agronomy

MDPI

Article

Effects of Organic Materials and Their Incorporation Depths on Humus Substances Structure and Soil Microbial Communities' Characteristics in a Chinese Mollisol

Jiawei Gan, Wenxiu Zou, Xiaozeng Han, Xu Chen, Jun Yan and Xinchun Lu *

Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Harbin 150081, China; ganjiawei2023@163.com (J.G.); zouwenxiu@iga.ac.cn (W.Z.); xzhan@iga.ac.cn (X.H.); chenxu@iga.ac.cn (X.C.); yanjun@iga.ac.cn (J.Y.)

* Correspondence: luxinchun@iga.ac.cn; Tel.: +86-0451-86691092

Abstract: Organic material incorporation are important agricultural practices, which can influence soil organic carbon (SOC) sequestration and stabilization. However, the response of interaction between SOC structure and soil microbial to organic material incorporation management are still poorly understood. In 2021, we conducted a three years field experiment in Guangrong country, northeastern China. Five treatments were established: conventional tillage (CK), conventional tillage with straw incorporation (T1); subsoil tillage with straw incorporation (T2); subsoil tillage with straw and organic manure incorporation (T3) and subsoiling tillage with organic manure incorporation (T4). Fulvic–like and protein–like components were found in fulvic acid (FA) in a 0–15 cm soil layer, while fulvic–like components in humic acid (HA) were found in 0–15 cm and 15–35 cm soil layers. In the 15–35 cm soil layer, the bacterial, fungal and total phospholipid fatty acid (PLFA) contents were significantly higher by 159.62%, 687.00%, and 139.02% in T3 than CK, respectively. The fungal to bacterial PLFA ratios (F/B) were significantly higher by 97.46% and the Gram–positive bacteria to Gram–negative bacteria PLFA ratios (G^+/G^-) were lower by 20.99% in T3 than CK in the 15–35 cm soil layer. Therefore, subsoil tillage with straw and organic manure incorporation could be recommended to improve soil quality in Mollisol.

Keywords: organic material return; tillage depths; PLFAs; humus substance; black soil

Citation: Gan, J.; Zou, W.; Han, X.; Chen, X.; Yan, J.; Lu, X. Effects of Organic Materials and Their Incorporation Depths on Humus Substances Structure and Soil Microbial Communities' Characteristics in a Chinese Mollisol. *Agronomy* **2023**, *13*, 2169. https://doi.org/10.3390/agronomy13082169

Academic Editor: Jiafa Luo

Received: 2 August 2023
Revised: 16 August 2023
Accepted: 17 August 2023
Published: 18 August 2023

1. Introduction

Soil organic carbon (SOC) plays a major role in the global carbon cycle, such as maintenance of soil fertility and support of plant growth [1,2]. Nowadays, the management of SOC is even more important than before because of high-intensity land use in terms of storing more carbon in the land and maintaining yields of crops. As the largest constituents of SOC, humic substances (HS) play a vital role in maintaining soil ecosystem services [3], which account for 85–90% of the total SOC, including fulvic acid (FA), humic acid (HA) and humin (HM). Compared to other soil compounds, HS have a unique structural pattern and its chemical composition can provide a good representation of natural organic matter [4]. In recent years, increasing attention was devoted to elucidate humic substances' structural characteristics [5]. These studies demonstrated that HS structural characteristics undergo significant changes upon shifts in soil management practices. Three-dimensional excitation emission matrix (3D–EEM) fluorescence spectroscopy has gradually been used to analyze the structure, configuration and kinetics of HS interactions with molecules and intramolecular interactions by researchers [6,7]. Furthermore, 3D–EEM has advantages of strong selectivity, high sensitivity and no damage to the sample, and its potential to analyze HS structures was demonstrated [8,9], while parallel factor analysis (PARAFAC) can mathematically quantify and qualitatively decompose complex fluorescence spectra into individual fluorescence components [10]. Therefore, 3D–EEM fluorescence spectroscopy

combined with PARAFAC was used for the description of HS structures, and provides a variety of spectral matrix data that can be excellent taxonomic resolution used to calculate specific indicators to characterize and evaluate the properties of HS components. Overall, understanding the chemical compositions of HS will help us clarify the response mechanism of SOC structure to soil management.

Soil microorganisms also play a key role in mediating changes in soil via mineralization of SOC [11]. Studies have shown that characterization of soil microbial communities is an early means of quantifying the increase and stability of SOC levels under different soil management systems [12,13]. Researchers have widely used phospholipid fatty acid (PLFA) method to analyze the overall composition and relative abundance of soil microbial communities. [14,15]. Although genomic methods have excellent taxonomic resolution, PLFA analysis is more relevant for quantitative characterization of the relative biomass of a group [12]. The PLFA analysis can help us to explore the effects of different organic material incorporation on microbial communities to investigate the role of microorganisms in soil management practices. Many studies have reported that soil management practices influence SOC contents by affecting soil microorganisms [16–18]. However, our study will explore the effects of soil microorganisms on the HS structure.

The region of Mollisol is an important soil resource for crop production and plays a unique role in food security in China [19]. However, SOC contents have decreased by 22.3% over the past three decades in the Mollisol region in Northeastern China because of intensive cultivation with lack of organic material inputs [20]. Moreover, HS structure characteristics have significantly changed because of improper soil management, such as a decrease of aliphatic C and hydrophobic C [21]. Tillage, as one of the most significant anthropogenic activities, significantly alters soil properties. However, conventional tillage practice decreases the contents of carbon and the soil microbial activities in the whole cultivated layer (0–35 cm soil layer) [22]. Moreover, long-term conventional tillage practice inhibits the exchange of heat, gas and water between the 0–15 cm soil layer and 15–35 cm soil layer [23]. Therefore, it is urgent to improve soil fertility after long-term conventional tillage without organic material incorporation [24]. In an agroecosystem, crop straw is a major source of organic carbon [25], and straw incorporation is considered as an important practice for offsetting carbon loss in agricultural soil [26]. Few data are available to date on the response of soil to different organic material incorporation depths, and changes in HS structural characteristics and soil microbial communities remain unclear. The aims of the study were to (I) identify the humus components according to PARAFAC under organic material incorporation, (II) examine the effects of organic material incorporation on soil microbial community composition characteristics, and (III) identify interaction between humus components and the soil microbial community.

2. Materials and Methods

2.1. Study Description and Experimental Layout

The field experiment was established in 2018 at Guangrong country (47.27° N, 126.41° E; 240 m a.s.l.) in Heilongjiang Province, China. The USDA Soil Taxonomy System classifies the soil at the site as a Mollisol [27]. The study site is in a moderate temperate continental monsoon climate zone with average annual rainfall of 550 mm and air temperature of 1.5 °C.

