



Forest management practices of *Pinus tabulaeformis* plantations alter soil organic carbon stability by adjusting microbial characteristics on the Loess Plateau of China



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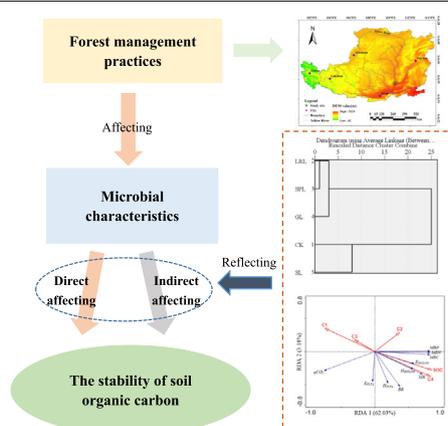
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HIGHLIGHTS

- Forest management changes the stability of soil organic carbon by altering microbes
- Different management practices affected microbial characteristics differently
- Natural shrub regeneration after clear-cutting increased the nonlabile carbon fraction
- Shrub contributed the most towards stabilizing soil organic carbon and enhanced the stability of soil organic carbon

GRAPHICAL ABSTRACT



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ABSTRACT

Sustainable management practices can enhance the capacity and potential for soil carbon (C) sequestration, significantly contributing towards mitigating regional climate change. Here, we investigated how the microbial characteristics of a *Pinus tabulaeformis* plantation responded to different management practices to identify the role of microbial characteristics in influencing the stability of soil organic carbon (SOC). We chose a *Pinus tabulaeformis* plantation on the Loess Plateau where forest management practices had been conducted since 1999. Five forest management practices were implemented: two at the forest level (*P. tabulaeformis* with and without ground litter), and three using different vegetation restoration approaches after clear-cutting (*P. tabulaeformis* seedlings, abandoned grassland, and natural shrub regeneration). Microbial biomass, soil respiration, microbial community structure, microbial metabolic function, and soil oxidizable organic carbon (OC) fractions were evaluated. Forest management practices changed SOC stability by adjusting the microbial characteristics (e.g. soil microbial community diversity and microbial metabolic function diversity). The result of path analysis was that the direct path coefficient of microbial biomass on soil oxidizable OC fractions was the largest, which was 1.499. Path analysis and redundancy analysis showed that microbial biomass had the largest direct influence on soil oxidizable OC fractions. Compared with other forest management practices, natural shrub regeneration increased the nonlabile carbon fraction by increasing soil microbial characteristics, and

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contributed the most towards stabilizing SOC, which enhanced the stability of the soil ecosystem on the plateau. In conclusion, microbial biomass was the biggest influence factor of SOC stability. In contrast, the stability of SOC may be most stable in the area of natural shrub regeneration.

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1. Introduction

Forest soils contain a huge portion of the carbon (C) stock in terrestrial ecosystems; consequently, forest soil C pools are important components of the global C cycle (Pan et al., 2011). Forest management practices contribute to the process of soil organic carbon (SOC) sequestration and, hence, soil C cycles, altering the extent to which soil C accumulates (Don et al., 2011). In general, forest soil C cycles are extremely sensitive to forestry management practices (Don et al., 2011). Management practices can alter the balance between the retention and release of soil C, potentially regulating the global climate (Shao et al., 2019). While the impact of forest management practices on soil C sequestration has been extensively studied (Jandl et al., 2007), knowledge on how they impact the C cycling, like the distribution and stability of SOC fractions, remains uncertain, with inconsistent results. Studying organic carbon (OC) pools, could help elucidate the mechanisms involved in SOC turnover under different management practices (Li et al., 2013).

SOC pools include a labile C pool and a nonlabile C pool (Liu et al., 2018; Majumder et al., 2008). The labile C pool has rapid turnover rates, enhancing nutrient cycling and soil quality (Chan et al., 2001). However, nonlabile C would not be easily decomposed by soil microbes, having an important C sequestration that is important for long-term C storage (Jiang et al., 2014) and SOC stability. Previous studies have shown that soil labile OC is strongly correlated to microbial biomass (Shrestha et al., 2015). In particular, it is easily affected by ecological restoration and management measures (Biederbeck et al., 1994). Oxidizable OC is part of the labile C pool, and is more sensitive to changes to the physical and chemical properties of soil than total OC (Xu et al., 2020b). Thus, oxidizable OC could be used as an indicator of early dynamic changes to SOC (Jiang et al., 2014; Ma et al., 2018). Blair et al. (1995) offered a method to divide the amount of OC oxidized in each concentration into four fractions with different degrees of lability: very labile oxidizable carbon (C1), labile oxidizable carbon (C2), less labile oxidizable carbon (C3), and nonlabile oxidizable carbon (C4). The fractions of C1 and C2 are the most easily oxidizable fractions in the oxidizable OC, consisting mainly of polysaccharides, decayed young organic matter, fungal hyphae and other microbial products, which contribute to the utilization and availability of nutrients (Maia et al., 2007; Liu et al., 2018). The fractions of C3 and C4 are correlated with compounds of high chemical stability, and are slowly decomposed by soil microorganisms (Chan et al., 2001; Sherrod et al., 2005; Liu et al., 2018). In addition, oxidizable OC is an important indicator of soil quality, SOC pool balance, soil chemistry, and biological stability (Ma et al., 2018; McLaughlan and Hobbie, 2004). However, few studies have investigated soil oxidizable OC fractions and the stability of SOC under different management practices, despite the importance of this parameter for enhancing quantity and quality of SOC (Liu et al., 2018) and increasing C sequestration.

