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Response of Functional Diversity of Soil Microbial Community to Forest Cutting and Regeneration Methodology in a Chinese Fir Plantation

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Abstract: With the expansion of pure forest planting area and the increase in the number of rotations used, soil activity and plant productivity have significantly reduced. The functional diversity of soil microorganisms plays a vital role in forest health and the long-term maintenance of productivity. Though the optimization of forest cutting and regeneration methodologies is necessary to improve the functional diversity of soil microorganisms, the effects of harvest residual treatment on the functional diversity of soil microorganisms remain unclear. During the period 2018–2020, we designed four harvest residual treatments—reference (RF), residual burning (RB), crushing and mulching (MT), and no residuals (NR)—to determine soil physical and chemical properties. We also used microbial biomass (MB) to evaluate the diversity in carbon source metabolism of soil microorganisms through Biolog microplate technology, and discussed the response mechanism of microbial functional diversity to the different forest cutting and regeneration methodologies used in Chinese fir plantations. The results indicated that RB significantly increased the carbon metabolic capacity of the microbial community, the community richness, and its dominance compared to RF, MT, and NR; however, they also showed that it decreased the uniformity of the soil microbial community. NR showed a poor carbon utilization capacity for microorganisms compared to RF and MT, while MT significantly increased the utilization capacity of carbohydrate and amino acid carbon compared with RF. Soil nutrients were the main driving factors of soil microbial carbon metabolic activity, and the different responses of microbial functional diversity to various forest cutting and regeneration methodologies were mainly due to the variation in the nutrient inputs of harvest residues. This study provides a practical basis for enhancing the functional diversity of soil microorganisms in plantations through the management of harvest residues.

Keywords: Chinese fir plantation; harvest residues; microbial functional diversity; soil nutrients; biolog microplate technology

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1. Introduction

With the expansion of the pure forest planting area and the increase in the number of rotations used, the continuous decline of soil fertility and the productivity of plantations has attracted national attention [1–4]. Ecosystem function depends largely on the functional diversity and activity of the underground microbial system [5]. Soil microbial functional diversity is extremely sensitive to changes in the soil microenvironment [6] and so can reflect changes in soil quality [7,8]. It is often used together with soil microbial biomass as an important indicator for evaluating soil fertility and health [9,10]. Deforestation and land management influence the function of soil microorganisms [11–13], with some

effects lasting for decades [14], and have nonnegligible impacts on forest health and the long-term maintenance of productivity [15]. Though the optimization of forest cutting and regeneration methodologies is necessary to improve the functional diversity of soil microorganisms, the effects of harvest residual treatment on the functional diversity of soil microorganisms remain unclear.

Traditional slash-and-burn cultivation is a common method of forest cutting and regeneration in plantations, but often has negative effects on the physicochemical and biological characteristics of soil [2,16]. Studies have shown that 93% of the total biomass is lost as gases and fly ash after burning, as well as nitrogen (N), phosphorus (P), and potassium (K), including losses of nutrients from dead plants [17]. In addition, runoff occurs easily and causes substantial soil erosion due to the loss of the surface protective layer [18]. In general, slash-and-burn cultivation is generally believed to change the structure of the soil food web, destroy soil organic matter [19], reduce soil total nutrient contents [17], and alter the soil microenvironment [20,21], thus disrupting the structure and affecting the functional diversity of soil microorganisms [16,22,23].

Compared with traditional slash-and-burn cultivation, the functional diversity of soil microorganisms is higher when there is little or no interference [22,24]. Moreover, the negative impact of wood and biofuel-oriented forestry practices on forest ecosystems can also be alleviated by mulching residues after harvesting [25–27]. However, some studies have indicated that the composition of animals and microorganisms needed for decomposing coniferous forest residues in woodland is single, resulting in the easy accumulation of residues, which leads to a low rate of nutrient cycling and thus a decline in soil fertility [28,29]. It is even possible to remove harvest residues while maintaining productivity [30]. Although there is no direct evidence that whole-tree harvesting reduces woodland productivity, mulching residues in woodland can reduce at least some of the direct nutrient losses of ecosystems [31]. One study showed that the rate of removal of N, P, K, calcium (Ca), and magnesium (Mg) with whole-tree harvesting increased by 216%, 304%, 152%, 254%, and 151%, respectively, compared to stem-only harvesting, and the removals and displacements of nutrients were unacceptably large [32]. Therefore, the appropriate removal and effective utilization of harvest residues is likely to affect the functional diversity of soil microorganisms and ultimately affect the forest productivity, especially in plantations with short-term rotations.

Mulching with crushing straw is widely used in agriculture, as it facilitates nutrient release, lessens nutrient loss, and reduces human disturbance to the ecosystem by avoiding the use of fire [33–36]. However, there have been few investigations on forestry management in the hilly areas of South China, and whether crushing residues can accelerate the nutrient transfer process, improve soil quality, enhance soil microbial functional diversity, and maintain a long-term high yield in the forest remains unclear.

Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook.) is a representative fast-growing tree species of plantations in the subtropical area of China [37], with an accumulative planting area of over 12 million hectares [38]. It has been used for large-scale afforestation for at least 1000 years and plays an important role in the construction of the timber forest base in southern China [39]. With the increase in the planting area of pure Chinese fir forest, the number of rotations, soil fertility, and plant productivity have significantly reduced, which has affected the construction of timber forest sites in this region [40]. Soil microbial change is the key to aboveground and underground ecological function [5]. In order to ensure the sustainability of high-productivity Chinese fir plantations, we hypothesized, based on the important role of harvest residues in nutrient cycling and soil fertility conservation in plantations, that the management of harvest residues could enhance the functional diversity of soil microorganisms. Our aim is to study the effects of three harvest residual treatments in Chinese fir plantations (RB, residual burning; MT, crushing and mulching treatment; and NR, no residuals) on the functional diversity of soil microorganisms and their changes over time, as well as to explore the response mechanism of soil microbial functional diversity to the three types of residual management in two years

(2018–2020). This will provide scientific evidence and a technological basis for solving the problem of soil fertility decline in Chinese fir plantations.

2. Materials and Methods

2.1. Study Areas

This study was conducted in a 38-year-old Chinese fir plantation in Guanshan forestry station (27°14′ N, 115°30′ E) with altitude of 64–109 m in Yongfeng County, Jian City of Jiangxi Province, which is one of the main production areas of Chinese fir plantations. The climate of this area is a subtropical humid monsoon climate and the main soil type is red soil. The annual average temperature is 18–22 °C, and the annual precipitation is 2100–2500 mm. The planting density used in this study was 1725 plants/ha, with an average diameter at breast height (DBH) of 19.79 cm and average tree height of 19.92 m in 2018. In August 2018, all trees with DBH > 6 cm were clear cut.