The experimental design was a completely randomized block with three replicates of five treatments: conventional tillage (CK, tillage depths to 15 cm with no straw incorporation), conventional tillage with straw incorporation (T1, tillage depths to 15 cm), subsoil tillage with straw incorporation (T2, tillage depths to 35 cm), subsoil tillage with straw and organic manure incorporation (T3, tillage depths to 35 cm) and subsoil tillage with organic manure (T4, tillage depths to 35 cm). The rate of straw incorporation in T1, T2 and T3 was 10,000 kg ha^{-1}, with the corn straw being cut by a machine into segments <5 cm long and evenly mixed into the 0–15 cm soil layer in T1 and the 0–35 cm soil layer in T2 and T3. The rate of straw incorporation was determined by the total straw production. The

rate of organic manure incorporation in T3 and T4 was 30,000 kg ha^{-1}. Furthermore, the organic carbon and total nitrogen contents of corn straw were 40.5% and 0.62%, and those of organic manure were 17.06% and 1.09%. Each plot was 12 m^2 (4 m × 3 m); the cropping system was a maize–soybean rotation. The rates of fertilization were 180 kg N ha^{-1} as urea, 70.0 kg P_2O_5 ha^{-1} as diammonium phosphate and 60 kg K_2O ha^{-1} as potassium sulphate in the maize field, and 27 kg N ha^{-1} as urea, 70 kg P_2O_5 ha^{-1} as diammonium phosphate, 60 K_2O kg as potassium sulphate in the soybean field. The field management practices adopted were the same as those used by local famers.

2.2. Soil Sampling

Soil samplings were collected in October 2021 from the 0–15 cm and 15–35 cm soil layers for each treatment at four randomly selected points, and then mixed into composite samples. These mixed samples were placed in sterile bags and transported to the laboratory for analysis. Visible stones, plant residues, and other organic debris were removed by hand, and we divided soil samples into two subsamples. The first set of subsamples were passed through a 2.00 mm sieve and stored in plastic bags at 4 °C prior to analyses of microbial biomass carbon (MBC), microbial biomass N (MBN) and PLFA. The other set of subsamples were air dried at room temperature and used for chemical analysis. The air-dried samples were sieved (0.25 mm) and milled for soil chemical properties, fulvic acid (FA) and humic acid (HA) analyses.

2.3. Soil Properties Testing

Soil total carbon and nitrogen contents were analyzed using an elemental analyzer (EA3000, Euro Vector, Pavia, Italy). Soil total carbon precisely represents SOC because the Mollisol are carbonate-free in the study area. Soil pH was measured using a pH meter (Delta 320, Mettler Toledo, Greifensee, Switzerland) on a 1:2.5 (w/v) mixture of soil and water. The contents of available nitrogen (AN), available phosphorus (AP) and available potassium (AK) were measured as described by Taylor and Francis [28]. Before and after drying at 105 °C for 24 h, soil–moisture content was measured by weighing the soil. We used chloroform fumigation-extraction to measure MBC and MBN [29].

2.4. Humic Substances Extraction, Fluorescence Spectra and EEM PARAFAC Analysis

Humus composition was analyzed following the method described by Zhang et al. [30]. Briefly, 3 g of air–dried soil was extracted with 30 mL distilled water (removed CO_2) under permanent shaking (180 r min^{-1}) at 50 °C for 1 h. The mixture was centrifuged for 15 min at 3000× g and filtered through a membrane filter. The supernatant was dissolved with organic carbon. The remaining soil was extracted with a 30 mL mixture of 0.1 M alkali solution (NaOH + $Na_4P_2O_7$) under permanent shaking (180 r min^{-1}) at 50 °C for 24 h. The mixture was centrifuged for 15 min at 3000× g and filtered. The supernatant was acidified to pH 1, then left standing at room temperature for 12 h, centrifuged, and filtered. This supernatant was fulvic acid (FA). The sediments were dissolved with 0.05 mol L^{-1} $NaHCO_3$ and filtered. The solution was humic acid (HA).

FA and HA concentrations were measured by a TOC analyzer (Elementar Analysen systeme, Hanau, Germany), and fluorescence spectra were obtained for aqueous solutions of FA and HA at a concentration of 10 mg L^{-1}. Spectra were recorded using a F7000 Fluorescence spectroscopy (Hitachi, Tokyo, Japan). Fluorescence spectra in the form of excitation/emission matrices (EEMs) were recorded over the emission (EM) wavelength range from 200 to 600 nm, and excitation (EX) wavelength range from 200 to 600 nm. The sampling interval with EM and EX was 5 nm and 10 nm, respectively, and the scan speed was 2000 nm min^{-1}. We used PARAFAC analysis with the DOMFluor toolbox [10], which includes all the tools used to identify outlier samples and perform split-half analysis and residual errors diagnostics.

The fluorescence index (FI) and humification index (HIX) were the indexes used for evaluating of changes of FA and HA. FI and HIX were calculated as [9,10];

$$\mathrm{FI} = \mathrm{Em}\,\frac{370\ \mathrm{nm}}{450\ \mathrm{nm}}\ (\mathrm{Ex} = 370\ \mathrm{nm}) \quad (1)$$

$$\mathrm{HIX} = \mathrm{Em}\,\frac{435 - 480\ \mathrm{nm}}{300 - 345\ \mathrm{nm}}\ (\mathrm{Ex} = 254\ \mathrm{nm}) \quad (2)$$

where the values of Ex and Em are the results in the fluorescence spectrogram after parallel factor analysis.

2.5. PLFA Analysis

PLFAs were extracted from 8 g of the freeze–dried soil samples to analyze microbial community structure [31]. The freeze–dried soil samples were taken and added with 30.4 mL of single-phase, citrate buffer–chloroform–methanol (volume ratio 0.8:1:2), shaken for 150 min away from light, and centrifuged for 7 min (4000× *g*). The phospholipid layer existed in the lower layer of the chloroform layer; the chloroform layer was transferred to a test tube and then the chloroform was blown dry with nitrogen at 30 °C. Then, phospholipids were separated from these glycolipids and neutral lipids by silica solid-phase extraction columns (Supelco, Bellefonte, PA, USA). Polar lipids were methylated and PLFA methyl esters were analyzed using an Agilent 6890A gas chromatograph (GC) (Agilent Tech, Santa Clara, CA, USA) equipped with an HP–5 capillary column and a flame ionization detector. Prior to GC analysis, the samples were dissolved in 150 µL of hexane, and methyl nonyl decanoate (19:0, Sigma-Aldrich, St. Louis, MO, USA) was added as an internal standard for quantification. Purified nitrogen was used as carrier gas at a 0.8 mL min^{-1} flow rate. Ultrapure nitrogen at a flow rate of 0.8 mL min^{-1} was used as the carrier gas. The Supelco 37 Component fatty acid methyl esters (FAMEs) were mixed, and bacterial acid methyl esters (Sigma-Aldrich) were used for peak identification and quantification. There were 27 PLFAs identified and used for data analysis.