Soil microorganisms participate in many vital soil biochemical reactions (Yang and Zhu, 2015), serving as important mediators of C cycling in nature (Houfani et al., 2019). Microorganisms have been identified as the drivers of nutrient turnover in soil. Changes to the structure and composition of microbial communities influence essential ecosystem functions and soil nutrient cycles and turnover (Pardo et al., 2011), especially for organic matter decomposition and C cycling (Garau et al., 2019). Consequently, it is important to elucidate how management practices affect soil microbiota (Jandl et al., 2007). Various studies have shown that forest management practices increase microbial

biomass that tend to enhance C accumulation (Shan et al., 2001; Drum et al., 2019). Management practices also affect soil microbial structure (Stevenson et al., 2014), and might alter the functional role of microorganisms, which play important roles in the soil C cycle (Xu et al., 2020a). Management practices of recovery years directly and indirectly affected changes to soil microbial activity, and changes in soil microbial activity are driven by plant characteristics and soil physicochemical parameters (Xu et al., 2020c). Soil microbial biomass C, N and C/N, crude dehydrogenase, glucosidase and phosphomonoesterase activity, and total and labile C concentration and C management index across aggregate fractions significantly increased with age in tropical ecosystems (Srivastava et al., 2020). Despite recent progress in the understanding of the responses of soil microbes to soil C, the mechanism of soil microbial properties on soil C stability remains poorly understood. Therefore, understanding the relationship between soil microbial properties and oxidizable OC could help towards elucidating the mechanisms that drive SOC stability in response to management practices (Li et al., 2013).

The Loess Plateau in China is one of the most vulnerable ecosystems in the world, thus requiring sustainable management practices to maintain SOC stability. *Pinus tabulaeformis* is a common forestry species in this region. Management practices have been previously shown to affect microbial populations relevant to nutrient cycling and plant health (Shrestha et al., 2015). Thus, microbial characteristics could also help improve the state of the regional ecosystem and SOC sequestration (Wang et al., 2017; Guo and Gifford, 2002). Thus, here, we investigated how different management practices of a *Pinus tabulaeformis* plantation impact soil microbial characteristics and the stability of SOC, and how soil microbial characteristics affect SOC stability. Specifically, we explored changes to microbial biomass, microbial community structure, and microbial metabolic function. We hypothesized that: (1) microbial characteristics and the stability of SOC would vary under different forest management practices, and (2) different forest management practices would affect the soil oxidizable OC fractions and the stability of SOC due to changes in microbial characteristics (including soil microbial biomass, soil microbial community structure diversity, and microbial metabolic function diversity). The results are expected to provide baseline information on the optimal forest management practices to enhance C sequestration and mitigate climate change.

2. Materials and methods

2.1. Site description

The study area is located at Tielongwan Forest Farm (35°39' N, 110°06' E) on the eastern margin of Huanglong Mountain forest, Yichuan County, northern Shaanxi Province, China. It is a typical *Pinus tabulaeformis* forest in a loess hilly region, with an altitude of 860–1200 m and a slope of 25°. The area has a continental climate, with an average annual temperature of 9.7 °C, an average frost-free period of 180 days, and an average annual precipitation of 584.4 mm, mainly between July and September. The soil type is calcareous brown forest soil (Ustalfs, FAO) (Fig. 1).

The *P. tabulaeformis* plantation was established in 1966, and covers an area of 600 ha. The zonal vegetation is a temperate deciduous broad-leaved forest. The main tree species is *Pinus tabulaeformis*. However, other vegetation is also present, including shrubs (e.g., *Elaeagnus pungens* Thunb., *Lonicera japonica* Thunb., *Rosa xanthine* Lindl., *Spiraea salicifolia* L.) and herbs (e.g., *Carex lanceolata* Boott).

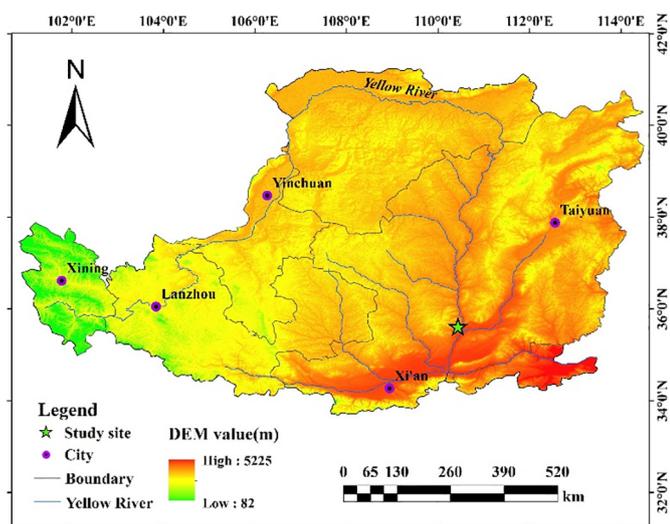


Fig. 1. Location of the study site (Tielongwan forest farm) on the Loess Plateau, China. Note: DEM, digital elevation model.

2.2. Experimental design and soil sampling

A *P. tabulaeformis* plantation was selected for this study because it had consistent basic conditions for climate, geographical location, topography, and stand density. After clear-cutting in 1999, the *P. tabulaeformis* plantation was separated into test plots of equal area delineated with wire which ensured that the environment of each plot was basically the same. Five forest management practices were implemented: two were forest-level practices (*P. tabulaeformis* without ground litter (LRL), *P. tabulaeformis* seedlings after clear-cutting (SPL), and three were different vegetation restoration practices (abandoned grassland after clear-cutting (GL) and natural shrub regeneration after clear-cutting (SL)). The artificial *P. tabulaeformis* forest without human interference was taken as the control (CK). Three plots were delineated for each treatment, each of which was $5 \times 10 \text{ m}^2$ (Table 1).

