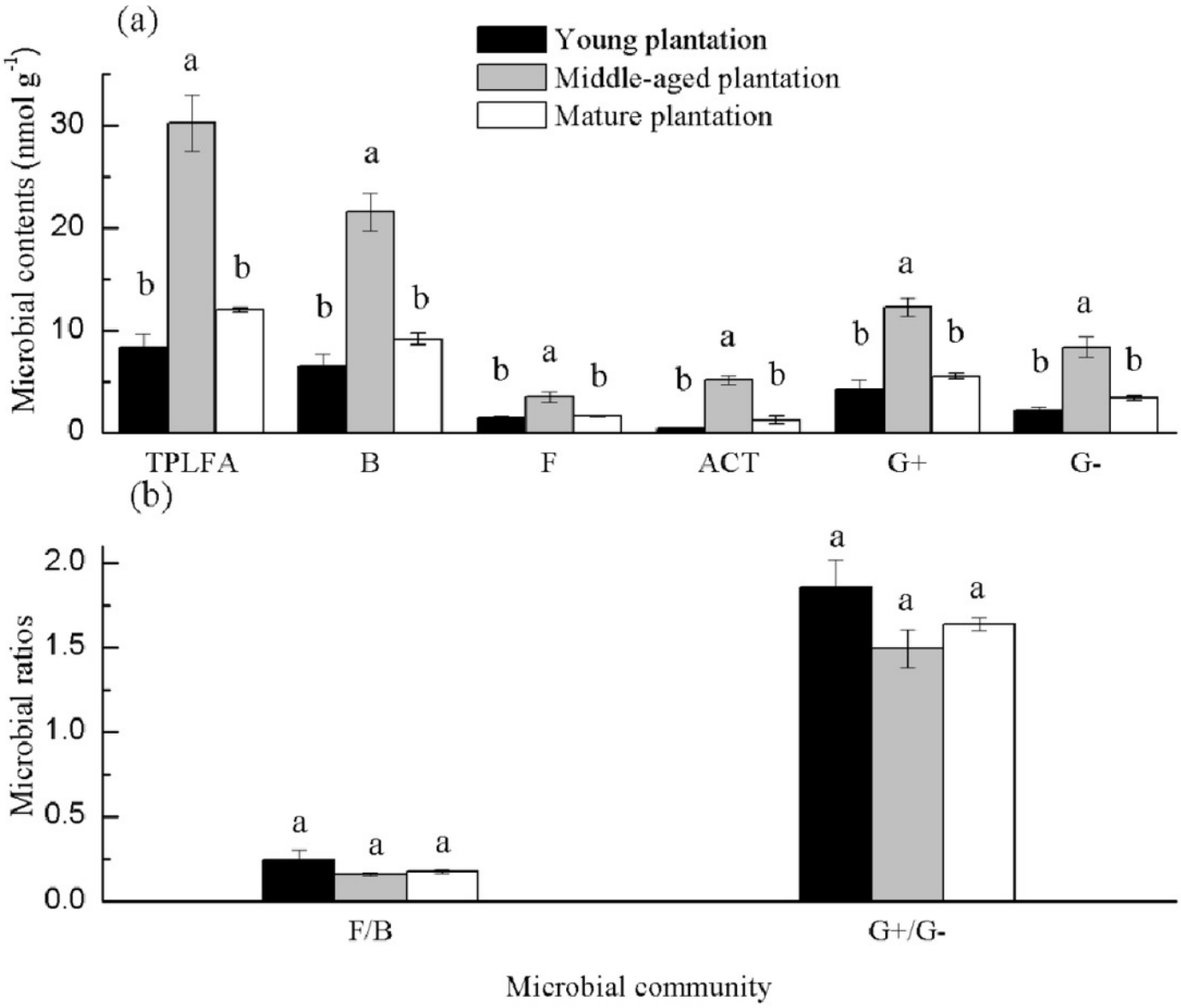

Figure 4

Principal component analysis (PCA) of soil microbial community structure in *Cunninghamia lanceolata* plantations of different ages

The red circles in the picture represent the young plantation, the blue triangles represent the middle-aged plantation, and the green stars represent the mature plantation