For the taxonomic categorization of PLFA biomarker data, we used fatty acids 14:0, a14:0, i14:0, 15:0, a15:0, 15:0DMA, i15:0, i15:1ω6c, 16:0, i16:0, 16:1ω7c, 17:0, a17:0, cy17:0ω7c, i17:0, 17:1ω8c, 18:0, 18:1ω5c, 18:1ω7c, cy19:0ω7c and 20:0 to represent bacterial PLFAs. Fatty acids 18:1ω9c and 18:2ω6c were used as indicators of fungal PLFAs [32]. We used fatty acids 10Me16:0, 10Me17:0, 10Me18:0 and 10Me18:1ω7c to represent actinomycetic PLFAs [33]. Fatty acids i14:0, a14:0, i15:0, a15:0, i16:0, i17:0, a17:0 and i15:1ω6c were used as biomarkers of Gram–positive (G^+) bacteria and 16:1ω7c, cy17:0ω7c, 17:1ω8c, 18:1ω5c, 18:1ω7c and cy19:0ω7c were used as biomarkers of Gram–negative (G^-) bacteria [34]. The ratio of Gram–positive to Gram–negative biomass (G^+/G^-) was considered to be a stress indicator based on PLFA [35]. The ratio of fungal to bacterial biomass (F/B) was used to assess the relative advantage of fungi over bacteria [36]. The sum of PLFAs represent the microbial lipid biomass.

2.6. Statistical Analysis

We used SPSS 17.0 (IBM, Armonk, NY, USA) to conduct statistical analyses. Significant differences among treatments were identified using a one-way analysis of variance (ANOVA) in combination with an LSD test ($p \leq 0.05$, $p \leq 0.01$) for all data. Figures for the principal component analysis (PCA) were created using Origin 2019b (IBM, Armonk, NY, USA). Figures for fluorescence spectra was performed using Matlab 2020a software (MathWorks, Natick, MA, USA). The partial least squares path modelling (PLS–PM) was used to evaluate the direct and indirect relationships among all indicators by using the R v4. 2. 3 software and PLS–PM packages. Direct effects (i.e., path coefficients) represent the direction and strength of linear relationships between variables [37].

3. Results

3.1. Soil Properties

The T1, T2, T3 and T4 significantly increased SOC contents by 6.16–15.38% I then 0–15 cm soil layer compared with CK ($p < 0.05$). The SOC and TN contents significantly

increased by 1.09–20.11% and 5.73–19.11% in T2, T3 and T4 in the 15–35 cm soil layer, compared with CK, respectively ($p < 0.05$). Soil pH did not differ significantly between CK and other treatments in the 15–35 cm soil layer, and no significant difference appeared in the AK contents in the 0–35 cm soil layer among the five treatments. The AN and AP contents were increased by 3.17–12.76% and 2.31–134.98% in T1, T2, T3 and T4 in the 0–15 cm soil layer, compared with CK. The AN and AP contents were 15.78–33.91% and 232.97–323.84% higher in T2, T3 and T4 than those in CK for the 15–35 cm soil layer. MBC contents of T3 were significantly higher by 79.31% than CK in the 0–15 cm soil layer ($p < 0.05$). In the 15–35 cm soil layer, T2, T3 and T4 significantly increased the MBC contents by 43.44–97.49%, compared with CK ($p < 0.05$). MBN contents were significantly higher by 19.95% in T3 than those in CK ($p < 0.05$) for the 0–15 cm soil layer. Compared with CK, the T2, T3 and T4 exhibited significantly increased MBN contents of 18.87–46.75% ($p < 0.05$) in the 15–35 cm soil layer (Table 1).

Table 1. Soil properties under different organic materials and their incorporation depths.

Treatments	Layers	SOC	TN	pH	AN	AP	AK	MBC	MBN
	(cm)	(g kg^{-1})	(g kg^{-1})		(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
CK	0–15	19.5 ± 0.02 d	1.72 ± 0.01 c	6.57 ± 0.01 a	101.1 ± 0.73 d	28.99 ± 1.84 c	130.5 ± 4.86 a	179.3 ± 8.40 c	12.88 ± 1.21 c
	15–35	18.4 ± 0.02 c	1.57 ± 0.01 c	6.60 ± 0.01 a	78.71 ± 0.41 d	7.34 ± 1.12 d	104.3 ± 2.87 a	111.8 ± 3.09 c	9.54 ± 0.39 c
T1	0–15	21.9 ± 0.10 b	1.74 ± 0.01 c	6.34 ± 0.01 b	108.7 ± 1.81 b	41.55 ± 1.99 b	140.0 ± 5.21 a	269.1 ± 8.10 b	13.12 ± 2.39 c
	15–35	19.2 ± 0.04 bc	1.61 ± 0.01 bc	6.61 ± 0.01 a	88.75 ± 0.59 c	14.88 ± 1.52 c	105.3 ± 2.87 a	148.4 ± 3.70 b	10.13 ± 1.23 c
T2	0–15	20.8 ± 0.02 c	1.78 ± 0.01 b	6.41 ± 0.01 b	104.4 ± 0.98 c	29.66 ± 0.53 c	139.5 ± 5.33 a	214.8 ± 8.68 c	12.39 ± 7.97 c
	15–35	19.9 ± 0.01 b	1.67 ± 0.01 b	6.61 ± 0.01 a	96.00 ± 1.11 b	22.71 ± 1.14 b	109.3 ± 6.45 a	204.7 ± 1.35 a	11.46 ± 0.096 b
T3	0–15	22.5 ± 0.01 a	1.94 ± 0.01 a	6.35 ± 0.01 b	114.0 ± 0.78 a	68.12 ± 1.24 a	149.0 ± 3.24 a	321.5 ± 7.97 a	15.45 ± 8.68 a
	15–35	22.1 ± 0.03 a	1.87 ± 0.01 a	6.61 ± 0.01 a	105.4 ± 0.38 a	31.11 ± 1.47 a	112.3 ± 4.68 a	220.9 ± 2.39 a	14.00 ± 2.92 a
T4	0–15	20.7 ± 0.01 c	1.78 ± 0.02 b	6.23 ± 0.01 d	104.3 ± 1.11 c	32.95 ± 1.31 c	139.0 ± 2.27 a	296.8 ± 4.70 b	14.34 ± 1.28 b
	15–35	18.6 ± 0.03 bc	1.66 ± 0.01 b	6.61 ± 0.01 a	91.22 ± 0.59 c	24.44 ± 3.00 b	109.8 ± 3.82 a	160.5 ± 1.28 b	11.34 ± 0.69 b
Two-way ANOVA results (p values)									
Treatment (T)		$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.01$	$p < 0.001$	$p < 0.01$	$p < 0.001$	$p < 0.001$
Layer (L)		$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
T × L		$p < 0.001$	N.A.	$p < 0.001$	$p < 0.001$	$p < 0.01$	$p < 0.001$	$p < 0.01$	N.A.