2.2. Treatments at the Experiment

Twelve plots of 8 m × 8 m (i.e., the block) were established in a randomized design with three replicates for each of the four treatments, with 5 m distances maintained between any two adjacent sampling plots (Figure 1). We first moved all the leaves and branches to a central collection site for weighing to ensure that there were the same amount of residuals between plots. Nine piles of residuals (each with a mixture of 600 kg of leaves and 133 kg of branches) were independently built for the three treatments: I, reference (RF)—all residuals were brought back to the plots and evenly distributed across the plots; II, residual burning (RB)—residuals were evenly distributed across the plots and manually burned using a homemade stove; III, mulching treatment (MT)—residuals were ground to ~1 cm mulches using a Pulverizer (DE WILD GTS900, Sulong Industrial Co., Ltd., Shanghai, China) before being evenly distributed across the plots; and IV, no residuals (NR)—no residuals were brought back to the harvested plots.

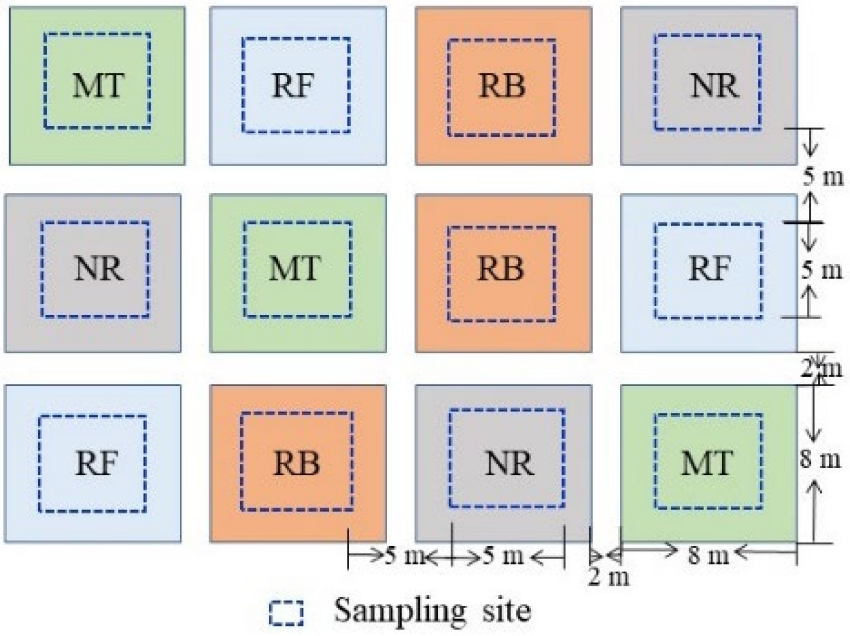

Figure 1. The experimental plot design. Sampling site, 5 m × 5 m in the central area of each plot. RF, reference; RB, residual burning; MT, crushing and mulching treatment; and NR, no residuals.

2.3. Collection and Determination of Soil Samples

Sampling period and frequency: soil samples were collected in September 2018 (fall), March 2019 (spring), June 2019 (summer), September 2019 (fall), and September 2020 (fall). Sampling method: 0–10 cm soil samples were drilled along the diagonal of each plot, and

six samples were pooled as a mixed soil sample. Fresh soil samples were taken back to the laboratory to remove fine roots and gravel, and screened using a 2 mm sieve. One part of each sample was used to determine the soil physical and chemical properties, and the other part was stored at 4 °C to measure the biomass and diversity of the soil microorganisms.

The soil water content (SWC) was determined by the drying and weighing method. Soil pH was determined in 1:2.5 soil:water slurry using a combination glass electrode. Soil organic carbon (SOC) was determined using the oil bath–$K_2Cr_2O_7$ titration method, available nitrogen (AN) was determined using the micro-diffusion technique after alkaline hydrolysis, available phosphorus (AP) was determined using 0.5 mol L^{-1} $NaHCO_3$ solution (pH 8.5), phosphate-P in solution was determined by the formation of the blue phosphomolybdate complex following reduction with ascorbic acid, and available potassium (AK) was determined by CH_3COONH_4 extraction [41].

Microbial biomass carbon (MBC) and nitrogen (MBN) were determined using chloroform fumigation [42,43]. In the fumigation group, ethanol and chloroform were fumigated for 24 h and extracted with 0.5 mol L^{-1} of K_2SO_4 solution. The control group was not fumigated, and the extraction process was the same as that of the fumigation group. The filtrate was analyzed using a total organic carbon analyzer (TOC-VcPH + TNM-1, Shimazu Inc.; Kyoto, Japan). MBC and MBN were calculated as follows: MBC = EC/0.45, MBN = EN/0.54, respectively, where EC and EN are the differences between the organic C and N extracted from the fumigated and non-fumigated soils, respectively [42,44].

The functional diversity of the soil microorganisms was evaluated for the utilization of 31 carbon sources using Biolog Ecoplates (Biolog Inc., Hayward, CA, USA) according to Classen et al. (2003) [45]. Briefly, 30 g soil was placed in a sterile triangular bottle, 270 mL of 0.85% NaCl sterile solution was added, and the bottle was sealed. Then, the bottle was shaken at 180 rpm for 30 min, and 3 mL of supernatant was added to 27 mL of NaCl solution, which was thoroughly mixed. Then, the diluted 3 mL of supernatant was added to another 27 mL of NaCl solution. After dilution, this soil solution was finally diluted to 10^{-3} and prepared for immediate reaction. Of the extract, 150 µL was added to each well of the microplate with a pipette gun and the mixture was then incubated at a constant temperature of 28 °C for 168 h. The absorbance at 590 nm (OD590) was read for each well using a Biolog microplate reader (Biolog Inc., Hayward, CA, USA) following incubation at 24, 48, 72, 96, 120, 144, and 168 h, respectively.

The average well color development (AWCD)—i.e., a measure of the catabolic potential of the microbial community—was calculated as follows:

$$\mathrm{AWCD} = \Sigma\,(Ci - R)/n \quad (1)$$

where C_i represents the absorbance of each hole; R represents the absorbance of the control hole; and n represents the number of carbon sources—i.e., 31 [46].

The classification of various carbon sources was carried out according to Insam (1997) [47]. Diversity parameters—i.e., the Shannon–Wiener diversity index (H')—were calculated according to Zak et al. (1994) [48]—i.e.:

$$H\prime = -\Sigma\, PilnPi \quad (2)$$

where P_i is calculated by n_i/N, n_i is the OD590 value on a specific substrate, and N is the sum of all the positive OD590 values in the plate.

The Simpson dominance index (D) was calculated as:

$$D = 1 - \Sigma(Pi)^2 \quad (3)$$

Additionally, the McIntosh uniformity index (E) was calculated as follows:

$$E = (N - \sqrt{\Sigma {n_i}^2})/(N - N/\sqrt{s}) \quad (4)$$

where S is the number of carbon sources catabolized (OD590 > 0.25) [49].