Soil samples were collected from the 0–20 cm soil layer using a multi-point method in each plot during September 2015. Soil cores with a 38-mm inner diameter were collected at a depth of 20 cm from eight randomly selected locations in each plot. Soil samples were thoroughly mixed in each layer, and were then transported to the laboratory in self-sealing bags for preservation. After removing rocks, roots, and other debris, the collected soil samples were screened in a 2-mm sieve and divided into three parts. One part was air-dried and sieved through a 0.25-mm mesh to analyze the basic physical and chemical properties of the soil and soil oxidizable OC fractions. A second part was stored at 4 °C, and was used to determine soil microbial biomass, soil respiration, and BIOLOG within two weeks. The third part was stored at –80 °C, and was used to determine phospholipid fatty acid (PLFA) content.

2.3. Analysis of soil physicochemical properties and microbial characteristics

SOC content and TN content were measured using the $\text{H}_2\text{SO}_4\text{-K}_2\text{Cr}_2\text{O}_7$ and Kjeldahl methods (Bremner and Mulvaney, 1982),

respectively. TP content was determined colorimetrically after digestion with $\text{HClO}_4\text{-H}_2\text{SO}_4$ (Schade et al., 2003). Soil $\text{NO}_3^- \text{-N}$ and $\text{NH}_4^+ \text{-N}$ content were extracted with water and determined by a flow injection autoanalyzer. Information about the soil chemical and physical properties is shown in the supplementary material (Table S1). Soil microbial biomass was measured by the chloroform fumigation extraction method (Zhu et al., 2017) and MBC was determined using a TOC analyzer (Liqui TOC II, elementar, Germany).

Basal respiration (BR) and the induced respiration (SIR) were measured by an infrared gas analyzer by a method adapted from Hueso et al. (2011). Phospholipid fatty acid (PLFA) was determined using a modified form of the Blight and Dyer method (Frostegard et al., 1993). The fatty acids were extracted from 3.0 g freeze-dried soil in a solution containing citric acid buffer, methane chloride, and methanol. Polysaccharides were isolated from neutral fatty acids and glycolipid fatty acids by solid phase extraction chromatography. The PLFAs were analyzed by a gas chromatography spectrometer (GC7890A, Agilent Technologies) provided with MIDI Sherlock software (Microbial ID, Inc., Newark, USA) after the slightly alkaline methane decomposition. External standard 19:0 methyl ester was used for quantitative analysis, and the dry soil was expressed as nmol g^{-1} .

Microbial metabolic function diversity was determined by the Biolog-ECO method (Garland and Mills, 1991; Zheng et al., 2005). Ten grams of fresh soil was placed in a 250 mL flask, and 90 mL of 85% NaCl solution was added. The mixture was shaken at $200 \text{ r}\cdot\text{min}^{-1}$ for 30 min at 25 °C, and the supernatant was removed. The solution was added to 9 mL of NaCl solution and diluted at a ratio of 1:1000. Then, 150 μL of the dilution was added to each well of a Biolog-ECO microplate, which was then inoculated for 240 h at 25 °C. The absorption value at 590 nm wavelength was read on the microporous plate reading instrument every 24 h.

2.4. Analysis of soil oxidizable OC fractions

Soil oxidizable OC fractions were determined by the Walkley and Black method (Blair et al., 1995; Chan et al., 2001). The content of easily oxidized OC in soil was determined by the oxidation methods of 333, 167, and 33 $\text{mmol}\cdot\text{L}^{-1} \text{KMnO}_4$, respectively (Wang et al., 2017). The soil oxidizable OC fractions was divided into four parts, including very labile fraction of oxidizable carbon (C1), labile fraction of oxidizable carbon (C2), less labile fraction of oxidizable carbon (C3), and nonlabile fraction of oxidizable carbon (C4).

2.5. Calculation of associations among parameters

The metabolite quotient ($q\text{CO}_2$) is the ratio of basic respiratory intensity to microbial biomass carbon, and is a sensitivity index of factors influencing microbial activities, such as environmental factors and changes to management practices.

$$q\text{CO}_2 = \text{BR} : \text{MBC} \quad (1)$$

In general, average color change rate (AWCD) is used to indicate the ability of a microbial community to utilize a single C source. This is an

Table 1
Basic information of sample plots.

| Management practices | Biomass ($\text{t}\cdot\text{hm}^{-2}$) | Sample number | Vegetations | Coverage(%) | Soil type |
|----------------------|---|---------------|--|-------------|------------------------------|
| SPL | 112.96 | 3 | <i>Pinus tabulaeformis</i> | 51 | Calcareous brown forest soil |
| GL | 8.28 | 3 | <i>Carex lanceolata</i> | 40 | Calcareous brown forest soil |
| SL | 3.56 | 3 | <i>Elaeagnus pungens</i> · <i>Spirea salicifolia</i> | 30 | Calcareous brown forest soil |

Notes: SPL, *P. tabulaeformis* seedlings after clear-cutting; GL, abandoned grassland after clear-cutting; SL, natural shrub regeneration after clear-cutting.

important indicator for describing the functional diversity of microbial metabolism.

$$AWCD = \sum_{i=1}^{31} (Ci - R) / n \quad (2)$$

where C_i is the optical density value of the first C source hole, R is the optical density value of the control hole, and n is the number of C sources, which is 31.