Different letters indicate significant differences between samples in the same soil layer ($p < 0.05$). Values are mean ± errors ($n = 4$). Abbreviations: SOC: Soil Organic Carbon; TN: Total Nitrogen; AN: Available Nitrogen; AP: Available Phosphorus; AK: Available potassium; MBC: Microbial Biomass Carbon; MBN: Microbial Biomass Nitrogen.

3.2. Fluorescence Spectra of Humus Substance

The T1, T2, T3 and T4 significantly increased FA–C and HA–C contents by 9.36–40.90% and 14.86–43.79% ($p < 0.05$) in the 0–15 cm soil layer, respectively. The FA–C and HA–C contents were significantly increased by 25.19–29.70% and 7.46–41.79% in T2 and T3, compared with CK ($p < 0.05$), in the 15–35 cm soil layer. The contents of FA–C and HA–C increased in the order of T3 > T1 > T2 > T4 in the 0–15 cm soil layer, and the FA–C and HA–C contents increased in the order of T3 > T2 > the T4 in 15–35 cm soil layer. FA–C and HA–C contents were higher in the 0–15 cm soil layer than the 15–35 cm soil layer under T1, T2, T3 and T4 (Figure 1).

We found three identified fluorescent components in the 0–15 cm soil layer and four components in the 15–35 cm soil layer (Figure 2). Components C1 and C2 were fulvic–like components associated with high-molecular weight (HMW) and aromatic organic compounds originating from some sources, such as straw incorporation, in the 0–15 cm soil layer. Components C3 were protein–like components (tyrosine or tryptophan-like), representing proteinaceous compounds from microbial activity, such as amino acids, peptide materials and free or bound proteins, in the 0–15 cm soil layer. In the 15–35 cm soil layer, components C1, C2 and C3 were fulvic–like components and components C4 were protein–like components. In the 0–15 cm and 15–35 cm soil layers, the HMW aromatic fluorophores from plant sources were abundant, while the protein–like components from microbial activity contributed little. The FA was comprised in HMW aromatic and LMW proteinaceous and condensed organic compounds in the 0–15 cm and 15–35 cm soil layers.

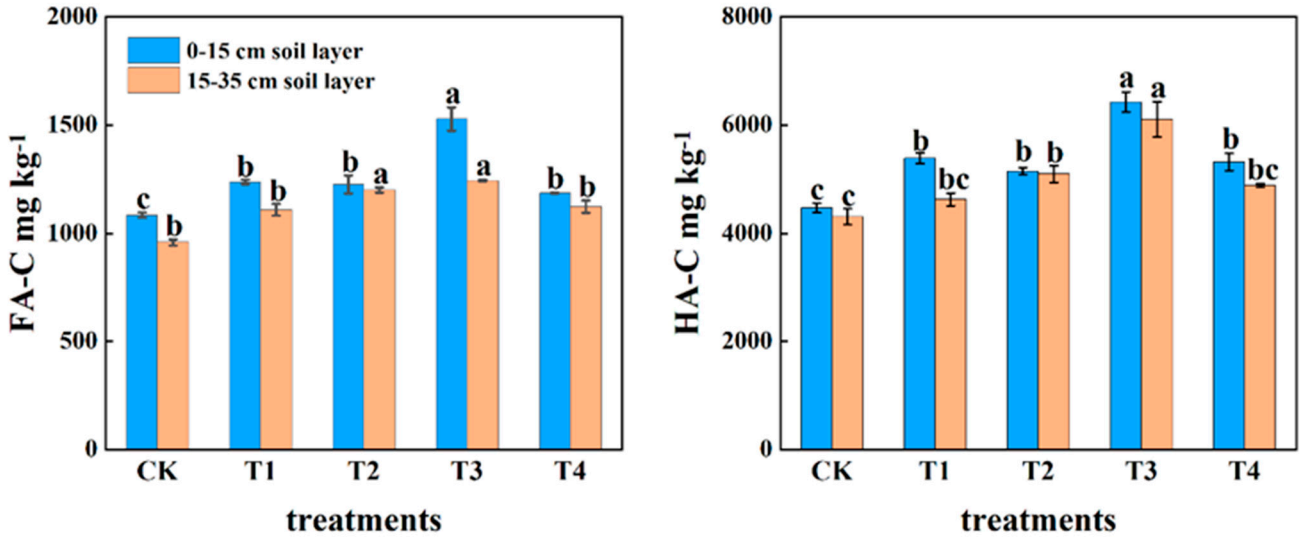

Figure 1. Effects of different organic materials and their incorporation depths on FA–C and HA–C. Different letters indicate significant differences between samples in the same soil layer ($p < 0.05$). Values are mean ± errors ($n = 4$). Abbreviations: FA–C: Fulvic Acid Carbon; HA–C: Humic Acid Carbon.

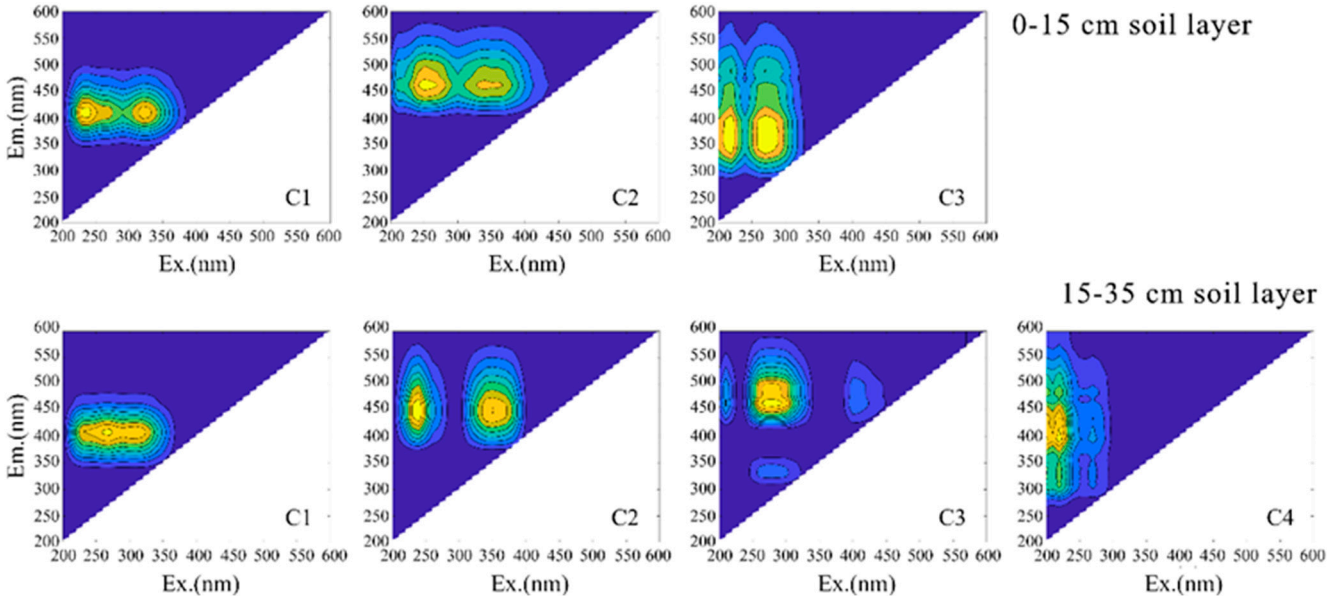

Figure 2. Components identified using PARAFAC analysis of FA under different organic materials and their incorporation depths. Abbreviations: C1: Component 1; C2: Component 2; C3: Component 3; C4: Component 4.