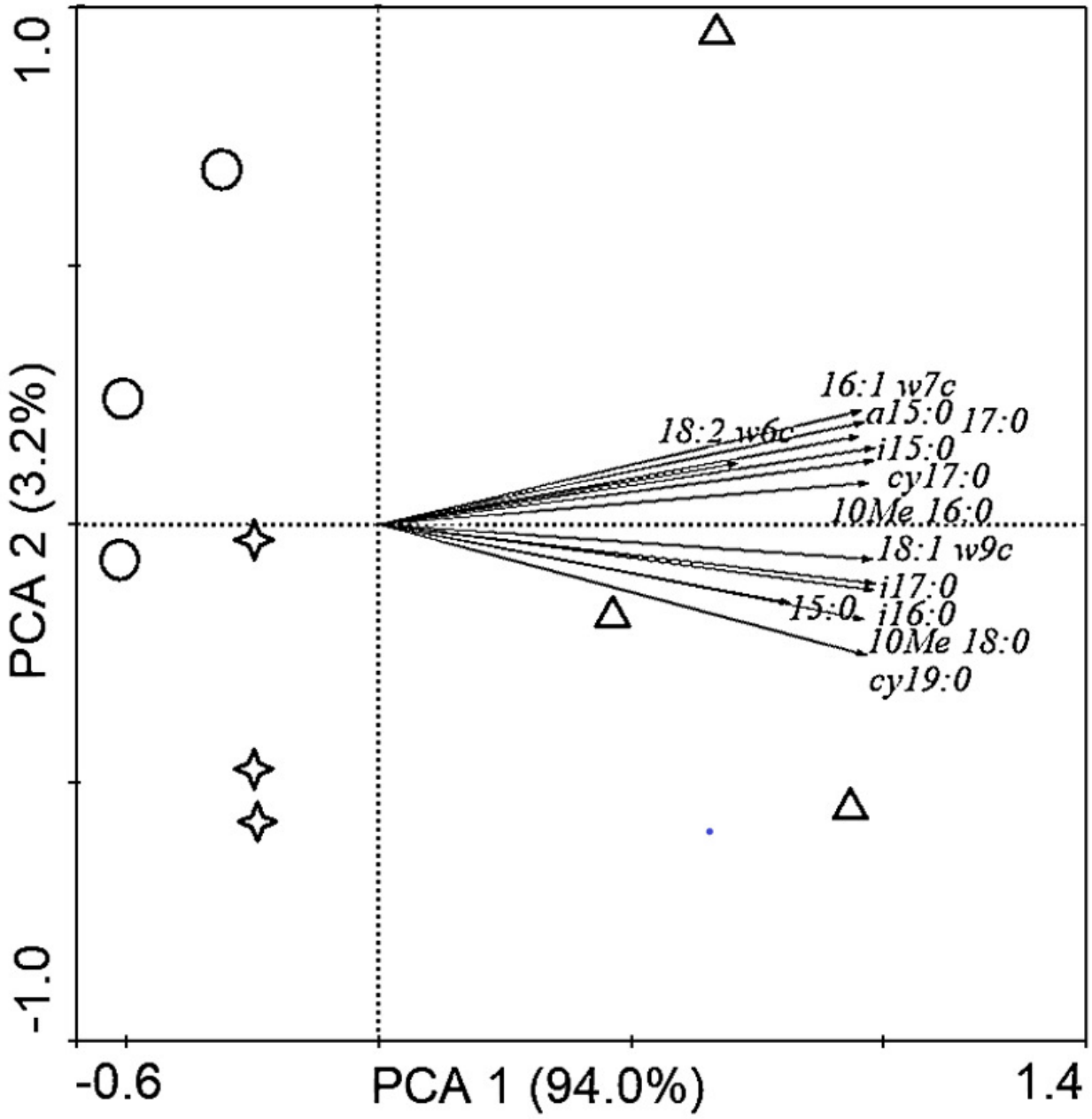
1.0
-1.0
PCA 2 (3.2%)
-0.6
PCA 1 (94.0%)
1.4
16:1 w7c
a15:0
17:0
18:2 w6c
i15:0
cy17:0
10Me 16:0
18:1 w9c
i17:0
15:0
i16:0
10Me 18:0
cy19:0