The diversities of PLFA and BIOLOG were calculated using the Shannon richness index (H), and the equitability of PLFA and BIOLOG were calculated with the Pielou evenness index (E).

$$H = -\sum_{i=1}^n Pi \ln Pi \quad (3)$$

$$E = H / \ln S \quad (4)$$

where P_i is the relative abundance of each fatty acid in all PLFAs or the relative abundance of each BIOLOG in the total BIOLOG, n is the number of fatty acids or BIOLOGs detected, and S is the total number of fatty acids or the total number of BIOLOGs tested in the community.

$$\text{Carbon Preference Index (CPI)} = \text{SOC} / \text{C}_{\text{CK}} \quad (5)$$

$$\text{Lability (L)} = \text{liable carbon content} / \text{nonlabile carbon content} \quad (6)$$

$$\text{Lability Index (LI)} = L / L_{\text{CK}} \quad (7)$$

$$\text{Carbon Management Index (CMI)} = \text{CPI} \times \text{LI} \times 100 \quad (8)$$

2.6. Statistical analysis

Pearson correlation analysis was used to determine the relationship between the environmental factors and diversity indices. Cluster analysis was used to evaluate the differences between different forest management practices, using the oxidizable OC fractions and SOC as variables. Excel 2013 software was used for the statistical analysis of the data. Variance analysis and multiple comparison of various indicators of different management practices were conducted by using a one-way ANOVA and Duncan method in SPSS 24.0 software ($P < 0.05$). All bar graphs were drawn in Origin 9.4. Both path analysis and Pearson correlation analysis were performed using SPSS 24.0. Redundancy analysis (RDA) was completed in CANOCO 5.0.

3. Results

3.1. Changes to soil microbial characteristics

BR and SIR were both highest in SL, with SIR in SL being significantly higher than that in all other forest management practices (Table 2). BR was significantly higher in SL compared to LRL, and did not differ significantly between SPL, CK, and GL. The lowest BR was recorded in LRL, while the lowest SIR was recorded in GL. SIR was significantly higher in SPL

Table 3

Changes in Shannon richness index (H_{PLFA} , H_{BIOLOG}) and Pielou evenness index (E_{PLFA} , E_{BIOLOG}) in the treatments.

| Treatments | H_{PLFA} | E_{PLFA} | H_{BIOLOG} | E_{BIOLOG} |
|------------|-------------------|-------------------|---------------------|---------------------|
| CK | 3.12 ± 0.15a | 0.93 ± 0a | 2.75 ± 0.03b | 0.82 ± 0.01b |
| LRL | 2.97 ± 0.02a | 0.92 ± 0.01ab | 2.75 ± 0.04b | 0.81 ± 0.01b |
| SPL | 2.93 ± 0.12ab | 0.95 ± 0.03a | 2.97 ± 0.04a | 0.88 ± 0.01a |
| GL | 2.69 ± 0.25b | 0.88 ± 0.04b | 2.77 ± 0.15b | 0.83 ± 0.05b |
| SL | 2.95 ± 0.04ab | 0.91 ± 0.03ab | 3.00 ± 0.04a | 0.88 ± 0.02a |

Notes: The results are reported as means ± standard deviation (SD). Different letters indicate significant differences at $P < 0.05$ level. CK, the artificial *P. tabulaeformis* forest without human interference was taken as the control; LRL, *P. tabulaeformis* without ground litter; SPL, *P. tabulaeformis* seedlings after clear-cutting; GL, abandoned grassland after clear-cutting; SL, natural shrub regeneration after clear-cutting; H_{PLFA} , Shannon-Weiner diversity index of the fatty acids; E_{PLFA} , Pielou evenness index of the fatty acids; H_{BIOLOG} , Shannon-Weiner diversity index of BIOLOG; E_{BIOLOG} , Pielou evenness index of BIOLOG.

compared to LRL and GL, and did not differ significantly between SPL and CK.

The Shannon Wiener diversity index (H_{BIOLOG}) and Pielou evenness index (E_{BIOLOG}) of BIOLOG were significantly different (Table 3). H_{BIOLOG} and E_{BIOLOG} were ordered across the treatments as: SL, SPL > GL, CK, LRL. The highest H_{BIOLOG} and E_{BIOLOG} were associated with SL, whereas the lowest values were associated with LRL. Thus, SPL and SL clearly affected soil microbial diversity, whereas no significant differences were detected in GL, CK, and LRL.

Compared with the other management practices, the H_{PLFA} was significantly higher for CK and LPL compared to GL, and did not differ significantly between CK and LRL (Table 3). E_{PLFA} was highest in SPL, and lowest GL. E_{PLFA} was significantly lower in GL compared to CK and SPL. There was no significant difference between H_{PLFA} in SPL and SL and the E_{PLFA} in LRL and SL.

3.2. Changes to soil oxidizable OC fractions and the stability of SOC

The four C fractions changed differently under the different management practices (Table 4). The C1 of the SL was significantly lower than that in other forest management practices. In contrast, the C4 of SL was significantly higher than that of other forest management practices. C2 was highest in GL and lowest in CK. C3 content changed in the order of: SPL, CK, GL, LRL, and SL. LI and CPI had different effects under different management practices. In comparison, L, LI, and CMI exhibited similar trends (Table 4), all being highest in GL and lowest in SL. CPI was significantly higher in CK and SL compared to LRL and GL; however, CK and SL were not significantly different. Cluster analysis delineated two major groups containing: (1) CK and SL; and (2) LRL, SPL, and GL (Fig. 2).