In the 0–15 cm soil layer, the fulvic–like components of FA (C1, C2) were the most abundant, accounting for 83.95% [80.58–87.26%] of the total fluorescence, whereas the protein–like component (C3) accounted for 16.05% [12.74–19.42%]. In the 15–35 cm soil layer, the fulvic–like components of FA (C1, C2, C3) and protein–like component (C4) accounted for 89.60% [83.73–91.48%] and 12.40% [8.52–16.27%] of the total fluorescence (Figure S1). The protein–like component contributed less than fulvic–like components in the whole layer of the fluorescent FA.

The PARAFAC modeling for HA fluorescence was conducted in the 0–15 cm and 15–35 cm soil layers (Figure 3). We found three identified fluorescent components in the 0–15 cm and 15–35 cm soil layers. Components C1, C2 and C3 were all fulvic–like components associated HMW in the 0–15 cm and 15–35 cm soil layers, and the HMW aromatic fluorophores were derived from plant sources. In the 0–15 cm soil layer, C1, C2

and C3 of HA accounted for 48.20% [42.59–53.81%], 28.30% [24.63–31.97%], and 23.50% [16.40–30.60%] of the total fluorescence, respectively. And in the 15–35 cm soil layer, C1, C2 and C3 of HA accounted for 55.51% [52.11–58.19%], 22.56% [20.46–24.66%] and 21.93% [17.67–26.19%] of the total fluorescence, respectively (Figure S2).

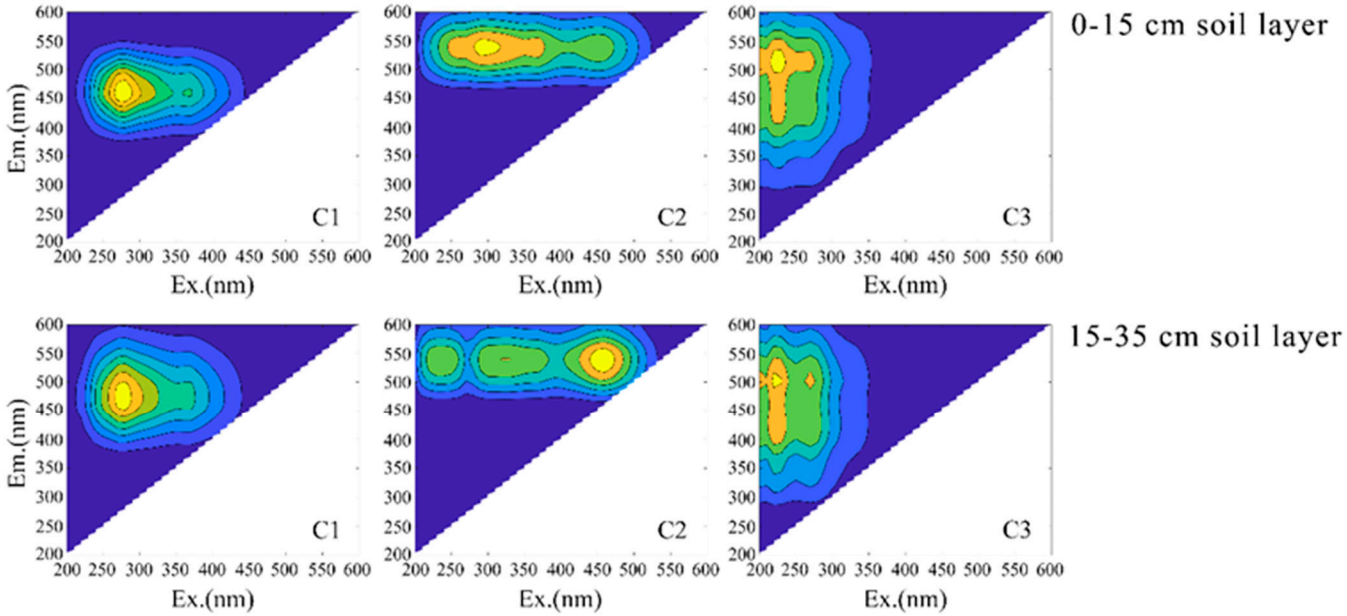

Figure 3. Components identified using PARAFAC analysis of FA under different organic materials and their incorporation depths. Abbreviations: C1: Component 1; C2: Component 2; C3: Component 3.

T1, T2 and T3 significantly increased the fluorescence index (FI) of FA in the 0–15 cm soil layer by 9.36–40.90% ($p < 0.05$), but the FI of FA did not differ significantly among all treatments in the 15–35 cm soil layer. The humification index (HIX) of FA were significantly increased by 4.35–20.11% in the 0–15 cm soil layer, and by 4.35–20.11% in the 15–35 cm soil layer in T1, T2, T3 and T4, compared with CK ($p < 0.05$). T1, T2 and T3 significantly increased the FI of HA by 9.36–40.90% ($p < 0.05$) in the 0–15 cm soil layer, but the FI of HA did not differ significantly among all treatments in the 15–35 cm soil layer. The HIX of HA also did not differ significantly among all treatments in the 0–15 cm soil layer, but significantly increased by 4.35–20.11% under the 15–35 cm soil layer in T2, T3 and T4, compared with CK ($p < 0.05$) (Figure 4).