2.4. Data Analysis

A nested mixed-model analysis was used to analysis the effect of the residual treatment, sampling season, and sampling year and their interactions with the soil physicochemical index, microbial biomass, carbon sources, and diversity index, which were analyzed using the "*aov*" function in the 'multcomp' package in RStudio. The sampling season was nested within the sampling year, and the blocks were considered as random effects. Principal component analysis (PCA) was performed for the AWCD of 31 carbon sources in soil samples of 96 h to evaluate the major carbon sources used by microorganisms. A redundancy analysis (RDA) was carried out with Canoco 5.0 to determine the effect of each independent variable on the response variable. The mean values and standard errors of three repeated samples were used for both bar and line plots. Data were summarized and calculated using Excel (Microsoft Office 2016) and SPSS 22.0 and plotted using Origin 2021. All statistical effects were considered significant at $p < 0.05$.

3. Results

3.1. Dynamic Characteristics of Soil Physical and Chemical Properties

The soil physicochemical properties were significantly influenced by residual treatments, and the effects differed by season and year (Figure 2, Table A1). The promotional effect of RB on soil nutrients was mainly manifested in the early stage of treatment, in which AP and AK were 88% and 31% higher than the RF in September 2018, respectively, while SOC and AN were 42% and 40% lower than the RF in September 2020, respectively (Figure 2). In addition, the pH of RB was higher in both June and September 2019, but in the dry fall (September 2019), RB reduced SWC by 20% compared to RF (Figure 2). The AK in MT was 17% higher than the RF one year after treatment (Figure 2). NR had negative effects on SOC, AN, AP, and AK, among which SOC was still 28% lower than RF after two years, and pH has been lower since June 2019 (Figure 2).

3.2. Dynamic Characteristics of Soil Physical and Chemical Properties

The results showed that different harvest residual treatments had significant effects on soil microbial biomass (MB), and a significant interaction was observed between the residual treatments and the sampling period (Figure 2, Table A1). Compared with RF, the MBC and MBN in RB were basically decreased throughout the whole observation period, while MBC decreased by 54% after one year of the treatment and MBN decreased by 46% after two years, but it had a higher C/N at the beginning stage (Figure 2). On the contrary, compared with RF, the MBC in MT increased by 63% after one year and 90% after two years (Figure 2). In addition, the MBC in NR increased in the early stage, though this effect weakened after two years, and MBN decreased by 50% compared with RF (Figure 2).

3.3. Dynamic Characteristics of Functional Diversity of Soil Microorganisms

3.3.1. The Average Well Color Development

The AWCD can comprehensively reflect the carbon source metabolism capacity of soil microbial community under different treatments of harvest residues. According to the development tendency of AWCD from 24 to 168 h, the value of 96 h was selected to compare the difference in the effects of different treatments and sampling periods on microbial carbon source utilization capacity, because this was an inflection point of the variation in the rising rate of AWCD value (Figure 3).

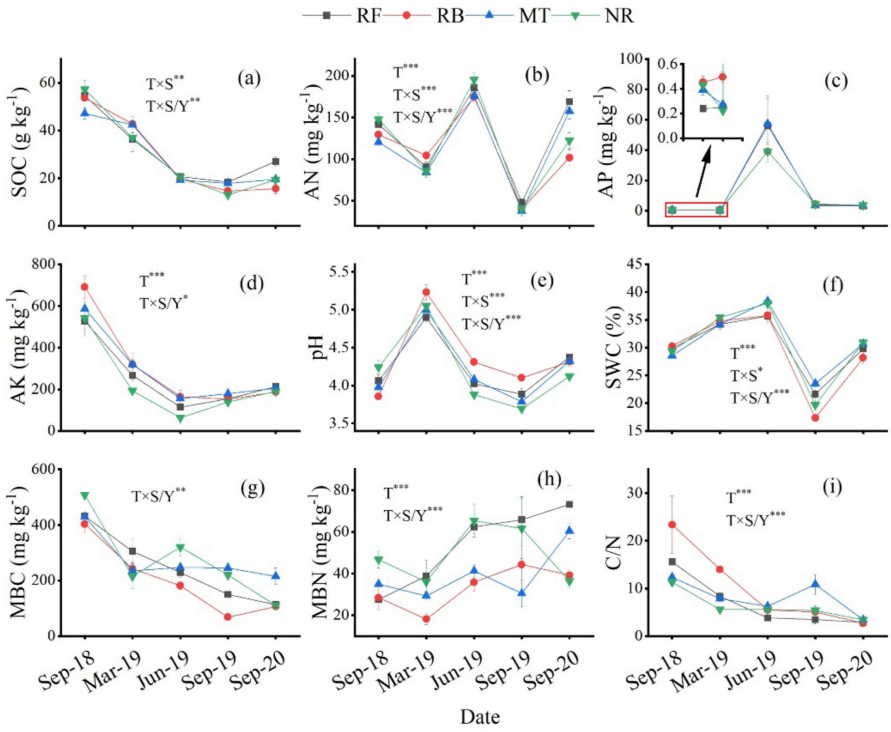

Figure 2. Dynamic characteristics of soil physical and chemical properties and microbial biomass under different harvest residue treatments from 2018 to 2020. RF, reference; RB, residual burning; MT: mulching treatment; NR, no residuals; (**a**) SOC, soil organic carbon; (**b**) AN, available nitrogen; (**c**) AP, available phosphorus; (**d**) AK, available potassium; (**e**) pH, potential of hydrogen; (**f**) SWC, soil water content; (**g**) MBC, microbial biomass carbon; (**h**) MBN, microbial biomass nitrogen; (**i**) C/N, MBC/MBN. Data are displayed as means ± standard errors. T, treatment; S, season; Y, year; T × S, the interaction between T and S; T × S/Y, the interaction between T and Y; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

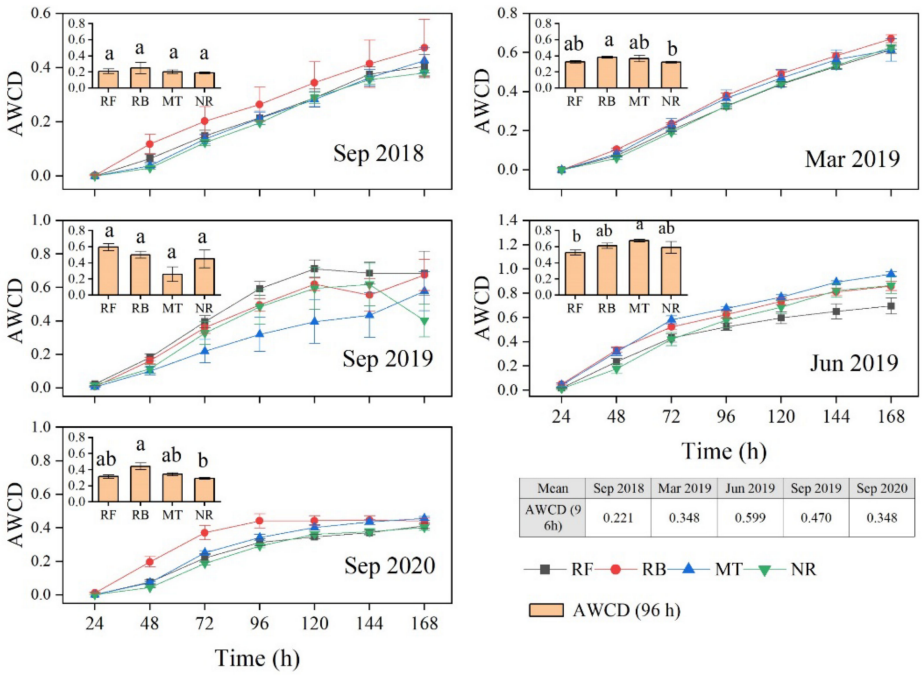

Figure 3. Effects of the use of different harvest residue management methods on average well color development (AWCD) from 2018 to 2020. Data are means ± standard errors, and different lower-case letters indicate significant differences among the residue treatments ($p < 0.05$).

The results showed that the interaction between residual treatment and sampling period had significant effects on AWCD (Figure 3, Table 1). The peak value of AWCD appeared in June 2019 in MT (0.676) and RB (0.612), respectively, and the trough value appeared in September in NR (0.188) during the study period. In March 2019, the AWCD value in RB was significantly higher than that in NR (18.7%) ($p < 0.05$); in June 2019, MT was significantly higher than RF (28.3%) ($p < 0.05$); and in September 2020, RB was significantly higher than NR (51.0%) ($p < 0.05$). In addition, all three treatments showed an increase after the first year of treatment (0.449–0.594) and a decrease after two years of treatment (0.292–0.441), but were still higher than the initial value (0.188–0.248), except for MT (continued to increase for 2 years) (Tables A2 and A3). These results indicated that the carbon source metabolic activity of the soil microbial community was higher in RB and MT than in RF and NR.

Table 1. The effect of residual treatments (T), study period (S, season; Y, year), and their interaction on the soil physicochemical index and microbial biomass, which were analyzed using repeated nested analysis.

Index	T	%	S	%	S/Y	%	T × S	%	T × S/Y	%
Carbon sources [1]										
AWCD	2.32	2.01	48.61 ***	28.02	29.74 ***	17.14	1.78	3.08	2.61 *	4.51
Carbohydrates	4.07 **	3.37	59.05 ***	32.59	21.96 ***	12.12	3.37 **	5.59	2.16	3.57
Phenolic compounds	1.16	1.31	9.87 ***	34.59	26.11 ***	17.55	0.86	2.95	1.77	1.61
Polymers	0.86	1.49	3.70 *	8.43	46.58 ***	22.29	0.81	2.21	4.24 ***	4.52
Carboxylic acids	2.68 *	2.48	35.49 ***	16.19	17.17 ***	11.63	2.18 *	2.69	2.67 *	14.99
Amines	0.18	0.90	36.77 ***	2.60	9.49 ***	32.75	4.00 ***	1.72	1.77	8.95
Amino acids	1.62	0.20	64.24 ***	26.49	32.59 ***	6.83	1.83	8.65	0.99	3.81
Diversity index [2]										
H′	2.34	2.78	22.87 ***	24.52	16.43 ***	11.86	1.27	4.52	7.06 ***	5.53
D	2.14 *	2.63	8.03 ***	6.58	15.80 ***	12.96	1.23	3.02	5.24 ***	12.90
E	6.43 ***	8.39	0.43	0.37	3.33 *	2.90	2.00	5.23	7.16 ***	18.70

[1] AWCD, average well color development. Carbohydrates ($n = 10$): H1 (α-D-L-lactose), A2 (β-methyl-D-glucoside), H2 (D, L-α-glycerol phosphate), G1 (D-cellobiose), D2 (D-mannitol), C2 (I-erythritol), G2 (glucose-1-phosphate), B2 (D-xylose), A3 (D-galactonic acid lactone), and E2 (N-acetyl-D-glucosamine); phenolic compounds ($n = 2$): C3 (2-hydroxy benzoic acid), and D3 (4-hydroxy benzoic acid); polymers ($n = 4$): F1 (glycogen), C1 (Tween 40), D1 (Tween 80), and E1 (α-cyclodextrin); carboxylic acids ($n = 7$): G3 (α-ketobutyric acid), B3 (D-galacturonic acid), E3 (γ-hydroxy butyric acid), F2 (D-glucosaminic acid), H3 (D-malic acid), F3 (itaconic acid), and B1 (pyruvic acid methyl ester); amines ($n = 2$): H4 (putrescine) and C4 (phenyl ethylamine); amino acids ($n = 6$): A4 (L-arginine), E4 (L-threonine), D4 (L-serine), G4 (L-phenylalanine), B4 (L-asparagine), and F4 (glycyl-L-glutamic acid). [2] *H′*, Shannon–Wiener diversity index; *D*, Simpson dominance index; *E*, McIntosh evenness index. Notes: Notes: F values and the contributions (%) are listed; $p < 0.1$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

3.3.2. Utilization of Various Carbon Substrates by Soil Microbial Community

Different residual treatments and sampling periods had different effects on the carbon source types used by soil microorganisms (Table 1, Figure 4). The carbohydrates (in September 2018 and September 2020), amino acids (in March 2019), and phenolic compounds (in September 2020) were higher in RB, while the carbohydrates (in June 2019), amino acids (in June 2019), and polymers (in September 2018 and September 2020) were higher in MT compared with in the other treatments (Figure 4). However, the different kinds of carbon sources in NR showed no significant advantages compared with the other treatments (Figure 4).

3.3.3. Diversity Index of Soil Microorganisms

The treatment and sampling period used and their interaction had significant effects on the diversity index (Table 1, Figure 5). Both *H′* and *D* showed that RB was significantly higher than NR in March 2019 and higher than the other three treatments in June 2019; however, *E* showed that RB was significantly lower than MT and NR in September 2018 and significantly lower than the other three treatments in September 2020 (Figure 5). *H′* in September 2018 and September 2020, *D* in September 2020, and *E* in March 2019 all showed that NR was significantly lower than RF (Figure 5). These results indicated that burning residues significantly increased the diversity and dominance of the soil microbial

community in the first year of treatment but decreased the evenness, which was still significant after two years of treatment.