3.3. Relationship between soil microbiological properties and the stability of SOC

To determine how soil microbial properties influence the stability of SOC, various soil microbial properties [SIR(X1), BR(X2), H_{PLFA} (X3), E_{PLFA} (X4), H_{BIOLOG} (X5), E_{BIOLOG} (X6), and MBC (X7)] were analyzed with soil carbon-related indices [CPI (Y1), LI (Y2), and CMI (Y3)] by stepwise

Table 2

Microbial biomass and soil respiration of the soils under different forest management practices.

| Treatments | MBC (mg kg ⁻¹) | BR (mg kg ⁻¹ h ⁻¹) | SIR (mg kg ⁻¹ h ⁻¹) | qCO ₂ (10 ³ h ⁻¹) |
|------------|----------------------------|---|--|---|
| CK | 86.19 ± 8.30bc | 1.05 ± 0.23ab | 4.45 ± 0.15bc | 12.45 ± 3.94a |
| LRL | 72.48 ± 16.41c | 0.91 ± 0.15b | 3.84 ± 0.46c | 12.81 ± 2.66a |
| SPL | 107.48 ± 8.98b | 1.11 ± 0.17ab | 4.88 ± 0.87b | 10.43 ± 2.19ab |
| GL | 85.86 ± 8.51bc | 0.97 ± 0.05ab | 3.66 ± 0.35c | 11.42 ± 1.37ab |
| SL | 160.41 ± 27.04a | 1.22 ± 0.13a | 5.84 ± 0.08a | 7.72 ± 1.05b |

Notes: The results are reported as means ± standard deviation (SD). Different letters indicate significant differences at $P < 0.05$ level. CK, the artificial *P. tabulaeformis* forest without human interference was taken as the control; LRL, *P. tabulaeformis* without ground litter; SPL, *P. tabulaeformis* seedlings after clear-cutting; GL, abandoned grassland after clear-cutting; SL, natural shrub regeneration after clear-cutting; MBC, microbial biomass carbon; BR, basal respiration; SIR, the induced respiration; qCO₂, metabolic quotient.

Table 4
Soil oxidizable OC fractions and soil carbon management index in the treatments.

| Treatments | C1 (mg/g) | C2 (mg/g) | C3 (mg/g) | C4 (mg/g) | CPI | L | LI | CMI |
|------------|--------------|--------------|----------------|---------------|---------------|--------------|--------------|----------------|
| CK | 1.12 ± 0.09a | 0.19 ± 0.09b | 0.65 ± 0.2ab | 13.37 ± 0.47b | 1 ± 0.02ab | 0.15 ± 0.02c | 1 ± 0.15c | 99.85 ± 13.18c |
| LRL | 1.32 ± 0.16a | 0.7 ± 0.1a | 0.32 ± 0.22bc | 11.33 ± 0.9c | 0.89 ± 0.06c | 0.22 ± 0.02b | 1.4 ± 0.15b | 124.54 ± 6.54b |
| SPL | 1.1 ± 0.06a | 0.44 ± 0.03b | 0.72 ± 0.11a | 11.71 ± 0.89c | 0.91 ± 0.06bc | 0.19 ± 0.02b | 1.31 ± 0.11b | 118.94 ± 3.09b |
| GL | 1.26 ± 0.17a | 0.82 ± 0.3a | 0.52 ± 0.29abc | 10.44 ± 0.73c | 0.85 ± 0.05c | 0.25 ± 0.01a | 1.69 ± 0.09a | 143.76 ± 1.23a |
| SL | 0.76 ± 0.23b | 1 ± 0.21a | 0.2 ± 0.02c | 14.86 ± 0.89a | 1.09 ± 0.07a | 0.12 ± 0.01c | 0.84 ± 0.04c | 91.53 ± 9.78c |

Note: The results are reported as means ± standard deviation (SD). Different letters indicate significant differences at $P < 0.05$ level. CK, the artificial *P. tabulaeformis* forest without human interference was taken as the control; LRL, *P. tabulaeformis* without ground litter; SPL, *P. tabulaeformis* seedlings after clear-cutting; GL, abandoned grassland after clear-cutting; SL, natural shrub regeneration after clear-cutting; C1, very labile fraction of oxidizable carbon; C2, labile fraction of oxidizable carbon; C3, less labile fraction of oxidizable carbon; C4, nonlabile fraction of oxidizable carbon; CPI, Carbon Preference Index; L, Lability; LI, Lability Index; CMI, Carbon Management Index.

regression (Table 5). Path analysis was used to determine the absolute value of direct action, with soil microbial properties on CPI being ordered as: $X7 > X5 > X6$. The direct path coefficient of X7 on CPI was 1.499, which was the maximum value. This result indicated that X7 was directly responsible for CPI in soil microbial properties, and that X7 had a greater indirect effect on CPI through X5 and X6. X7 and X5 directly affected LI, and had greater indirect effects on X6. Soil microbial properties affecting CMI were ordered: $X3, X1, X7 > X4, X6, X5 > X2$; thus, X3, X1 and X7 were likely directly responsible for the SOC pool management index in soil microbial properties.

RDA showed a positive correlation for C2 and C4 with SOC. C4 had a strong mutual influence with SOC (Fig. 3). Yet, C1 and C3 were negatively correlated with SOC. SOC and C4 were positively correlated with MBC, MBN, MBP, BR, SIR, H_{PLFA} , and H_{BIOLOG} , and were negatively correlated with qCO_2 . Out of these parameters, C4 and microbial biomass (MBC, MBN, MBP) had the greatest influence.