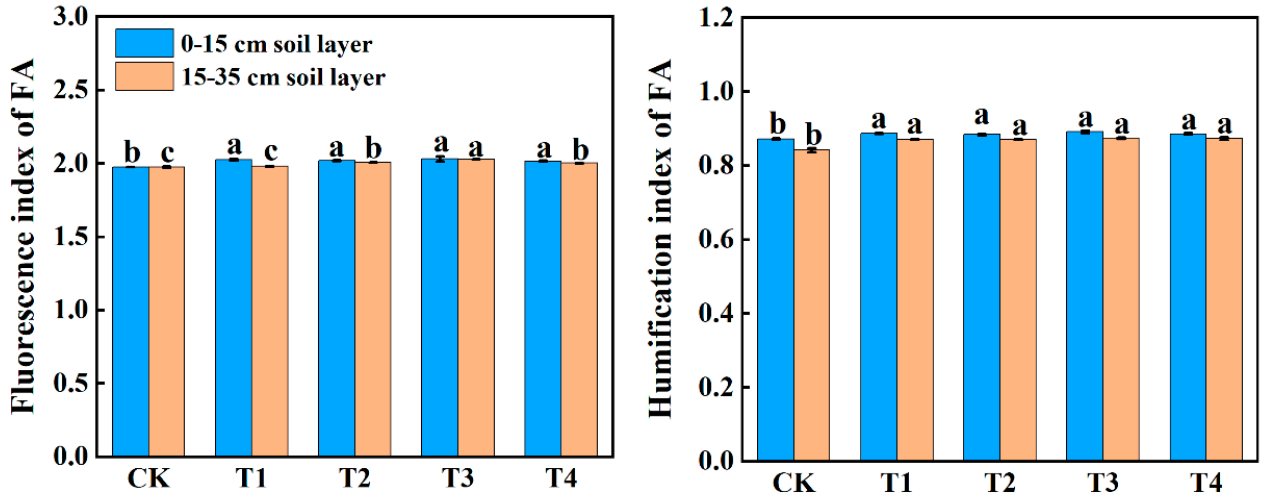

Figure 4. *Cont.*

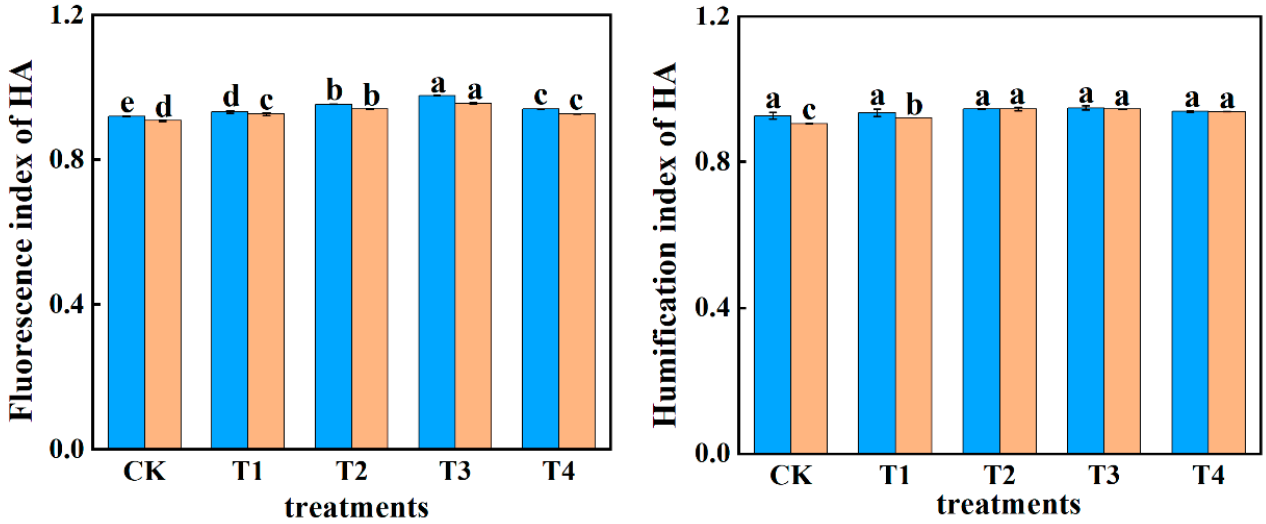

Figure 4. Fluorescence spectrum indices of FA and HA under different organic materials and their incorporation depths. Different letters indicate significant differences between samples in the same soil layer ($p < 0.05$). Values are mean ± errors ($n = 4$).

3.3. Soil Microbial Community Structure Characteristics

Bacterial PLFA, fungal PLFA, actinomycetic PLFA and total PLFA contents increased by 20.32–56.86%, 1.17–99.30%, 1.69–31.22% and 11.97–48.98% in the 0–15 cm soil layer under the T1, T2, T3 and T4, compared with CK, respectively (Table 2). Bacterial PLFA, fungal PLFA, actinomycetic PLFA and total PLFA contents increased in the order of T3 > T2 > T4 > T1 > CK in the 15–35 cm soil layer. And compared with CK, the T2, T3 and T4 significantly increased bacterial PLFA, fungal PLFA, actinomycetic PLFA and total PLFA contents by 79.57–159.62%, 133.74–687.00%, 52.21–96.21% and 81.77–139.02% ($p < 0.05$) in the 15–35 cm soil layer, respectively.

Table 2. Bacterial PLFA, fungal PLFA, actinomycete PLFA and total PLFA contents under different organic materials and their incorporation depths.

Treatment	Layer	Bacteria	Fungi	Actinomycetic	G^+	G^-	Total PLFAs
	(cm)	noml g^{-1}	noml g^{-1}	noml g^{-1}	noml g^{-1}	noml g^{-1}	noml g^{-1}
CK	0–15	34.84 ± 0.75 d	4.28 ± 0.13 c	7.11 ± 0.32 b	12.12 ± 1.41 c	13.47 ± 0.38 d	47.53 ± 1.85 d
	15–35	20.42 ± 0.42 d	1.66 ± 0.06 d	4.75 ± 0.11 d	7.1 ± 0.12 d	7.24 ± 0.19 d	26.83 ± 0.57 d
T1	0–15	45.52 ± 0.29 b	6.00 ± 0.29 b	8.92 ± 0.23 a	14.54 ± 0.58 b	17.25 ± 0.21 b	60.45 ± 0.53 b
	15–35	29.53 ± 0.70 c	2.6 ± 0.07 c	6.6 ± 0.13 c	9.76 ± 0.12 c	10.69 ± 0.26 c	38.74 ± 0.89 c
T2	0–15	43.91 ± 1.37 bc	4.66 ± 0.08 c	8.61 ± 0.75 a	13.19 ± 0.12 bc	16.57 ± 0.28 bc	55.39 ± 2.65 bc
	15–35	39.08 ± 0.16 b	4.06 ± 0.04 b	8.25 ± 0.03 b	12.9 ± 0.17 b	14.53 ± 0.08 b	51.39 ± 0.14 b
T3	0–15	54.65 ± 0.48 a	8.53 ± 0.3 a	9.33 ± 0.7 a	16.96 ± 1.29 a	20.38 ± 0.65 a	70.81 ± 2.09 a
	15–35	52.94 ± 1.77 a	8.52 ± 0.30 a	9.32 ± 0.35 a	16.96 ± 0.65 a	21.01 ± 0.15 a	64.13 ± 2.09 a
T4	0–15	41.92 ± 0.48 c	4.33 ± 0.18 c	7.23 ± 1.14 b	13.06 ± 0.49 bc	14.8 ± 1.1 cd	53.22 ± 1.33 cd
	15–35	37.67 ± 3.59 b	3.88 ± 0.49 b	7.22 ± 0.57 bc	13.03 ± 0.25 b	14.8 ± 1.1 b	48.77 ± 4.64 b
	Two-way ANOVA results (p values)						
Treatment (T)		$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
Layer (L)		$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
T × L		$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$

Different letters indicate significant differences between samples in the same soil layer ($p < 0.05$). Values are mean ± errors ($n = 4$).