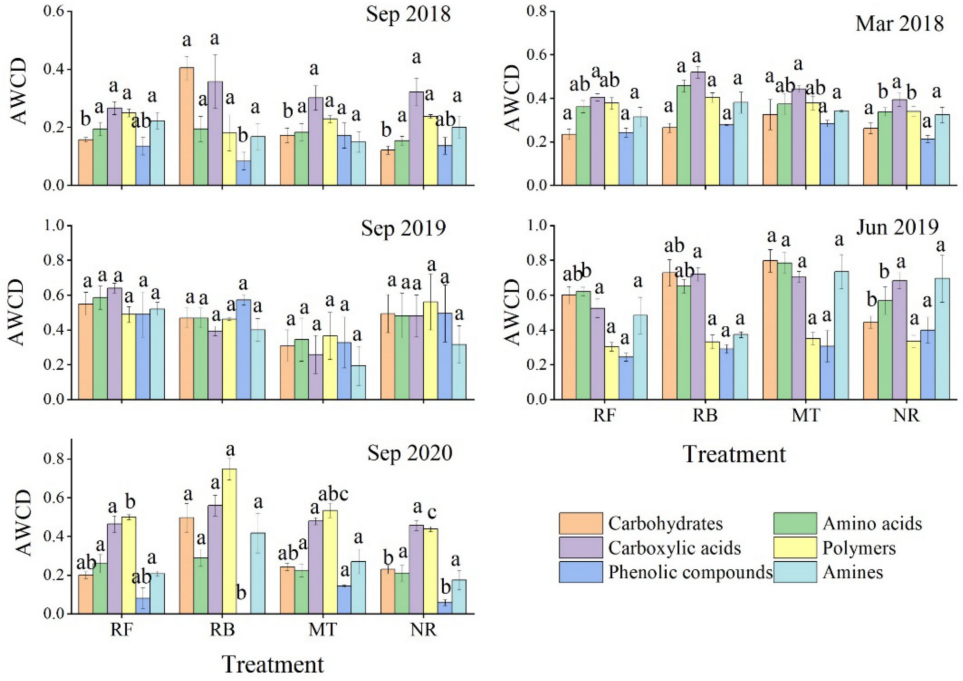

Figure 4. Effects of different harvest residue managements on the AWCD of six carbon sources (carbohydrates, polymers, carboxylic acids, phenolic compounds, amines, and amino acids) at 96 h. Data are means ± standard errors, and different lower-case letters indicate significant differences among residue treatments ($p < 0.05$).

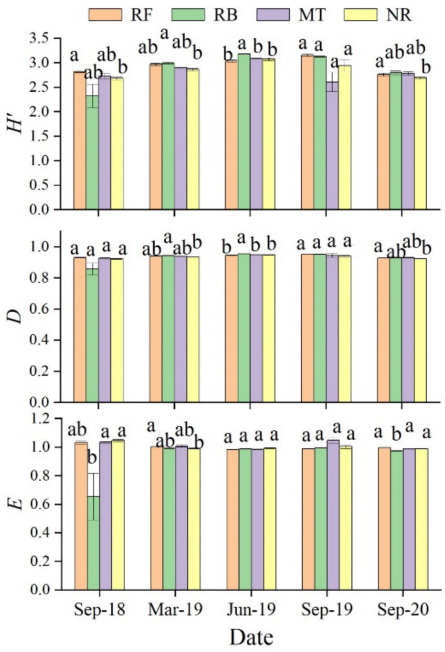

Figure 5. Soil microbial community functional diversity indexes in different treatments of harvest residue at 96 h. *H′*, Shannon–Wiener diversity index; *D*, Simpson dominance index; *E*, McIntosh evenness index. Data are means ± standard errors. Different lower-case letters indicate significant differences among the residue treatments ($p < 0.05$).

3.3.4. PCA of Carbon Utilization by Soil Microorganisms

The PCA was used to screen out the types of carbon sources which the soil microbial communities used more (Table 2). The first four principal components (PCs) explained 33.98%, 9.25%, 6.97%, and 5.27% of the variation, while 55.47% of the variation was explained cumulatively (66.77% was explained cumulatively for the first seven PCs). PC1, with an absolute value of loading value greater than 0.5, consisted of nine types of carbon: one carbohydrate, five amino acids, two phenols, and one amine. PC2 consisted of seven types: two carbohydrates, three carboxylic acids, one polymer, and one amine. PC3 consisted of six types: three carbohydrates, two carboxylic acids, and one polymer. Finally, PC4 included two polymers and one carbohydrate. All these indicate that amino acids, carbohydrates, and carboxylic acids were the most common carbon sources used by soil microbial communities in Chinese fir plantations.

Table 2. Loading factors of principal components (PCs) of different carbon sources at 96 h.

PC	Chemical Type	Carbon Source	Loading Value
PC1 33.98%	Amine	L-Phenylalanine C4	0.835
	Phenolic compounds	4-Hydroxy benzoic acid D3	0.792
	Amino acid	L-Serine D4	0.772
	Amino acid	L-Asparagine B4	0.742
	Carbohydrates	D-Mannitol D2	0.645
	Phenolic compounds	2-Hydroxy benzoic acid C3	0.624
	Amino acid	Phenylethylamine G4	0.56
	Amino acid	L-Threonine E4	0.546
	Amino acid	L-Arginine A4	0.546
PC2 9.25%	Carboxylic acids	D-Glucosaminic acid F2	0.808
	Polymers	Tween 40 C1	0.747
	Amine	Putrescine H4	0.709
	Carbohydrates	D-Galactonic acid lactone A3	0.689
	Carboxylic acids	Pyruvic acid methyl ester B1	0.65
	Carboxylic acids	γ-Hydroxybutyric butyric acid E3	0.648
	Carbohydrates	N-Acetyl-D-glucosamine E2	0.565
PC3 6.97%	Carboxylic acids	D-Malic acid H3	0.684
	Carbohydrates	D-Xylose B2	0.641
	Polymers	Tween 80 D1	−0.568
	Carboxylic acids	D-Galacturonic acid B3	0.567
	Carbohydrates	Glucose-1-phosphate G2	0.558
	Carbohydrates	D-Cellobiose G1	0.524
PC4 5.27%	Polymers	Glycogen F1	0.786
	Polymers	α-Cyclodextrin E1	0.689
	Carbohydrates	I-Erythritol C2	0.617

Note: only loading values greater than 0.5 are listed.

3.4. Contribution of Soil Factors to Soil Microbial Community Functional Diversity Variations

The RDA was used to evaluate the relationship between the diversity of the soil microbial community carbon source metabolism and soil environmental parameters under four treatments of harvest residues (Figure 6, Table A4). The explanation rates for all variables to dependent variables in different treatments reached 36.81% (RF), 40.76% (RB), 88.95% (MT), and 40.27% (NR), respectively. The major factors contributing to RF included AK, pH, MBC, and SWC, where AK and pH were significantly negatively correlated. The major factors influencing RB included AK, AN, and SOC, where AK was a significant negative correlation factor. In MT, the major influencing factors were SOC (explanatory rate, 58.8%), AP, and SWC, where SOC was positively correlated with D3, E2, and E3. The major factors influencing NR included MBN, SWC, MBC, and AP, where MBN, SWC, and AP were significant positive correlation factors. Soil nutrients explained most of the

variation in the soil microbial carbon source metabolic diversity, which indicated that soil nutrients were the main factor influencing the microbial carbon source metabolic activity.