4. Discussion

4.1. Effects of management practices on microbiological properties

Our study showed that the type of management practice used significantly impacts microbial biomass, soil respiration, soil microbial

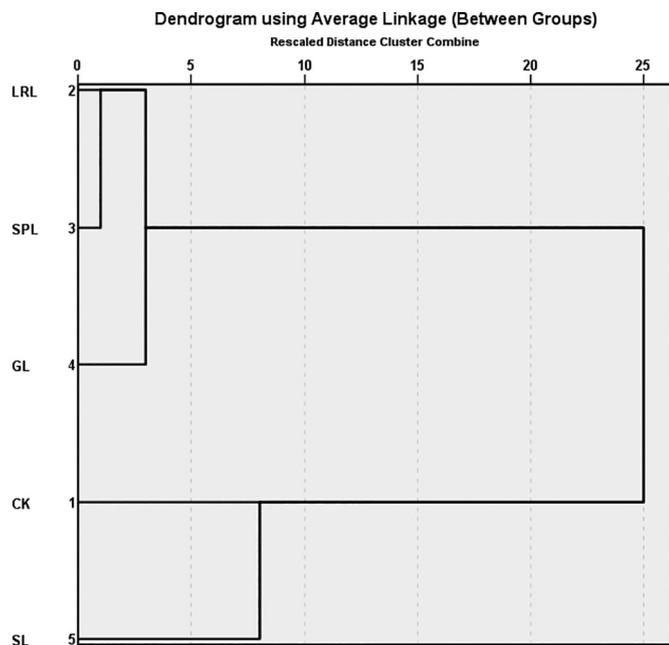


Fig. 2. Cluster analysis for different management practices with the soil organic carbon (SOC) and oxidizable organic carbon (OC) fractions. Notes: CK, the artificial *P. tabulaeformis* forest without human interference was taken as the control; LRL, *P. tabulaeformis* without ground litter; SPL, *P. tabulaeformis* seedlings after clear-cutting; GL, abandoned grassland after clear-cutting; SL, natural shrub regeneration after clear-cutting.

community diversity, and microbial metabolic function diversity. Previous studies also demonstrated that management practices are important factors influencing the soil microbial community (Galazka et al., 2017). Our study showed that MBC differed for LRL compared to SPL and SL. Compared with CK, natural shrub regeneration and *P. tabulaeformis* seedlings generate more litter on the soil surface containing a variety of substrates. These substrates cause the belowground biomass and root secretion to increase, promoting the decomposition of microorganisms in the soil (Zhang et al., 2017) and resulting in high microbial biomass. Furthermore, the removal of litter would reduce C input to this area (Traore et al., 2007). Snajdr et al. (2011) showed that the presence of shrubs in plantations significantly increased C accumulation and biomass in the organic layer C, which was supported by our study.

Our study also showed that shrubs increase soil respiration and decrease qCO_2 (Drum et al., 2019). Xu and Qi (2001) showed that the intensity of soil respiration was positively correlated with soil microbial biomass (Fig. 3). Changes to microbial biomass cause changes to respiration (Zhang et al., 2017). In general, high efficiency of qCO_2 resulted in less CO_2 exhaled per unit microbial mass and less qCO_2 . The low efficiency of qCO_2 indicated that the amount of microorganisms formed by using the same energy is small, while qCO_2 is large, releasing more CO_2 , and the turnover rate of microorganisms is fast. The rate of soil microbial respiration is relatively high, whereas the proportion of microbial respiration is relatively small. Thus, this phenomenon ensures that the soil has higher metabolic efficiency and, consequently, sufficient active organic matter. It is beneficial to enhance the quality of the soil and to maintain sustainable utilization potential (Mao et al., 1992).

The current study showed that the AWCD of all C sources was significantly higher in SL compared to other management practices after 240 h (Fig. S1). Under different management practices, the values obtained for AWCD, H_{BIOLOG} , and E_{BIOLOG} showed that soil microbial communities were characterized by different catabolic potentials and versatility; thus, the same microbial community utilized C substrate differently depending on the management practice implemented (Garau et al., 2019). C source utilization was positively correlated with soil microbial biomass, positively impacting the proliferation and activity of microorganisms. In addition, the higher the metabolic potential and catabolic diversity, the faster and more effective the turnover of organic matter, which significantly increases the metabolic activity of microorganisms (Garau et al., 2019; Jastrow et al., 2007). In particular, microbial metabolic function diversity significantly increased in SL through changes to the microbiomes (Huang et al., 2019). Our results supported this phenomenon.

Our study showed that total PLFAs, G^- -PLFAs, G^+ -PLFAs, fungal PLFAs, and actinomycetic PLFAs were significantly affected by forest management practices, with the highest values being recorded for SL, except for actinomycetic PLFAs (Table S2). CK also significantly increased soil microbial community diversity, whereas GL reduced it (Table S2). Shao et al. (2019) found that SOC storage was positively correlated with total microbial lipids. The increase in microbial community structure diversity might be related to the higher content of total SOC in CK

Table 5
Path coefficients and correlation coefficients between soil microbiological properties and soil carbon management index.

| | | Direct | | Indirect | | | | | | | Correlation |
|----|----|--------|--------|----------|--------|--------|--------|--------|--------|----------|-------------|
| | | | | X1 | X2 | X3 | X4 | X5 | X6 | X7 | |
| Y1 | X1 | 0.271 | - | - | -0.320 | -0.012 | 0.055 | -0.781 | 0.394 | 0.848 | 0.455 |
| | X2 | -0.43 | 0.202 | - | - | 0.065 | 0.124 | -0.900 | 0.436 | 1.201 | 0.698** |
| | X3 | 0.265 | -0.012 | -0.105 | - | - | 0.325 | -0.248 | 0.118 | 0.019 | 0.362 |
| | X4 | 0.51 | 0.0298 | -0.104 | 0.169 | - | - | -0.654 | 0.253 | -0.210 | -0.008 |
| | X5 | -1.431 | 0.148 | -0.270 | 0.046 | 0.233 | - | - | 0.669 | 0.959 | 0.354 |
| | X6 | 0.712 | 0.150 | -0.264 | 0.044 | 0.182 | -1.345 | - | - | 0.899 | 0.377 |
| | X7 | 1.499 | 0.153 | -0.344 | 0.003 | -0.071 | -0.916 | 0.427 | - | - | 0.751** |
| Y2 | X1 | -0.392 | - | 0.113 | 0.018 | -0.044 | 0.479 | -0.194 | -0.511 | -0.511 | -0.532* |
| | X2 | 0.152 | -0.292 | - | -0.096 | -0.100 | 0.552 | -0.215 | -0.723 | -0.723** | -0.723** |
| | X3 | -0.394 | 0.018 | 0.037 | - | -0.261 | 0.152 | -0.058 | -0.012 | -0.012 | -0.518 |
| | X4 | -0.41 | -0.042 | 0.037 | -0.251 | - | 0.401 | -0.125 | 0.126 | 0.126 | -0.263 |
| | X5 | 0.877 | -0.214 | 0.096 | -0.068 | -0.187 | - | -0.329 | -0.578 | -0.578 | -0.404 |
| | X6 | -0.35 | -0.217 | 0.093 | -0.065 | -0.146 | 0.824 | - | -0.542 | -0.542 | -0.402 |
| | X7 | -0.903 | -0.222 | 0.122 | -0.005 | 0.057 | 0.561 | -0.21 | - | - | -0.6* |
| Y3 | X1 | -0.445 | - | -0.048 | 0.021 | -0.018 | 0.067 | 0.086 | -0.228 | -0.228 | -0.565* |
| | X2 | -0.065 | -0.332 | - | -0.114 | -0.040 | 0.077 | 0.096 | -0.322 | -0.322 | -0.701** |
| | X3 | -0.468 | 0.020 | -0.016 | - | -0.105 | 0.021 | 0.026 | -0.005 | -0.005 | -0.527* |
| | X4 | -0.165 | -0.048 | -0.016 | -0.298 | - | 0.056 | 0.056 | 0.056 | 0.056 | -0.36 |
| | X5 | 0.122 | -0.243 | -0.041 | -0.081 | -0.075 | - | 0.147 | -0.257 | -0.257 | -0.429 |
| | X6 | 0.156 | -0.246 | -0.040 | -0.078 | -0.059 | 0.115 | - | -0.241 | -0.241 | -0.394 |
| | X7 | -0.402 | -0.252 | -0.052 | -0.006 | 0.023 | 0.078 | 0.094 | - | - | -0.518* |

Notes: X1, represents SIR, the induced respiration; X2, represents BR, basal respiration; X3, represents H_{PLFA} , Shannon-Weiner diversity index of the fatty acids; X4, represents E_{PLFA} , Pielou evenness index of the fatty acids; X5, represents H_{BIOLOG} , Shannon-Weiner diversity index of BIOLOG; X6, represents E_{BIOLOG} , Pielou evenness index of BIOLOG; X7, represents MBC, microbial biomass carbon; Y1, represents CPI, Carbon Preference Index; Y2, represents LI, Lability Index; Y3, represents CMI, Carbon Management Index.

and SL (Bastida et al., 2019). Increases in biomass are often associated with diverse microbial substrates (Ghimire et al., 2019). Increased diversity in microbial community structure might be attributed to a greater availability of substrate for microbial growth. Thus, management practices likely influence the structure of the soil microbial community (Das and Varma, 2011; Zhou et al., 2017), and might change the functional role of microorganisms in soil C cycling.

4.2. Effects management practices on soil oxidizable OC fractions and the stability of SOC

This study showed that C4 increased more in SL compared to CK, whereas C4 declined in GL, LRL, and SPL. This difference might reflect the higher input of aboveground litter in natural shrub regeneration compared to abandoned grassland and *P. tabulaeformis* seedlings (Kurganova et al., 2019). Compared to steppe litter, forest litter contains more lignin and lipids (stable components) and less carbohydrates (Kogel-Knabner, 2000; Bonanomi et al., 2013; Guo et al., 2006), resulting in the quality of aboveground litter differing. Removing litter and *P. tabulaeformis* seedlings lowers litter levels, reducing C input.

Previous studies showed that higher L and LI increase the lability of the SOC pool, increasing its vulnerability to decomposition and transformation (Liu et al., 2013). In this study, C4 was highest in SL, and L and LI were lowest in SL. Thus, shrubs appear to stabilize the SOC pool. Ma et al. (2018) showed that forest management practices, such as thinning, strongly influence SOC sequestration. Forest soils typically have high levels of SOM (Guo et al., 2006; Marti-Roura et al., 2019). Previous studies also showed that the type of forest management practice implemented influences both the concentration and storage of SOC (Jandl et al., 2007; Li et al., 2013). For instance, SOM is higher in thinned plots of *P. tabulaeformis* plantations (Dang et al., 2018), in turn, enhancing microbial biomass (Kim et al., 2018), which was similar to the results of our study. Thus, reasonable management practices could promote the accumulation of nonlabile C and enhance SOC storage and conversion.