T1, T2, T3 and T4 significantly increased the F/B ratios by 13.58–53.37% compared with CK in the 0–15 cm soil layer ($p < 0.05$) (Figure 5). Compared with CK, the F/B ratios significantly increased by 43.44–97.46% in T2, T3 and T4 ($p < 0.05$) in the 15–35 cm soil layer. The contents of G^+/G^- ratios decreased in the order of CK > T1 > T2 > T4 > T3 in the 0–15 cm soil layer, and the G^+/G^- ratios decreased in the order of CK > T1 > T2 > T4 > T3 in the 15–35 cm soil layer. The G^+/G^- ratios were significantly lower by 11.44–17.5% in

T1, T2, T3 and T4 than CK ($p < 0.05$) in the 0–15 cm soil layer. T2, T3 and T4 significantly decreased the G^+/G^- ratios by 7.69–20.99% compared with CK ($p < 0.05$) in the 15–35 cm soil layer.

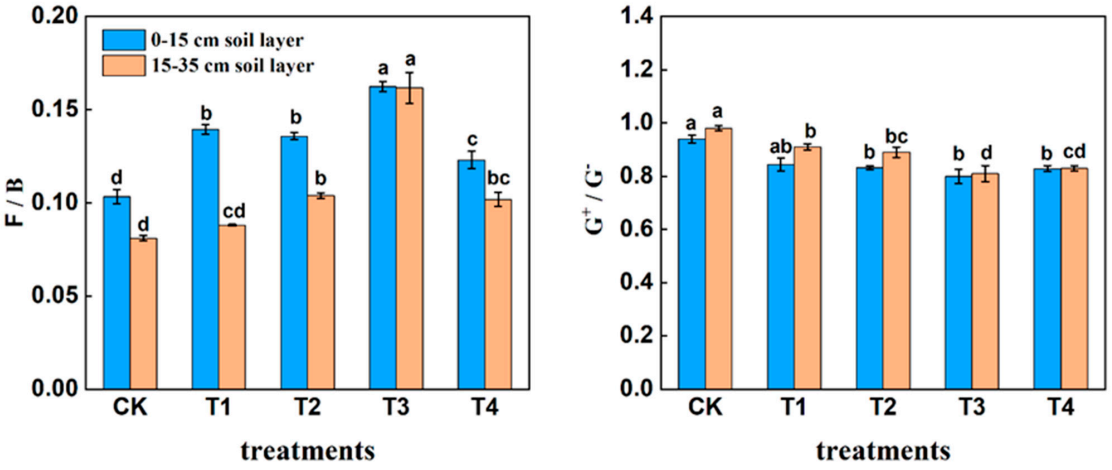

Figure 5. F/B and G^+/G^- under different organic materials and their incorporation depths. Abbreviations: F/B: ratio of fungi and bacteria; G^+/G^-: ratio of Gram–positive bacteria and Gram–negative bacteria. Different letters indicate significant differences between samples in the same soil layer ($p < 0.05$). Values are mean ± errors ($n = 4$).

Principal component analysis (PCA) showed the changes in soil microbial community structures under different organic material incorporation depths. Differences in microbial community structures between T1, T3 and T2, T4, CK were mainly found in PC1, and the difference between the T1, T2, T4 and T3 and CK was mainly reflected in PC2. PCA 1 and PCA 2 explained 87.4% and 5.5% of the total variance for the 0–15 cm soil layer (Figure 6). Differences between T1, T3, CK and T2, T4 were mainly found in PC1, and the difference between the T1, T3 and T2, T4, CK was mainly reflected in PC2. In the 15–35 cm soil layer, PCA 1 and PCA 2 explained 87% and 5.7% (Figure 6).

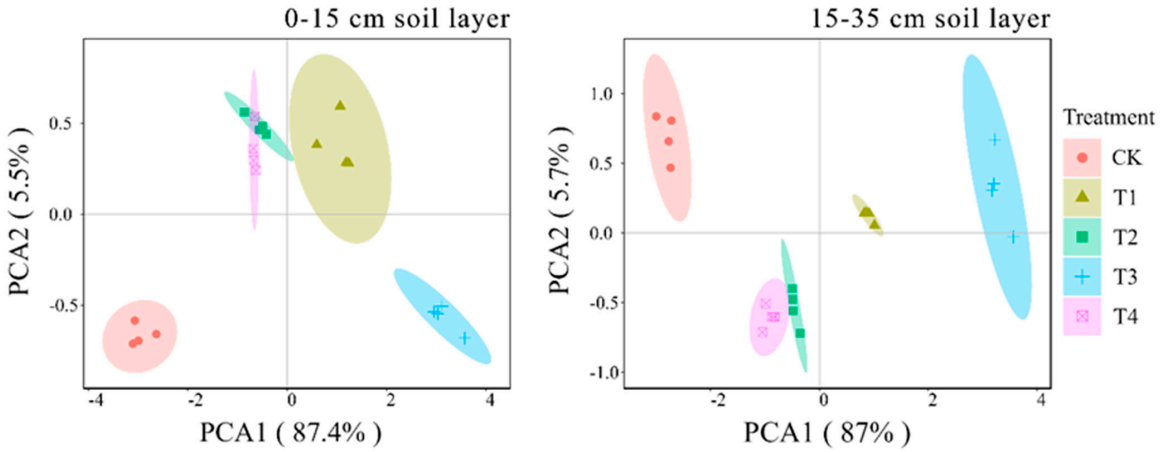

Figure 6. Principal component analysis (PCA) of PLFA profiles under different organic materials and their incorporation depths.

3.4. Partial Least Squares Path Modeling of the Relationships between Humus Substance Structure and Soil Microbial Community

Partial least squares path modeling (PLS–PM) explores the relationship between organic material incorporation, soil properties, soil microbial community (F/B and G^+/G^-) and humus substance's (HS) structure (a combination of six indicators about HS) (Figure 7). This model indicates that organic materials and their incorporation depths had a signifi-

cantly and directly positive effect on TN (0.558) and AK (0.784) in the 0–15 cm soil layer. Moreover, the F/B and the G^+/G^- had a directly negative effect on the HS structure (−0.750 and −0.284) in the 0–15 cm soil layer. FAHIX was the most important factor in the HS structure (0.836) in the 0–15 cm soil layer. In the 15–35 cm soil layer, the organic materials and their incorporation depths had a significantly and directly positive effect on AN (0.668) and AP (0.813). The F/B had a directly positive effect on the HS structure (0.696) in the 15–35 cm soil layer. F/B and G^+/G^-, and the HS structure were all affected by organic material incorporation, proving that it was critical in regulating these factors in the 15–35 cm soil layer. The HA–C was the most important factor in the HS structure (0.919) in the 15–35 cm soil layer. We found that F/B and G^+/G^- directly affected the HS structure differently in two soil layers, and the most important factors in the HS structure were FAHIX and HA–C, respectively.