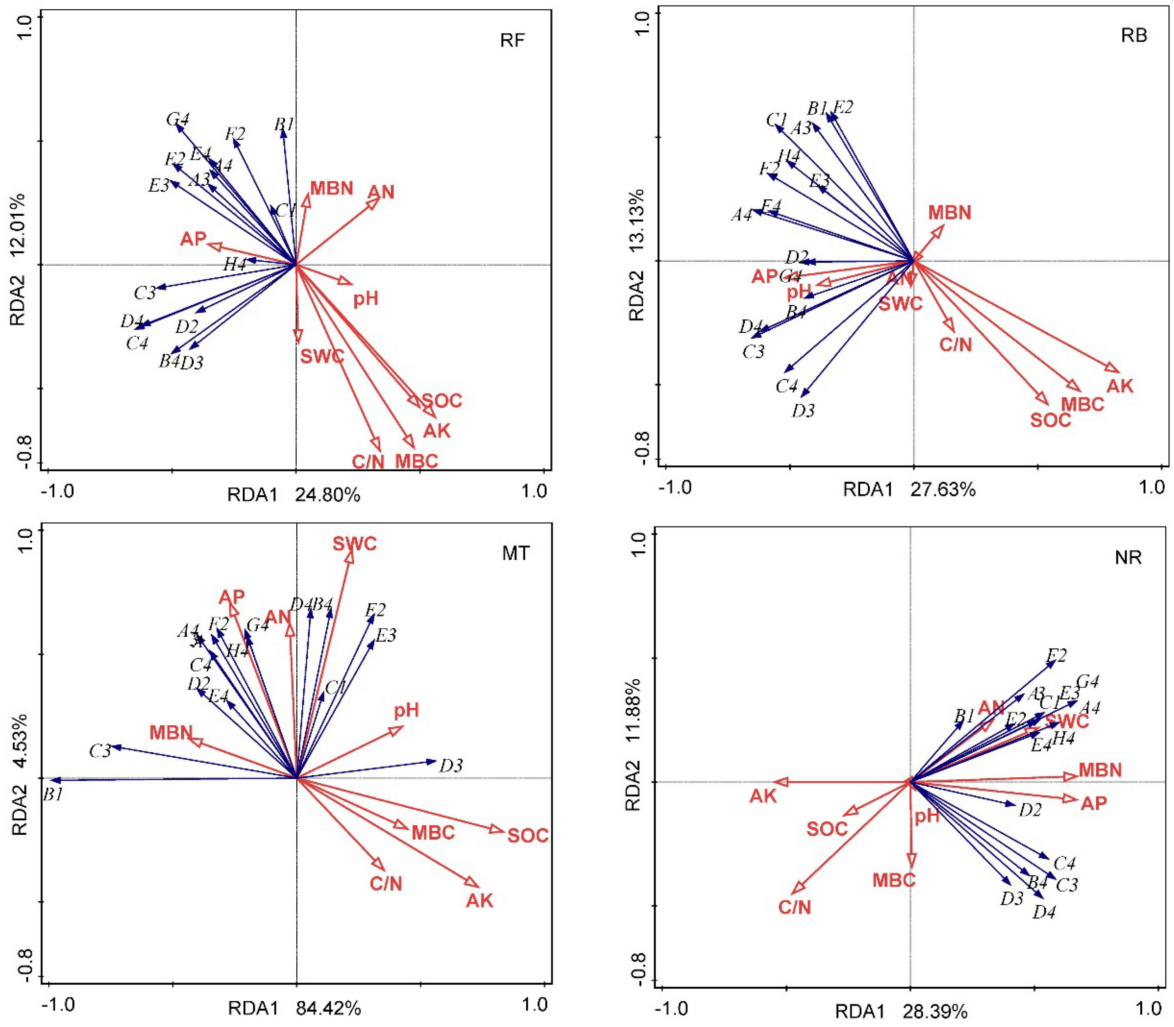

Figure 6. Double sequence diagram of the redundancy analysis in the soil microbial community constrained by soil data. AK, available potassium; AN, available nitrogen; AP, available phosphorus; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; C/N, MBC/MBN; pH, potential of hydrogen; SOC, soil organic carbon; and SWC, soil water content. Carbohydrates: D2 (D-mannitol), A3 (D-galactonic acid lactone), and E2 (N-acetyl-D-glucosamine); phenolic compounds: C3 (2-hydroxy benzoic acid), and D3 (4-hydroxy benzoic acid); polymers: C1 (Tween 40); carboxylic acids: E3 (γ-hydroxy butyric acid), F2 (D-glucosaminic acid), and B1 (pyruvic acid methyl ester); amines: H4 (putrescine) and C4 (phenyl ethylamine); amino acids: A4 (L-arginine), E4 (L-threonine), D4 (L-serine), and G4 (L-phenylalanine), B4 (L-asparagine).

4. Discussion

Based on two years of observation, we studied the characteristics of the variation of the functional diversity of soil microorganisms under different cutting and regeneration methodologies in a Chinese fir plantation and analyzed the driving factors in order to understand the mode of response of the soil microorganisms to plantation management. Our study showed that the modes of response of soil microbial functional diversity in a forest ecosystem to various cutting and regeneration methodologies varied, and that they were also affected by soil physical and chemical properties, as well as the sampling period.

Forest management can affect the metabolic activity of the soil microbial community by changing the soil physical and chemical properties [50–52]. In our study, the AWCD

values (Figures 3 and 4) and diversity index (Figure 5) were combined to show that the soil microbes in the burned area had a higher metabolic activity, including dominance and richness, than those in the unburned area, but that the community evenness was poor. Rosaria et al. (2005) [53] reported that in the first week after a fire disturbance, the carbon source substrate used by soil microorganisms changed slightly and transiently, but the microbial community quickly restored its function and diversity. They also showed that, compared to unburning, fire disturbance increases the functional diversity of soil microorganisms. The nutrients in organic matter are returned to the soil in a relatively short time after residual burning [54,55]. We found that, especially at the early stage, the content of available nutrients such as AP and AN was higher (Figure 2), was positively correlated with the metabolic activity of the soil microbial carbon source, and could effectively improve the metabolic activity of the soil microbial community. However, the comparison of soil samples collected 5 years after burning and unburned soil samples demonstrated that burning significantly reduced the microbial functional diversity [56], suggesting that severe forest disturbances, such as significant burning, should be avoided in order to reduce their effect on the soil microbial community. This may because soil nutrients are only increased for a short period of time after slash-and-burn cultivation and become substantially reduced after many years of using this technique, especially in the rainy, hilly regions of the south of China [57]. A large number of nutrients are directly lost in the form of gas or dust in the instant of slash burning, and the ash left after burning is easily lost by erosion caused by rain in rainy seasons [17]. Unexpectedly, no significant decrease in the metabolic capacity of soil microorganisms in the burning area was observed during our 2-year study, and some still showed a significant increase after two years of treatment (Figure 3), although the soil nutrients in the soil given this treatment had begun to decrease (Figure 2), which may have been caused by the time lag between microbial response and substrate reduction or other factors not observed in this study. However, the community evenness in soil given the burning treatment was always poor (Figure 5). Slash burning can kill and weaken certain plants, animals, and microorganisms, creating an ecological niche [58–60]. An adequate supply of nutrients would help microorganisms to perform all their functions, encourage some microorganisms to compete successfully and come to occupy a dominant position, and thus improve the richness and dominance of the soil microbial community [21,61,62]; however, it would also destroy the microbial community structure and lead to the deterioration of community evenness.

The soil microbial community structure and diversity have been found to be related to the variation in soil nutrients caused by organic mulch [63,64]. Studies have shown that the soil microbial functional diversity in Chinese fir plantations was significantly affected by silvicultural treatments, which was related to the microclimate and soil substrate input [65,66]. Chinese fir could supply a huge amount of litterfall, which was found to be significantly correlated with the input of soil C, N, etc. [67,68]. Therefore, litter may be an important factor supporting soil microorganisms [69]. Once harvest residues reach the ground, they can also be treated as litterfall to an extent.

In our study, the soil given the crushing and mulching treatment had a higher metabolic capacity for microbial carbon sources in summer, especially for amino acids and carbohydrates, which are two major carbon sources used by microorganisms (Figures 3 and 4). Huang et al. (2008) argued that plant residues such as mulch significantly increased the diversity of soil microbial functional communities in Australian subtropical plantations compared with communities given no mulch [70]. When topsoil is mulching, the evaporation rate will be reduced, higher water contents and smaller soil temperature changes will be maintained for a longer period of time [71–73], soil loss will be effectively controlled [74,75], and more suitable habitats can be provided for microbial metabolism activities. This can also increase the substrate required for microbial metabolic activities through the decomposition and leaching of organic matter [76,77], and even help to control weed growth [78,79] and reduce the loss of nutrients [80]. The addition of organic matter can also increase the microbial biomass, change the composition of the microbial community, and lead it

to develop a higher abundance and activity, thus enhancing the functional diversity of microorganisms [81]. Compared with untreated soil, mulching crushed residues can cause the non-synchronous decomposition of organic matter, providing different habitats and nutrients for microorganisms and thus changed the metabolic mechanism of microorganisms. As the host of microorganisms, fresh organic matter, after being subjected to crushing, not only greatly increased the area available for microbial lodging but also increased the contact area of organic matter with air, water, and sunlight and accelerated the decomposition rate of organic matter, especially in the hot and rainy summer. This provided a more favorable habitat and greater amount of substrate for microorganisms [77] and in turn improved the carbon source metabolic activity of soil microorganisms.

A plot with no residues will have a reduced soil quality [4], which may be the main reason for the low metabolic activity of microorganisms seen in this treatment. Large amounts of organic matter were removed from the ecosystem in this treatment, leading to the direct loss of large amounts of nutrients [4,82]. Although the SOC was higher in the initial stage of treatment, this was only due to the rapid decomposition of the surface humus layer due to the exposure of the surface and direct sunlight [83]. At a later stage, having no residues to reduce the nutrients, SWC, and pH affected the functional diversity of soil microorganisms [82–85].

Overall, the use of burning and crushing treatments showed certain advantages for the functional diversity of soil microorganisms. However, the microbial biomass seen in the burning treatment was lower throughout the whole observation process, which may have had a profound impact on the sustainability of the soil ecosystem and had no sustained effect on promoting the supply of soil nutrients [21]. Moreover, it has been proven that burning can only increase the functional diversity of microorganisms for a short period [56]. Therefore, we prefer the use of the crushing treatment, which may be applied for the future management of Chinese fir plantations. Of course, longer-term data are needed to verify the effects of this process.

5. Conclusions

In our study, we found the functional diversity of soil microorganisms to be significantly affected by the use of different forest cutting and regeneration methodologies, which varied with the sampling period. Compared with the non-burning treatments (RF, MT, and NR), the burning treatment (RB) increased the amount of nutrients in the soil temporarily, but led to them decreasing after 2 years of treatment. This also elevated the microbial functional diversity, but reduced the MBC and MBN and the evenness of the microbial distribution. The crushing and mulching treatment (MT) significantly increased the AK and MBN levels at the early stage compared to the reference treatment (RF), elevated MBC and MBN compared with RB and NR after one year, and increased the utilization capacity of carbohydrates and amino acids in summer compared with RF. Compared with residual retention (RF and MT), residual removal (NR) had a negative impact on the soil available nutrients, which showed a poorer carbon utilization capacity in soil microorganisms. Soil nutrients are key factors in the metabolic capacity of carbon sources in the soil microbial community in Chinese fir plantations. Amino acids, carboxylic acids, and carbohydrates are the major carbon sources used by soil microorganisms. Moreover, the carbon utilization capacity was lower at the early stage following treatment and gradually rose to a maximum in summer. Overall, considering the short-term soil nutrient and soil microbial functional diversity and biomass in MT showed certain advantages and is expected to have positive effects on saplings during the growth stage.

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Appendix A

Table A1. The effect of residual treatments (T), study period (S, season; Y, year), and their interaction on the soil physicochemical index and microbial biomass, which were analyzed using repeated nested analysis.

Index	T	%	S	%	S/Y	%	T × S	%	T × S/Y	%
Physicochemical index [1]										
SOC	1.91	0.46	100.75 ***	16.10	420.61 ***	67.20	3.56 **	1.70	3.68 **	1.76
AN	31.58 ***	5.90	341.39 ***	42.49	248.06	30.88	7.89 ***	2.95	20.98 ***	7.84
AP	0.51	0.43	91.34 ***	51.89	0.42	0.24	1.17	1.99	0.00	0.01
AK	6.55 ***	2.34	60.39 ***	14.36	260.25 ***	61.89	0.94	0.67	2.42 *	1.72
pH	18.57 ***	3.39	612.58 ***	74.44	30.32 ***	3.68	7.97 ***	2.91	16.08 ***	5.86
SWC	6.69 ***	1.20	464.76 ***	55.62	252.28 ***	30.19	2.65 *	0.95	6.87 ***	2.47
Microbial biomass [2]										
MBC	1.10	1.00	1.78	1.07	66.68 ***	40.28	1.62	2.94	3.52 **	6.37
MBN	10.38 ***	11.45	11.71 ***	8.61	9.46 ***	6.96	0.75	1.67	5.64 ***	12.45
MBC/MBN	6.19 ***	4.83	7.09 **	3.69	71.07 **	37.00	1.50	2.35	6.70 ***	10.47

[1] SOC, soil organic carbon; AN, available nitrogen; AP, available phosphorus; AK, available potassium; pH, potential of hydrogen; SWC, soil water content. [2] MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; MBC/MBN, microbial biomass C/N ratios. Notes: F values and their contributions (%) are listed. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table A2. The mean AWCD at 96 h in different treatments from 2018 to 2020.