4.3. Effects of microbiological properties on the stability of SOC

Soil microbes regulated how SOC was sequestered and stored under different management practices. The two analytical methods of path analysis and redundancy analysis used here showed that microorganisms were an important and direct factor influencing the stability of SOC. Different management practices influence soil microbial processes by changing various microbial characteristics, including microbiological activity, soil microbial community structure, and microbial metabolic function. Some studies showed that changes to soil oxidizable OC fractions are related to soil microbes (Yuan et al., 2018). Microorganisms

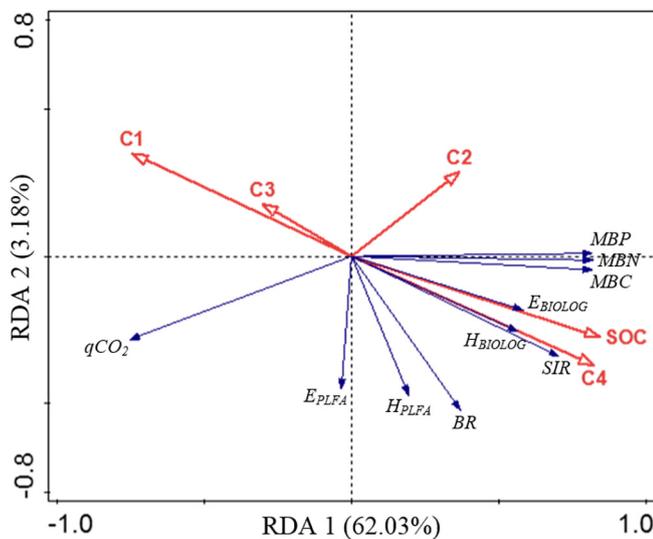


Fig. 3. Relationship of microbial characteristics and soil carbon fraction based on redundancy analysis (RDA). Notes: the red arrows represent the species variables; C1, very labile fraction of oxidizable carbon; C2, labile fraction of oxidizable carbon; C3, less labile fraction of oxidizable carbon; C4, nonlabile fraction of oxidizable carbon; SOC, soil organic carbon; H_{PLFA} , Shannon-Weiner diversity index of the fatty acids; E_{PLFA} , Pielou evenness index of the fatty acids; H_{BIOLOG} , Shannon-Weiner diversity index of BIOLOG; E_{BIOLOG} , Pielou evenness index of BIOLOG; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; MBP, microbial biomass phosphorus; BR, basal respiration; SIR, the induced respiration; qCO_2 , metabolic quotient.

mediate many processes, including SOM cycling and C sequestration (Clemmensen et al., 2013; Tian et al., 2016). Shao et al. (2019) showed that microbial communities facilitate the formation and retention of SOM belowground, with microorganisms being important for immobilizing C through the decomposition and turnover of SOM (McGuire and Treseder, 2010). This phenomenon occurs because soil carbon mainly originates from litter and root exudate, and is offset by the assimilation, dissimilation, respiration, and decomposition of microbes. Moreover, the increase of labile OC in the surface soil promotes the accumulation of OC (Huang et al., 2019).

The soil surface of *P. tabulaeformis* and shrub is rich in litter, with C from litter and humus accounting for a large proportion of DOM (Kooch et al., 2019). Litters increased plant and root C input, improved microbial activity and microbial biomass, leading to increased soil organic matter decomposition. After thinning, the decomposition rate of shrubs might increase, enhancing SOM content (He and Barclay, 2000). When C input increases, microbial activity and microbial biomass also increase, enhancing the storage and conversion capacity of SOC (Leon-Sanchez et al., 2016). Higher microbial biomass results in more substrate being available for microbial growth, which enhances the structure of the soil microbial community, altering labile C pools in surface soils (DuPont et al., 2010). Therefore, compared with CK, the management practice of SL might enhance carbon fixation, even though this effect was not significant.

5. Conclusions

The current study demonstrated that different management practices affect the stability of SOC differently. Microbial biomass was the major factor driving the stability of SOC, with the type of forest management practice significantly influencing microbial characteristics. Compared with CK, the microbial characteristics of *P. tabulaeformis* without ground litter, *P. tabulaeformis* seedlings, and abandoned grassland were not significantly different. Compared with CK, the forest management practice of natural shrub regeneration increased the nonlabile fraction of oxidizable carbon, enhanced carbon fixation and maintained the stability of SOC pools, even though this effect was not significant. This study confirmed that forest management practices alter microbial characteristics, which regulate soil oxidizable OC fractions and the stability of SOC. These findings have important implications for forest management, advancing our understanding on how soil oxidizable OC fractions and the stability of SOC pool alter in *P. tabulaeformis* plantations under different management practices. In conclusion, adopting sustainable and effective management practices could increase the stability of SOC pool and facilitate the ecological restoration of the Loess Plateau.

CRedit authorship contribution statement

Yahui Song: Writing – original draft, Writing - review & editing. **Jiaying Zhai:** Investigation. **Jiaoyang Zhang:** Formal analysis, Data curation. **Leilei Qiao:** Software, Visualization. **Guoliang Wang:** Methodology, Validation. **Lihui Ma:** Supervision, Project administration, Funding acquisition. **Sha Xue:** Conceptualization, Resources.

Declaration of competing interest

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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Appendix A. Supplementary data

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