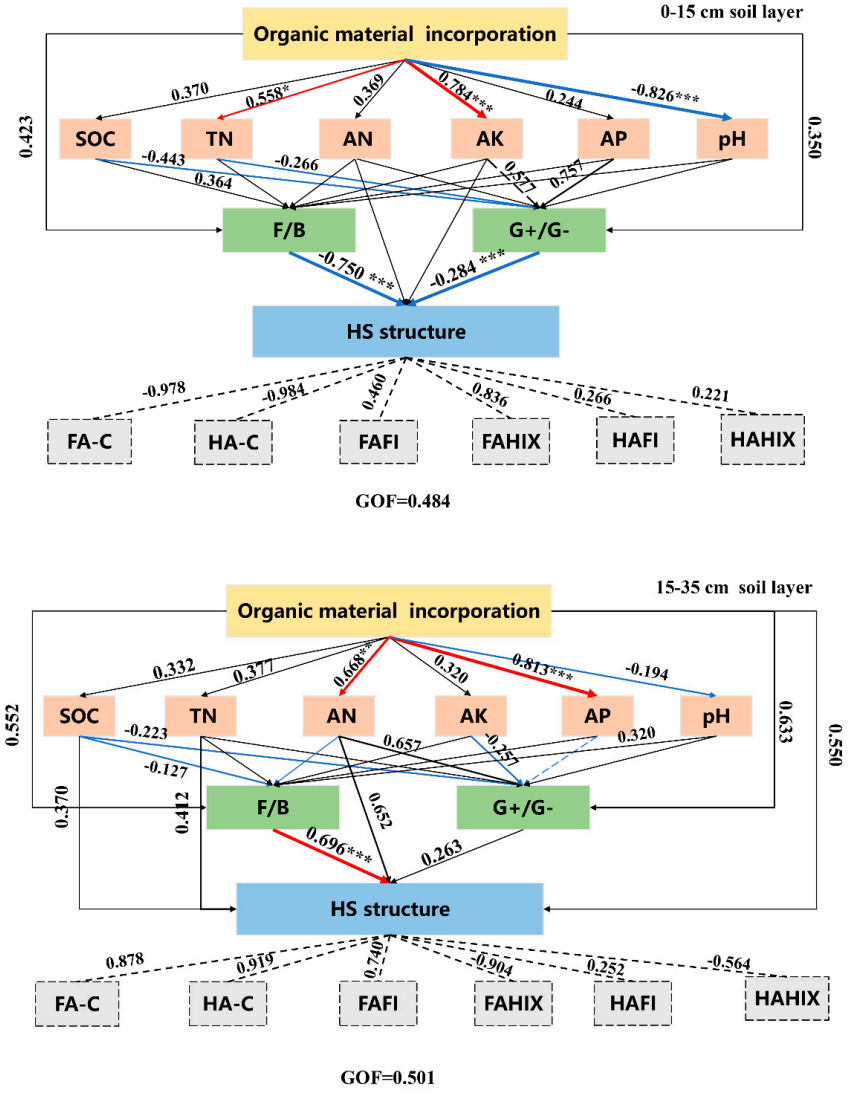

Figure 7. Directed figure of the partial least squares path model (PLS–PM). Dashed rectangles indicate the load of the HS structure and six HS structure indictors that produce potential variability. Red lines indicate positive effects, wider lines indicate greater effects, and blue lines indicate negative effects. Solid lines indicate no significant effect ($p > 0.05$). Numbers associated with the lines mean correlation coefficients, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. GoF, goodness-of-fit, numbers are 0.484 in the 0–15 cm soil layer and 0.501 in the 15–35 cm layer. The abbreviations of soil properties, soil microbial community and HS structures are located in Tables 1 and 2, Figures 4 and 5, respectively. Abbreviation for fluorescence index, FAFI: fulvic acid fluorescence index; FAHIX: fulvic acid humification index; HAFI: humic acid fluorescence index; HAHIX: humic acid humification index.

4. Discussion

4.1. Soil Properties

Organic material incorporation is an important measure to increase soil microbial activity and nutrient effectiveness [38], and plays an important role in maintaining and increasing SOC levels and improving soil structure. Wang et al. [39] showed that SOC contents increased by 13.97% on average after organic material incorporation, compared to no organic material incorporation. Our study has found similar results. The subsoil tillage and organic material incorporation increased the activity and quantity of soil microorganisms, which accelerated the decomposition of organic material and the sequestration of SOC in the 0–15 cm soil layer. While the T2, T3 and T4 increased the SOC and TN contents in 15–35 cm soil layer, this indicates that the subsoil tillage and organic material incorporation homogenized in the 0–35 cm soil layer, and thus promoted the increase of SOC and TN contents in the 15–35 cm soil layer [40]. Meanwhile, subsoil tillage and organic material incorporation improved the aeration of the15–35 cm soil layer and broke the plough pan, which promoted the growth of crop roots in the 15–35 cm soil layer and increased the input of root carbon [41]. Huang et al. [38] showed that organic material incorporation was beneficial to increase the contents of AN, AP and AK in the 0–15 cm soil layer, compared with no organic material incorporation. Our study is consistent with them. Organic material incorporation can improve the contents and effectiveness of soil nutrients [42], promote soil nutrients uptake and organic material fixation, and reduce the leaching loss of effective soil nutrients [8]. The microbial biomass in the 0–15 cm soil layer is usually higher than that in the 15–35 cm soil layer [43], while MBC and MBN contents respond to the activity status of soil microorganisms. In this study, T3 exhibited the greatest increase in MBC contents in the 0–15 cm and 15–35 cm soil layers, compared with CK, because the subsoil tillage and organic material incorporation provided rich substrate for soil microorganisms. The subsoil tillage and organic material incorporation transferred soil nutrients to the 0–35 cm soil layer, which in turn increased soil fertility in the 15–35 cm soil layer. Therefore, subsoil tillage and organic material incorporation can further improve the nitrogen supply capacity of the soil.

4.2. Effects of Different Organic Materials and Their Incorporation Depths on Humus Substance

As a constituent of HS, FA is closely related to HA, and is a precursor and degradation product of HA. FA contains hydroxyl, carboxyl and aliphatic hydrocarbons and other reactive functional groups, which are highly bioavailable and have an important impact on the carbon cycle in soil [44]. Cui et al. [45] showed that the carbon contents in FA and HA increased by 26.39% and 9.20%, respectively, in the 0–20 cm soil layer under subsoil tillage and organic material incorporation, compared to no organic material incorporation. Our study obtained similar results. The FA–C and HA–C contents increased further in our study, indicating that the combined application of straw and organic manure promotes the increase of C contents in HS, compared to organic manure alone.

The fluorescence index (FI) and humification index (HIX) characterize the origin of the humic substance and the level of humification, respectively [46]. FI and HIX play an important role in evaluating HS properties [47]. