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Effects of Soil Microbiological Properties on the Fractional Distribution and Stability of Soil Organic Carbon under Different N Addition Treatments

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Abstract: Soil organic carbon (SOC) fractions are influenced by inputs of nitrogen (N) from globally rising N deposition; however, the mechanisms of how soil microbiological properties are influenced by N deposition and its impact on the fractional distribution and stability of SOC remain unclear. In this study, we assessed the effects on SOC fraction distribution and stability from four aspects of soil microbiological properties: soil microbial biomass (SMB), soil microbial activity, structure diversity, and functional diversity of soil microbial community in a *Pinus tabuliformis* plantation, which received four N addition levels (0 g N m^{-2} y^{-1} (N0), 3 g N m^{-2} y^{-1} (N3, low N addition), 6 g N m^{-2} y^{-1} (N6, mid-N addition), and 9 g N m^{-2} y^{-1} (N9, high N addition)) for 2 years. The N inputs did significantly affect some soil microbiological properties, like SMB, soil phospholipid fatty acid (PLFA), and soil microbial functional diversity. Mid- and high N addition decreased the richness (H_{PLFA}) and evenness (E_{PLFA}) index of the soil microbial community, from 3.24 to 2.91 and 0.93 to 0.87, respectively. In addition, the low N addition promoted the carbon management index (CMI) to 141.35, i.e., higher than the CMIs in the mid- and high-level treatments. The SOC stability also showed significant differences among N addition treatments, and SOC could be the most stable at the mid-N addition level. Regarding the effects of the four soil microbiological attributes on the CMI and stability, SMB and soil respiration positively impacted the CMI, but did not significantly affect the stability. In addition, E_{PLFA} had positive effects, but E_{BIOLOG} had negative effects on CMI and lability. Our findings indicate that soil microbiological properties are essential in SOC fractional distributions and stability. Further identification and study of soil microbial species used to change SOC fractions would help to clarify the detailed mechanisms involved.

Keywords: nitrogen deposition; microbiological properties; SOC stability; SOC fractions

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1. Introduction

Soil ecosystems are the largest carbon sinks on land, and have the potential to enhance carbon sequestration and offset the release of fossil fuels [1], which is essential in soil carbon storage and sequestration [2]. Small fluctuations in the equilibrium of import and export of this huge carbon pool will have a significant source–sink effect on the atmospheric carbon pool [2–4]. Therefore, increasing the sequestration potential, quality, and stability of soil organic carbon (SOC) has been widely recognized to alleviate the pressure from global climate change as well as ensure the sustainable development of agriculture [1], which

will also help us achieve the carbon peak and neutrality targets. However, SOC is very susceptive to global climate change and regional environmental change, like N deposition, due to a close coupling linkage between the C and N cycles of ecosystem changed by N addition. For example, N addition may increase plant and microbial demands for soil sources, like C, N, P, and S, before N saturation, thus promoting soil element cycles [5,6]. As we know, global anthropogenic nitrogen (N) contents increased tenfold from the 1860s to the 1990s, with contents ranging from 15 Tg N y^{-1} to 156 Tg N y^{-1}, and is anticipated to achieve 270 Tg N y^{-1} in 2050 [7]. The deposition of N caused by the burning of fossil fuels and the production and use of chemical fertilizers, as a main contributor to the worldwide enhancement in N inputs, is gradually impacting the turnover of SOC [1,8]. Therefore, the key scientific problem to be solved is to understand how N addition would affect the sequestration potential, quality, and stability of SOC and mechanisms under N addition.

SOC was divided into four separate fractions: very labile organic C (OC), labile OC, less labile OC, and non-labile OC [9], which have different chemical stability and turnover rates [10]. Soil labile OC pools, including very labile OC, labile OC, and less labile OC, as the energy sources for soil food webs, had relatively rapid turnover rates [9]. Therefore, they have been considered to be an early sensitivity index to the impacts of environmental changes on the dynamics of the SOC [11,12]. Although the non-labile OC pool had a lower turnover rate and was relatively stable and slowly altered by microbial activity [13], it is still essential in C sequestration to relieve climate change [14]. Some studies illustrated the impacts of N addition on SOC fractions [10,15]. Chen et al. [10] revealed that both soil labile OC pools as well as non-readily oxidizable OC contents went up with N addition. In contrast, Wang et al. [15] found N addition did not alter the soil labile OC content. However, these results were not consistent. Therefore, the C management index (CMI), as a sensitive indicator of the renewal degree and the change in the quality of the C pool [16] and lability as a stability indicator of SOC [17] under N addition, still need to be further explored.

Soil microbes are closely correlated with changes in SOC fractions, thus influencing the stability of SOC fractions and the CMI. In ecosystems, the factors affecting SOC fraction contents could include the inputs of plant litter, root exudates, and the offsetting of organic C inputs [11,18], which was put forward with the theory "microbial carbon pump, MCP". In this theory, one method of microbial control of soil carbon could be called "microbial ex vivo modification", which means the decomposition and transformation of organic C as mediated by soil microbes (soil microbial respiration and enzymatic activities) [18,19]. The other method could be "microbial in vivo turnover", which consists of the soil microbial assimilation of small molecule plant-derived carbon substrates into soil microbial biomasses [19,20], i.e., a contributor of soil labile OC. Moreover, the changes in the structure and functional diversity of the soil microbial community would also influence the decomposition ability of soil microbes due to the different C metabolism capacities of individual microorganisms. Some studies have revealed the effect of SMB [21] and soil enzymatic activity [22], as well as soil respiration [23], on the distribution and stability of SOC fractions. However, mass previous studies mainly concentrated on separate aspects of soil microbial control on the stability of SOC fractions and the CMI, which is too one-sided for us to fully understand the mechanism. Further, some studies concentrated on the impacts of the increasing N inputs on soil microbial community and SOC in terrestrial ecosystems [24,25]. Fang et al. [21] found N addition can influence the SOC by decreasing the microbial biomass C via "microbial ex vivo modification". Hok et al. [26] revealed that soil enzymatic activity via "microbial in vivo modification" had a closely positive relationship with soil labile OC. However, the mechanism of how microbes could have an impact on the quality and stability of SOC under N addition has not been fully illustrated based on the MCP theory.

To investigate how N addition influences the CMI and stability of SOC via soil microbial control from a comprehensive viewpoint, we, therefore, conducted an N addition experiment in the *Pinus tabuliformis* plantation in Yichuan County. Previously, this place was in a state of N deficiency but has recently received gradually increasing N deposition

from 16.12 kg ha^{-1} in 2010 [27] to 28.89 kg ha^{-1} in 2014 [28] in Shaanxi province. We hypothesized that: (1) the soil microbiological properties would vary along the N-addition gradient; (2) the SOC fractions and CMI and SOC stability would also vary along the N-addition gradient; and (3) N addition would affect the CMI and stability of SOC based on aspects of the MCP theory, such as SMB, soil microbial activity, structure diversity, and functional diversity of the soil microbial community.

2. Materials and Methods

2.1. Site Description

We carried out this N addition experiment at the TIELONGWAN plantation (36°04′ N, 110°15′ E; 860–1200 m a.s.l.), Yichuan county, Shaanxi province, China. This area has a continental climate, with a mean annual precipitation and temperature of 584.4 mm and 9.7 °C, respectively. In addition, the zonal vegetation is temperate deciduous broad-leaved forests. The soil type is a gray forest soil (Gray Luvisol, FAO soil classification) with a landscape containing rolling hills with slopes ranging from 20° to 25°. The artificial *P. tabuliformis* forest in our experiment was established in 1966, with an area of 600 ha. The main trees are *P. tabuliformis* and some other shrubs and herbaceous plants are included. The climatic, soil, and vegetation conditions are also described by Zhang et al. [29].

2.2. Experimental Design and Soil Sampling

This experiment was designed as a completely random experiment. Each plot was established with an area of 10 × 10 m^2, with a 5 m buffer zone separating the plots. This experiment contained four N addition treatments: 0 g N m^{-2} y^{-1} (N0), 3 g N m^{-2} y^{-1} (N3, low N addition), 6 g N m^{-2} y^{-1} (N6, mid-N addition), and 9 g N m^{-2} y^{-1} (N9, high N addition), which were set based on the amount of global N deposition [24]. Each treatment had four replicates. N was added with urea ($CO(NH_2)_2$) in April, June, August, and October, four times a year from 2014. Treated plots (N3, N6, and N9) were added with urea solution one day before a rain in case of ammonia volatilization. CK was added using the same volume of water without urea.

Soil cores were collected from the 0–20 cm layer via random sampling from the soil plots included in the five treatments in September 2015 with 2 years of N addition. The collected soil cores from each plot were mixed as a soil sample. After the manual removal of roots and stones, every soil sample was sieved through a 2 mm mesh sieve. After sieving, each sample was separated into three subsamples, the first subsamples were air-dried and then passed through a 0.25 mm mesh sieve. This subsample was used for determining the soil physicochemical properties and soil sucrase, soil cellulase, soil urease, and soil carbon fractions. The second subsamples were stored at 4 °C for later determination of soil microbial biomass, soil respiration, and Biolog analysis. The third ones were stored at −80 °C for soil enzymatic activities and soil phospholipid fatty acid (PLFA).

2.3. Analyses of Soil Chemical Properties

Soil chemical properties were measured using standard procedures. The SOC was measured using the H_2SO_4-$K_2Cr_2O_7$ method. The TN was measured using the Kjeldahl method [30]. The soil TP was determined calorimetrically after digestion with H_2SO_4 and $HClO_4$ [31]. The soil NH_4^+-N and NO_3^--N in filtered 2.0 mol L^{-1} extracts of fresh soil sample were measured with a flow injection autoanalyzer. The soil pH was determined in 1:2.5 (*w*:*v*) solutions. The soil aP was measured via molybdenum–antimony colorimetry with $Na(HCO_3)_2$ extracts. The soil chemical properties are described in detail in the Supplementary Materials (Table S1).

2.4. Analyses of Soil Microbiological Properties

Soil microbiological properties were divided into four groups: SMB (Figure 1); microbial activity, including extracellular enzyme activities (EEA) and soil respiration (Figure 2); microbial community structure with PLFA; and soil microbial metabolic diversity with the

Biolog technique, which were measured with relevant methods and are described in the Supplementary Materials.

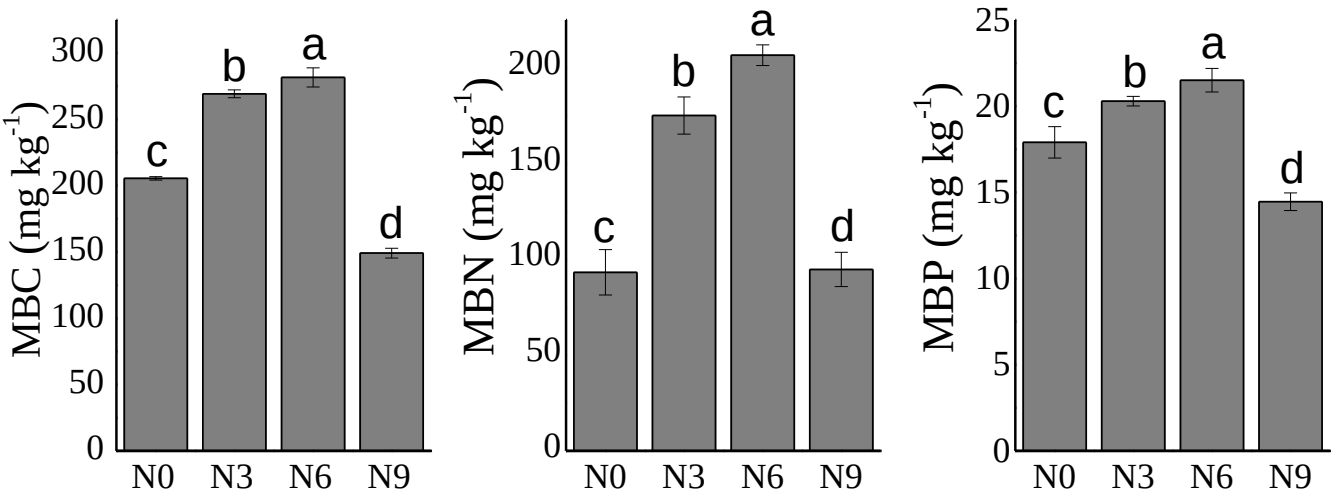

Figure 1. Soil microbial biomass (MB) under different N addition treatments: 0 g N m^{-2} y^{-1} (N0), 3 g N m^{-2} y^{-1} (N3, low-level N addition), 6 g N m^{-2} y^{-1} (N6, mid-level N addition), and 9 g N m^{-2} y^{-1} (N9, high-level N addition). Different letters represent significant differences between means under different N addition treatments ($p < 0.05$). Error bars represent standard errors.

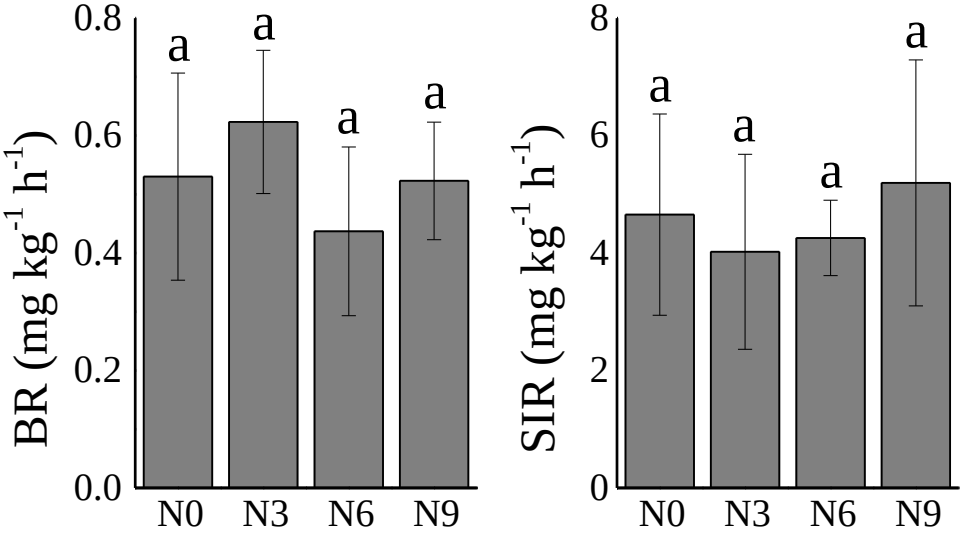

Figure 2. Soil microbial basal respiration (BR) and induced respiration rates (SIR) under different N addition treatments: 0 g N m^{-2} y^{-1} (N0), 3 g N m^{-2} y^{-1} (N3, low-level N addition), 6 g N m^{-2} y^{-1} (N6, mid-level N addition), and 9 g N m^{-2} y^{-1} (N9, high-level N addition). Different letters represent significant differences between means under different N addition treatments ($p < 0.05$). Error bars represent standard errors.

2.5. Analyses of Soil C Fractions and CMI

Labile organic C (OC) was measured with the method described by [16]. Soil including 15 mg of C was sieved (0.25 mm), weighed, and placed into 50 mL centrifuge tubes, and then processed with 25 mL 333 mM $KMnO_4$. Soil extraction solutions were shaken for 1 h and centrifuged at 2000 r min^{-1} for 5 min. Three labile OC fractions were measured using different concentrations of $KMnO_4$. Very labile OC: fraction I (C1) was oxidized using 33 mM $KMnO_4$. Labile OC: fraction II (C2) was determined as the difference between oxidized C with 167 mM $KMnO_4$ and C1. Less labile OC: fraction III (C3) was determined as the difference between oxidized C with 333 mM $KMnO_4$ and SOC fractions measured

with 167 mM $KMnO_4$. The non-labile soil oxidizable C fraction (C4) was determined as the content difference between SOC and soil labile OC. Absorbance values of the supernatants and standards were determined at 565 nm to indicate the soil labile OC. In this study, different SOC fractions were used to calculate the CMI and the stability index.

Compared with N0, the CMIs were calculated along the different N gradients using the following equations:

$$\text{Carbon pool index (CPI)} = \text{SOC (Ni)}/\text{SOC (N0)} \quad (1)$$

$$\text{Lability (L)} = \text{labile carbon}/\text{non-labile carbon} \quad (2)$$

$$\text{Lability index (LI)} = \text{L (Ni)}/\text{A (N0)} \quad (3)$$

$$\text{Carbon management index (CMI)} = \text{CPI} \times \text{AI} \times 100 \quad (4)$$

$$\text{Sensitivity index (SI)} = (\text{C fraction (Ni)} - \text{C fractions (N0)})/\text{C fractions} \quad (5)$$

$$\text{Stability} = 1/\text{lability} \quad (6)$$

Ni represents each N treatment; lability (L) represents soil C stability. The higher the lability, the lower the SOC stability, indicating that the soil labile OC was easily decomposed and transformed by interference [17].

2.6. Statistical Analyses

The Shannon richness and evenness indices of soil microbial community structure and metabolic diversity were calculated using soil PLFA and Biolog parameters, respectively, and the equations are listed in the Supplementary Materials.

Differences in the responses of soil chemical parameters, soil microbiological parameters, SOC fractions, and soil CMIs along the N-addition gradient were analyzed using one-way ANOVA. The mean values were compared using Duncan's tests at $p < 0.05$. The ANOVA and principal component analyses (PCAs) were processed with SPSS 20.0. The figures were prepared using OriginPro 9.0.

A structural equation model (SEM) was established to assess the impacts of N addition on the soil microbiological properties and further on the SOC fractions. Firstly, the number of variables for SMB, soil enzymatic activities, and soil respiration were reduced using PCAs [32]. In the PCAs, SMB explained 94.14% of MBC, MBN, and MBP; EEA1 explained 77.01% of the β-1,4-glucosidase (BG), β-1,4-N-acetylglucosaminidase (NAG), alkaline phosphatase (AP), cellulase, arylsulfatase, and β-xylosidase activities; EEA2 explained 52.89% of sucrase, amylase, and urease activities; and SR explained 57.39% of soil basal respiration and soil-induced respiration.

3. Results

3.1. Soil Microbiological Properties under N Addition

3.1.1. Soil Microbial Activity under N Addition

Low N addition significantly decreased soil arylsulfatase activity (Figure 3E), while soil urease activity was significantly enhanced by low N addition (Figure 3I). Other N addition levels along the gradient had no significant influence on the soil arylsulfatase and urease activities. Soil β-xylosidase activity showed similar tendencies with the soil arylsulfatase activity, with a numerically minimum value measured at the low N (N3) addition treatment (Figure 3F). Mid- (N6) and high (N9) N addition reduced soil sucrase activity significantly compared with the N0 (control) and N3 treatments. Compared with

N0, the soil amylase activity was significantly reduced by the N9 addition, but was not significantly impacted by the N3 and N6 treatments.

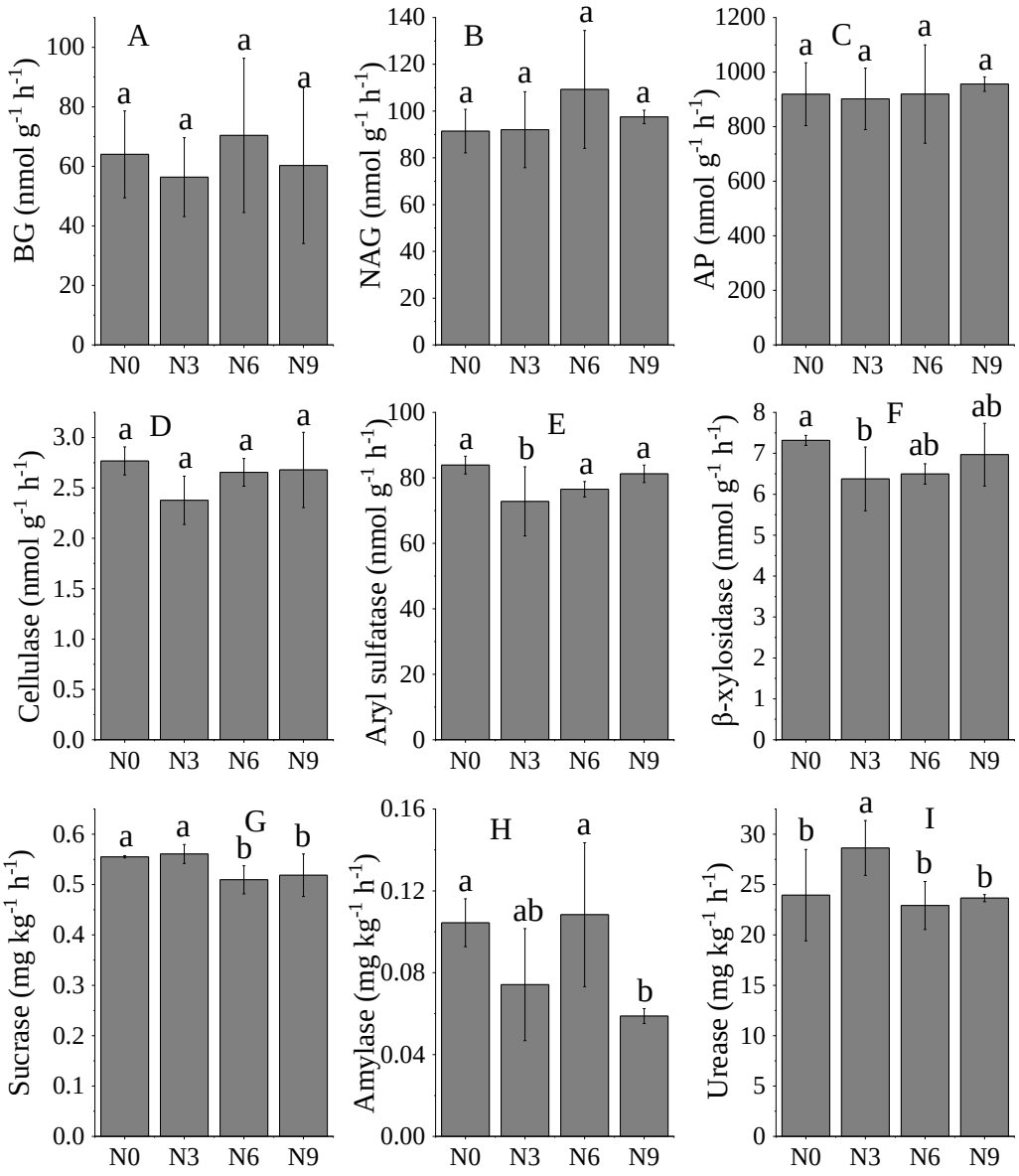

Figure 3. Soil enzymatic activities under different N addition treatments: 0 g N m^{-2} y^{-1} (N0), 3 g N m^{-2} y^{-1} (N3, low-level N addition), 6 g N m^{-2} y^{-1} (N6, mid-level N addition), and 9 g N m^{-2} y^{-1} (N9, high-level N addition). (**A**–**I**) represents the serial number of the subfigures showing each enzymatic activities. Different letters in each subfigure represent significant differences between means under different N addition treatments ($p < 0.05$). Error bars represent standard errors.

3.1.2. Soil Microbial PLFAs under N Addition

Soil G^+ PLFA and G^-PLFA showed different trends along the N-addition gradient (Figure 4). Soil G^+PLFA contents increased at first and then decreased, peaking at the N6 treatment. G^-PLFA contents enhanced with the N-addition gradient, with the N6 and N9 treatments significantly higher than the N0 and N3. In addition, the richness and evenness indices of the soil microbial community structure had similar trends, with the indices in the N0 and N3 treatments being higher than the N6 and N9 treatments (Table 1).

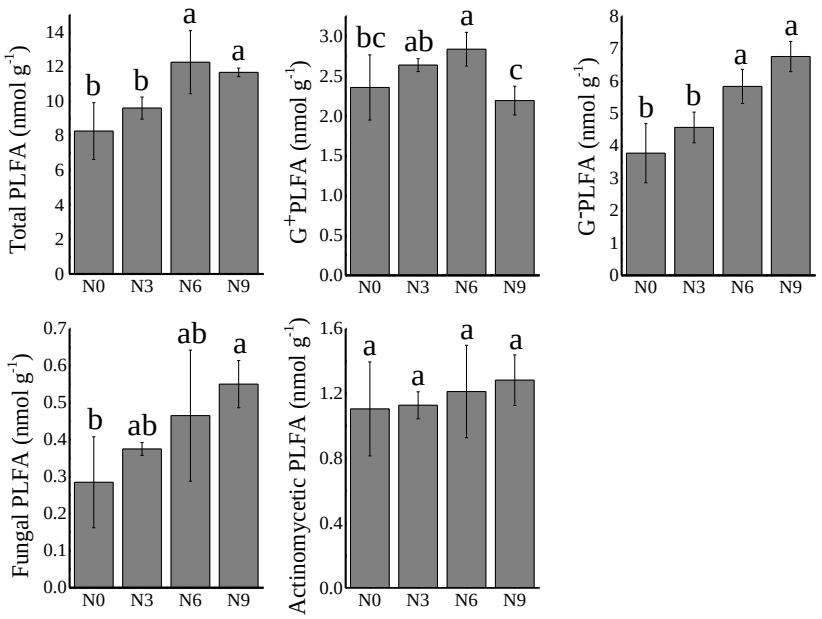

Figure 4. Soil phospholipid fatty acid under different N addition treatments: 0 g N m^{-2} y^{-1} (N0), 3 g N m^{-2} y^{-1} (N3, low-level N addition), 6 g N m^{-2} y^{-1} (N6, mid-level N addition), and 9 g N m^{-2} y^{-1} (N9, high-level N addition). Different letters represent significant differences between means under different N addition treatments ($p < 0.05$). Error bars represent standard errors.

Table 1. Changes in Shannon richness (*H*) and Shannon evenness (*E*) of the soil microbial community structure and metabolic diversity in the N addition treatments, as determined by the soil phospholipid fatty acids (PLFA), and soil microbial metabolic diversity using the Biolog technique (BIOLOG).

Treatment [a]	H_{PLFA}	E_{PLFA}	H_{BIOLOG}	E_{BIOLOG}
N0	3.15 ± 0.12 a [b]	0.93 ± 0.01 a	2.78 ± 0.05 a	0.82 ± 0.01 ab
N3	3.24 ± 0.10 a	0.92 ± 0.01 a	2.71 ± 0.05 a	0.80 ± 0.02 b
N6	2.94 ± 0.05 b	0.88 ± 0.04 b	2.78 ± 0.11 a	0.85 ± 0.03 a
N9	2.91 ± 0.16 b	0.87 ± 0.02 b	2.77 ± 0.10 a	0.81 ± 0.03 ab

[a] Rates of 0 g N m^{-2} y^{-1} (N0), 3 g N m^{-2} y^{-1} (N3, low-level N addition), 6 g N m^{-2} y^{-1} (N6, mid-level N addition), and 9 g N m^{-2} y^{-1} (N9, high-level N addition). [b] Data represent means ± standard deviations. Different letters indicate significant differences at $p < 0.05$.

3.1.3. Soil Microbial Metabolic Diversity under N Addition

The AWCD of all C sources for all N treatments almost followed the same trend during the culture period of 240 h (Figure 5), i.e., increased with the increase in culture time and almost reached a stable plateau towards the end of the measurement period. In the first 24 h, the AWCDs were observed to be at a similar level. After 120 h, the AWCDs started to deviate among the treatments. The richness indices of the soil microbial metabolic diversity had no significant differences among each treatment. The evenness index of soil microbial metabolic diversity peaked in the N6 treatment and was significantly higher than the N3 treatment (Table 1).

3.2. SOC Fractions under N Addition

Contents and proportions of SOC fractions under different N addition treatments are shown in Figure 6. Low N addition significantly enhanced the CMI, thus increasing soil quality (Table 2). Moreover, lability and the lability index decreased at first and then increased, with minimum values at N6 as well as maximum numerical values at N9. The sensitivity indices of C1, C2, and C3 showed different trends with the N-addition gradient. The N3 and N6 treatments significantly decreased the C1 sensitivity index compared with

the N0 and N9 treatments. In addition, the N3 treatment increased the C2 and C3 sensitivity indices, with maximum values observed in the N3 treatment.

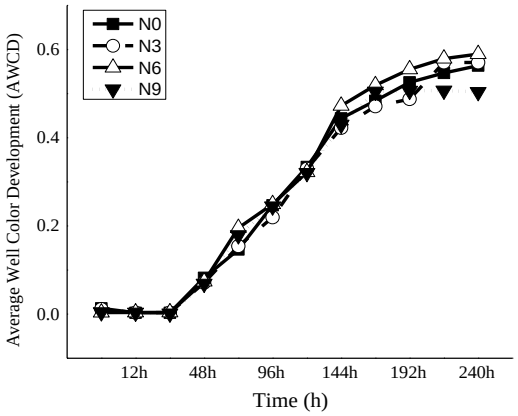

Figure 5. Cumulative changes in bacterial AWCD in the 0–20 cm soil layer under different N addition treatments: 0 g N m^{-2} y^{-1} (N0), 3 g N m^{-2} y^{-1} (N3, low-level N addition), 6 g N m^{-2} y^{-1} (N6, mid-level N addition), and 9 g N m^{-2} y^{-1} (N9, high-level N addition) throughout the culture period.

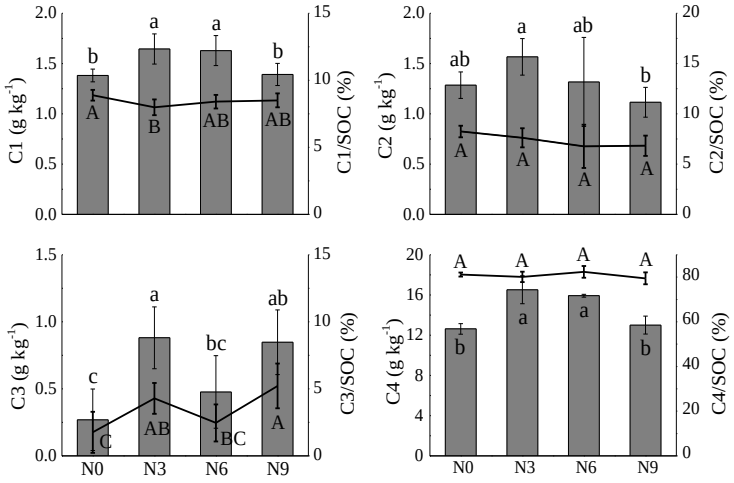

Figure 6. Contents and proportions of soil organic carbon (SOC) fractions under different N addition treatments: 0 g N m^{-2} y^{-1} (N0), 3 g N m^{-2} y^{-1} (N3, low-level N addition), 6 g N m^{-2} y^{-1} (N6, mid-level N addition), and 9 g N m^{-2} y^{-1} (N9, high-level N addition). Lowercase letters represent significant differences among the means of SOC fraction contents. Uppercase letters represent significant differences among the means of SOC fraction proportions ($p < 0.05$). Error bars represent standard errors.

Table 2. Soil carbon pool index (CPI), carbon management index (CMI), lability index (LI), and sensitivity index (SI) under different levels of N addition.

Treatment [a]	CPI	Lability (%)	Lability Index	CMI	SI [b] (L) (%)	SI [c] (C1) (%)	SI (C2) (%)	SI (C3) (%)
N0	1 b	23.29 ± 1.36 ab [d]	1ab	100	0 ab	0 b	0 ab	0 c
N3	1.32 ± 0.09 a	24.92 ± 3.55 ab	1.07 ± 0.15 ab	141.35 ± 19.77 a	7.00 ± 15.26 ab	19.06 ± 10.87 a	21.91 ± 14.18 a	227.57 ± 85.80 a
N6	1.24 ± 0.04 a	21.49 ± 2.27 b	0.92 ± 0.10 b	115.01 ± 15.71 b	−7.73 ± 9.77 b	17.88 ± 10.77 a	2.48 ± 23.43 ab	77.21 ± 100.75 bc
N9	1.05 ± 0.05 b	25.97 ± 2.70 a	1.12 ± 0.12 a	117.00 ± 9.64 b	11.53 ± 11.59 a	0.68 ± 7.93 b	−13.20 ± 11.57 b	215.14 ± 89.88 ab

[a] Rates of 0 g N m^{-2} y^{-1} (N0), 3 g N m^{-2} y^{-1} (N3, low-level N addition), 6 g N m^{-2} y^{-1} (N6, mid-level N addition), and 9 g N m^{-2} y^{-1} (N9, high-level N addition). [b] Sensitivity index of lability. [c] Fraction I (C1) was oxidized using 33 mM $KMnO_4$. Fraction II (C2) was determined as the difference between oxidized C with 167 mM $KMnO_4$ and C1. Fraction III (C3) was determined as the difference between oxidized C with 333 mM $KMnO_4$ and SOC fractional contents measured with 167 mM $KMnO_4$. Non-labile soil oxidizable C fraction (C4) was determined as the difference between SOC and soil labile OC. [d] Data represent means ± standard deviations. Different lowercase letters indicate significant differences at $p < 0.05$.

3.3. Integrated Effect of Microbiological Properties on SOC Fractions and Stability in Response to N Addition Treatments

The SEM explained 92.3% of the variance in CMI and 87.1% of the variance in the lability of SOC (Figure 7) with the parameters chosen in this study. N addition had both indirect as well as direct impacts on the CMI and lability (Figure 7), showing directly positive effects on both CMI and lability. Soil microbiological properties, SMB, SR, and E_{PLFA}, had positive effects on the CMI; SMB and SR did not show any significant effects on lability; and E_{PLFA} had a positive effect on lability. In addition, the sensitivity indices of the different SOC fractions as influenced by the four groups of biomicrobial properties are shown in Figure S1.

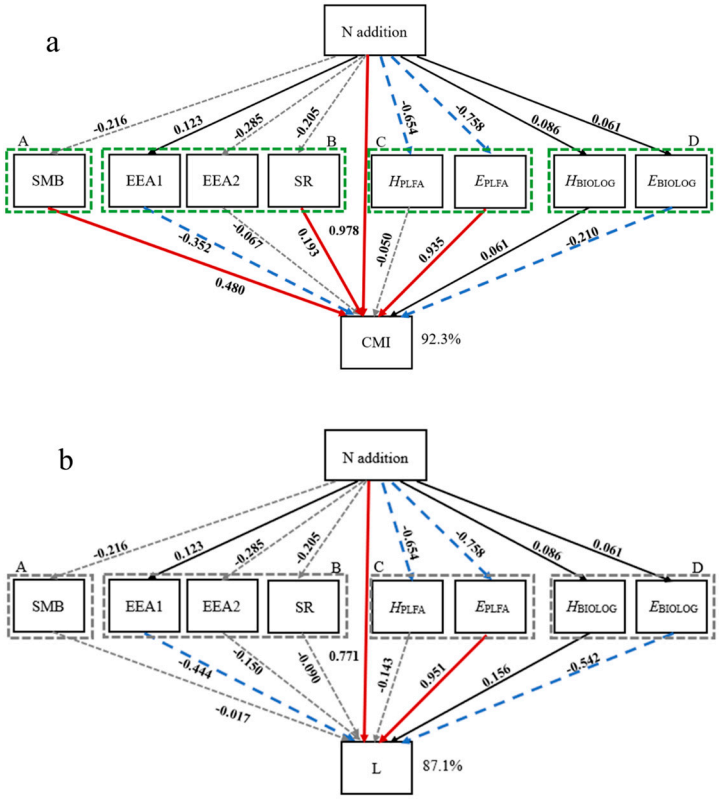

Figure 7. Structural equation model of the effects of soil microbiological factors on CMI (**a**) and lability (L) (**b**). These figures indicate the relationships between N addition (exogenous variable) and SMB, SR, H_{PLFA}, E_{PLFA}, H_{BIOLOG}, and E_{BIOLOG}. The final model (**a**) showed a good fit of the data: Chi-square = 36.273, $p = 0.068$. The final model (**b**) showed a good fit of the data: Chi-square = 36.269, $p = 0.052$. The numbers near the arrows represent the standardized path coefficients. The red arrows indicate significant positive relationships and the blue dashed arrows indicate significant negative relationships ($p < 0.05$). The solid arrows indicate positive relationships and the grey dashed arrows indicate negative relationships. Percentages near endogenous variables indicate the variance explained by the model. SR represents soil basal respiration and soil-induced respiration; H_{PLFA} represents the richness index of soil using the PLFA; E_{PLFA} represents the evenness index of soil using the PLFA; H_{BIOLOG} represents the richness index of the soil Biolog analysis; E_{BIOLOG} represents evenness index of the soil Biolog analysis.

4. Discussion

4.1. Response of Soil Microbiological Properties to N Addition

Enzymatic activities indicate the adaptation of the microbes to changes in the soil resources. According to previous studies, N addition could enhance the demand of soil microbes for soil sources, such as C, N, P, and S, before N saturation, thus the activities of different enzymes involving soil element cycling should be increased because of N

addition [5,6]. Some studies also showed different responses of enzymes along an N-addition gradient, as expected [33,34]. In this study, BG, NAG, and AP participated partially in the cycling processes involving C, N, and P; however, they did not show significant differences under each N addition treatment [29]. Apart from BG, cellulase involving C decomposition also did not vary along the N-addition gradient, which is supported by the findings of Gong et al. [5]. This may be because N addition did not change the N-limited status for microbes; therefore, the microbial community did not enter into a C-limited status, which is supported by our previous finding [29]. Moreover, enzymatic activities were also influenced by environmental materials, which could interact with enzymes released from cells [35]. In our manuscript, the urease activity involving the N cycling process reached the highest level at low N addition, which is inconsistent with the findings of Liu et al. [36] and Gong et al. [5] but consistent with those of Song et al. [37]. This is also supported by the highest soil C:N ratio being observed in the treatment with low N addition, where the urease activity was increased to alleviate this N-limited status. Amylase activity, involving S mineralization, was decreased at low N addition treatment, and no significant differences were observed at mid- and high N addition treatments compared with the control, which is supported by the results that N addition decreased arylsulfatase activity [38].