Date	RF	RB	MT	NR
18 September	0.209 ± 0.027	0.248 ± 0.070	0.201 ± 0.022	0.188 ± 0.010
19 March	0.325 ± 0.015	0.381 ± 0.012	0.367 ± 0.041	0.321 ± 0.010
19 June	0.527 ± 0.034	0.612 ± 0.031	0.676 ± 0.015	0.591 ± 0.071
19 September	0.594 ± 0.041	0.500 ± 0.043	0.258 ± 0.091	0.449 ± 0.111
20 September	0.314 ± 0.022	0.441 ± 0.042	0.343 ± 0.018	0.292 ± 0.012

Table A3. Mean ± standard error values for carbon sources and diversity index in different seasons (S) and years (Y).

Index	S			Y		
	Spring	Summer	Fall	0	1	2
AWCD	0.348 ± 0.012 ^{c}	0.599 ± 0.021 ^{a}	0.470 ± 0.042 ^{b}	0.221 ± 0.018 ^{b}	0.470 ± 0.042 ^{a}	0.348 ± 0.016 ^{c}
Carbohydrates	0.272 ± 0.020 ^{c}	0.649 ± 0.035 ^{a}	0.456 ± 0.043 ^{b}	0.196 ± 0.027 ^{b}	0.456 ± 0.043 ^{a}	0.276 ± 0.024 ^{b}
Phenolic compounds	0.285 ± 0.016 ^{a}	0.365 ± 0.037 ^{a}	0.398 ± 0.058 ^{a}	0.150 ± 0.017 ^{b}	0.398 ± 0.058 ^{a}	0.064 ± 0.014 ^{b}
Polymers	0.375 ± 0.013 ^{ab}	0.335 ± 0.017 ^{b}	0.420 ± 0.042 ^{a}	0.233 ± 0.020 ^{c}	0.420 ± 0.042 ^{b}	0.562 ± 0.025 ^{a}
Carboxylic acids	0.422 ± 0.014 ^{b}	0.659 ± 0.024 ^{a}	0.482 ± 0.048 ^{b}	0.305 ± 0.023 ^{b}	0.482 ± 0.048 ^{a}	0.491 ± 0.015 ^{a}
Amines	0.356 ± 0.014 ^{b}	0.598 ± 0.051 ^{a}	0.383 ± 0.044 ^{b}	0.186 ± 0.019 ^{b}	0.383 ± 0.044 ^{a}	0.262 ± 0.030 ^{b}
Amino acids	0.383 ± 0.017 ^{c}	0.687 ± 0.030 ^{a}	0.493 ± 0.051 ^{b}	0.181 ± 0.015 ^{b}	0.493 ± 0.051 ^{a}	0.246 ± 0.021 ^{b}
H′	2.934 ± 0.014 ^{b}	3.090 ± 0.013 ^{a}	2.961 ± 0.067 ^{b}	2.632 ± 0.073 ^{b}	2.961 ± 0.067 ^{a}	2.760 ± 0.017 ^{b}
D	0.940 ± 0.001 ^{b}	0.949 ± 0.001 ^{a}	0.947 ± 0.004 ^{a}	0.909 ± 0.011 ^{c}	0.947 ± 0.004 ^{a}	0.928 ± 0.001 ^{b}
E	0.999 ± 0.002 ^{ab}	0.988 ± 0.001 ^{b}	1.010 ± 0.007 ^{a}	0.934 ± 0.053 ^{a}	1.010 ± 0.007 ^{a}	0.988 ± 0.002 ^{a}

AWCD: average well color development. Carbohydrates (n = 10): H1 (α-D-L-lactose), A2 (β-methyl-D-glucoside), H2 (D, L-α-glycerol phosphate), G1 (D-cellobiose), D2 (D-mannitol), C2 (i-erythritol), G2 (glucose-1-phosphate),

B2 (D-xylose), A3 (D-galactonic acid lactone), and E2 (*n*-acetyl-D-glucosamine); phenolic compounds (n = 2): C3 (2-hydroxy benzoic acid) and D3 (4-hydroxy benzoic acid); polymers (n = 4): F1 (glycogen), C1 (Tween 40), D1 (Tween 80), and E1 (α-cyclodextrin); carboxylic acids (n = 7): G3 (α-ketobutyric acid), B3 (D-galacturonic acid), E3 (γ-hydroxy butyric acid), F2 (D-glucosaminic acid), H3 (D-malic acid), F3 (itaconic acid), and B1 (pyruvic acid methyl ester); amines (n = 2): H4 (putrescine) and C4 (phenyl ethylamine); amino acids (n = 6): A4 (L-arginine), E4 (L-threonine), D4 (L-serine), G4 (L-phenylalanine), B4 (L-asparagine), and F4 (glycyl-L-glutamic acid). Diversity index: H', Shannon–Wiener diversity index; D, Simpson dominance index; E, McIntosh evenness index. Time: spring (March 2019); summer (June 2019); fall (September 2019); 0 (September 2018); 1 (September 2019); 2 (September 2020). Statistically significant differences for each parameter within each factor (treatment, season, and year) are shown in different letters ($p < 0.05$).

Table A4. Explanatory rate and contribution rate of soil environmental factors to the change in soil microbial carbon source metabolic activity.

Treatment	Variables	Explains %	Contribution %	Pseudo-F	*p*
RF	AK	12.7	27.9	6.3	0.002
	pH	8.7	19.1	5.0	0.004
	MBC	5.8	12.6	3.6	0.008
	SWC	5.1	11.2	2.7	0.012
	AN	4.4	9.6	2.2	0.028
	MBN	3.9	8.5	2.5	0.012
RB	AK	21.5	43.3	11.8	0.002
	SOC	5.9	11.8	3.4	0.006
	AN	7.7	15.4	5.0	0.002
MT	SOC	58.8	64.6	61.4	0.002
	AP	8.9	9.8	19.1	0.002
	SWC	7.6	8.3	9.5	0.002
	MBN	6.0	6.6	8.9	0.002
	MBC	6.0	6.6	18.3	0.002
	AK	2.0	2.2	7.1	0.002
	AN	0.8	0.9	3.1	0.03
NR	MBN	13.4	25.9	6.7	0.002
	SWC	10.6	20.5	5.9	0.004
	MBC	6.3	12.2	3.7	0.004
	AP	5.7	11.0	3.5	0.014
	pH	5.1	9.8	3.4	0.014
	SOC	4.5	8.7	3.3	0.008

AK, available potassium; SWC, soil water content; SOC, soil organic carbon; AP, available phosphorus; pH, potential of hydrogen; AN, available nitrogen; MBN, microbial biomass nitrogen; MBC, microbial biomass carbon. Variables arere listed when $p < 0.05$.

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