Figure 5

Results of variation partitioning analyses illustrating the relative contribution of soil microbial community and soil physicochemical properties to soil organic carbon

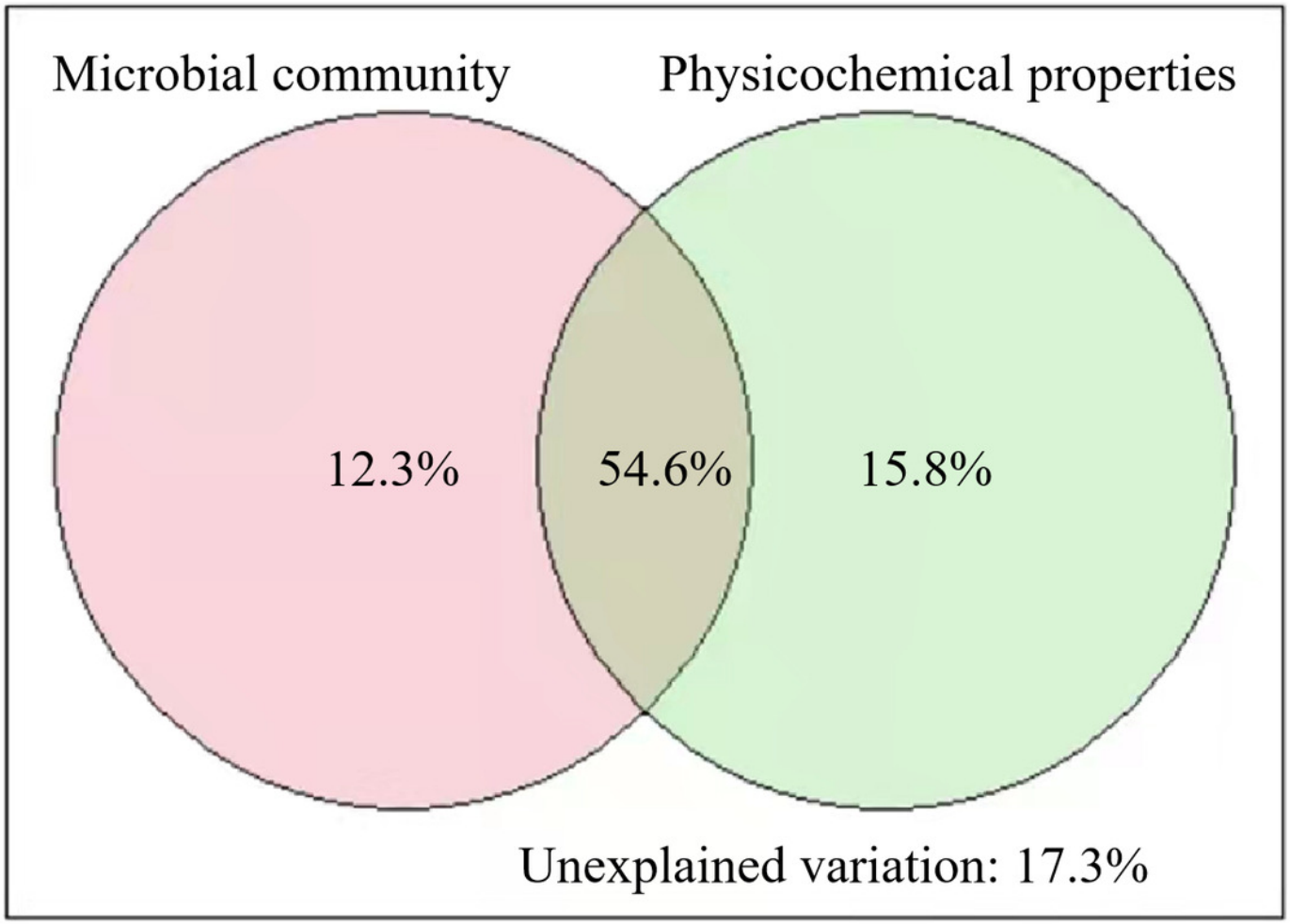

Table 1(on next page)

Soil physicochemical properties in *Cunninghamia lanceolata* plantations of three different stand ages

The data shown in the table is the mean ± standard error (n=3). Different lower case letters indicated a significant difference among plantations of different stand ages at 0.05 level□SWC, Soil water content; BD, Bulk density; TN, Total nitrogen; C/N, the ratio of organic carbon to total nitrogen; NH_4^+-N, Ammonium nitrogen; NO_3^--N, Nitrate nitrogen; DOC, Dissolved organic carbon.

Table 1. Soil physicochemical properties in *Cunninghamia lanceolata* plantations of three different stand ages

Soil physicochemical properties	Young plantation	Middle-aged plantation	Mature plantation
pH	4.88±0.03b	5.10±0.08a	4.67±0.06b
SWC（%）	20.83±0.46ab	22.50±0.77a	19.74±0.41b
BD（g cm^{-3}）	1.07±0.14a	1.30±0.03a	1.33±0.04a
TN（g kg^{-1}）	0.60±0.06b	1.07±0.03a	0.67±0.07b
C/N	9.35±1.50a	9.96±0.48a	11.34±0.35a
NH_4^+-N（mg kg^{-1}）	12.37±0.98b	15.27±2.10b	22.13±1.25a
NO_3^--N（mg kg^{-1}）	0.68±0.13a	1.55±0.34a	1.40±0.28a
DOC（mg kg^{-1}）	17.67±6.13a	14.74±2.92a	6.44±1.15a

The data shown in the table is the mean ± standard error (n=3). Different lower case letters indicated a significant difference among plantations of different stand ages at 0.05 level. SWC, Soil water content; BD, Bulk density; TN, Total nitrogen; C/N, the ratio of organic carbon to total nitrogen; NH_4^+-N, Ammonium nitrogen; NO_3^--N, Nitrate nitrogen; DOC, Dissolved organic carbon.

Table 2 (on next page)

Pearson correlations between SOC and soil microbial/physicochemical properties.

TPLFA, Total phospholipid fatty acids; B, Bacteria; F, Fungi; ACT, Actinobacteria; G^+, Gram-positive bacteria; G^-, Gram-negative bacteria; F/B, Ratio of fungi to bacteria; G^+/G^-, Ratio of gram-positive bacteria to gram-negative bacteria;SWC, Soil water content; BD, Bulk density; TN, Total nitrogen; C/N, the ration of organic carbon to total nitrogen; NH_4^+-N, Ammonium nitrogen; NO_3^--N, Nitrate nitrogen; DOC, Dissolved organic carbon; SC, Mineral-associated organic carbon; SA, Organic carbon protected within microaggregates; POM, Particulate organic matter.

Table 2. Pearson correlations between SOC and soil microbial/physicochemical properties.

Microbial community			Physicochemical properties					
	r-value	*P*-value		*r*-value	*P*-value		*r*-value	*P*-value
TPLFA	**0.87**	**<0.001**	pH	0.48	0.11	SC	-0.22	0.50
B	**0.87**	**<0.001**	SWC	0.49	0.10	**SA**	**0.62**	**0.03**
F	**0.72**	**0.008**	BD	0.44	0.15	POM	-0.22	0.50
ACT	**0.89**	**<0.001**	**TN**	**0.90**	**<0.001**			
G^+	**0.89**	**<0.001**	C/N	0.32	0.31			
G^-	**0.85**	**<0.001**	NH_4^+-N	0.34	0.29			
F/B	**-0.68**	**0.02**	**NO_3^--N**	**0.63**	**0.03**			
G^+/G^-	-0.34	0.28	DOC	0.12	0.72			

TPLFA, Total phospholipid fatty acids; B, Bacteria; F, Fungi; ACT, Actinobacteria; G^+, Gram-positive bacteria; G^-, Gram-negative bacteria; F/B, Ratio of fungi to bacteria; G^+/G^-, Ratio of gram-positive bacteria to gram-negative bacteria; SWC, Soil water content; BD, Bulk density; TN, Total nitrogen; C/N, the ration of organic carbon to total nitrogen; NH_4^+-N, Ammonium nitrogen; NO_3^--N, Nitrate nitrogen; DOC, Dissolved organic carbon; SC, Mineral-associated organic carbon; SA, Organic carbon protected within microaggregates; POM, Particulate organic matter.