The effect of N inputs on the C emissions largely affected the direction and degree of the soil C balance response [39]. In this study, the N addition did not have a significant impact on the soil basal and induced respiration, which is consistent with Samuelson et al. [40]. However, studies have illustrated that N addition can either inhibit [41] or promote soil respiration [42]. This may be because soil microbial respiration is influenced by substrate quality and quantity, EEA, temperature, moisture, and microbial biomass [43].

In the present study, the mid-N addition increased the AWCD of all C at 72 h (Figure S1). The changes in AWCD among the different C substrates caused by N addition led to no significant changes in H_{BIOLOG} but a maximum numerical value for E_{BIOLOG} at N6. Zhong and Cai [44] found that N application had no direct impact on the soil microbial functional diversity, which partially supports our findings. Fang et al. [45] revealed that N inputs stimulated microbial metabolic activity and the preferential utilization of organic acids. He et al. [46] revealed that N inputs of 5 g N m^{-2} y^{-1} also significantly enhanced microbial functional diversity, while high N inputs of 20 g N m^{-2} y^{-1} reduced the microbial functional diversity because of belowground plant biomass. In addition, communities with a high plant species richness generated more various litter types and root exudates, thus resulting in a higher microbial functional diversity [47].

4.2. Response of Different SOC Fractions to N Addition

Labile organic carbon, C1, C2, and C3, consist of amino acids, simple carbohydrates, a portion of SMB, and other simple organic compounds [11], which are easily altered by environmental factors. Both labile organic carbon as well as non-labile OC increased at low N addition in this study. However, Wang et al. [15] illustrated that N addition did not alter the labile OC content. Wang et al. [15] reported no alternations in the labile OC ascribed to the decomposition offset of organic C generated from higher inputs of net primary production (NPP). In high N addition, labile and non-labile OC did not show significant differences with CK. However, Chen et al. [10] found an increase in labile OC with the addition of high N above the N saturation level because excessive N addition would inhibit the extracellular ligninolytic enzyme activities, leading to a decrease in the decomposition rate of litter. In addition, the high N addition may also result in excess soil N availability and reduce the N requirements of litter decomposers [23], thus affecting C fractions.

The maximum SOC fractions, including labile and non-labile OC, were observed in the treatment with a low level of N addition in this study, consistent with Chen et al. [10]. This was because appropriate N addition promoted plant litter and root exudates, increasing C input, while microbial respiration did not change significantly, inducing no changes in the offset of OC, thus the SOC content increased. In addition, the low N addition treatment

significantly increased the CMI compared with the mid- and high N addition treatments. This indicated that appropriate N input was capable of increasing soil quality and enhancing the function of soil nutrient cycling, which is supported by Liu et al. [48]. Moreover, the stability of SOC did not show significant differences at N0, low, and mid-N addition, which is supported by Ghosh et al. [13] who also found that N addition (12 g N m^{-2} y^{-1}) would not change SOC stability. However, high N addition increases the stability of SOC, which is supported by previous findings that high-level addition would inhibit the extracellular ligninolytic enzyme activities and lead to a decrease in litter decomposition rate.

4.3. Driving Factors of Soil Microbiological Properties on CMI and SOC Stability

SEM was conducted to analyze the impact of soil microbiological properties under N addition on CMI and the stability of SOC based on modified MCP theory. According to the results, N addition showed direct impacts on the CMI and SOC stability. In addition, "microbial in vivo modification" and "microbial ex vivo modification" played more vital roles in microbial control of CMI than the stability of SOC under N addition. SMB, as a "microbial in vivo modification" method to control SOC, was found to have a positive correlation with CMI and no significant relationship with SOC stability. This was mainly because soil microbes, as the composition of soil labile OC, positively impacted CMI, while the stability of SOC was not only affected by soil labile organic carbon but also soil non-labile organic carbon, inducing no significant relationship between them. Soil enzymes play significant roles in decomposing plant-derived macromolecule carbon substrates in soil into soil labile and non-labile organic carbon [22,23], which depend on the available soil element contents and environmental factors [35]. In this study, enzymatic activities via "microbial ex vivo modification" had a negative influence on the CMI and stability of SOC, showing different functional trends as seen in other studies [10,23]. This was mainly because N addition higher than N saturation would inhibit enzymatic activities or soil acidity would lower enzymatic activities, thus having a negative influence. From a microbial community structure perspective, E_{PLFA} was decreased by N addition and CMI increased under N addition. This is attributed to the increase in functional taxa or fungi that would have capacities to decompose recalcitrant substrates, thus decreasing E_{PLFA} and increasing CMI.

5. Conclusions

Our data indicated that appropriate N addition would increase sequestration potential and the quality of SOC in soil ecosystems in plantations. Specifically, "microbial in vivo modification" and "microbial ex vivo modification" played more vital roles in microbial control of CMI than the stability of SOC under N addition. SMB and SR had a positive relationship with CMI under N addition, while some enzymatic activities had the opposite effect on CMI. Moreover, E_{PLFA} could be decreased by N addition, and it enhanced CMI and the stability of SOC. The present study furthers our understanding of the influence of N addition on the sequestration potential, quality, and stability of SOC based on MPC theory. Further identification and study of soil functional microbial taxa that can play a vital role in decomposing recalcitrant substrates may be helpful in determining the detailed mechanisms involved.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f14081540/s1, Table S1: Soil chemical properties in different N addition level. Figure S1. Bacterial AWCD at 72 h and 240 h under different N addition. References [30,31,49–54] are cited in supplementary materials.

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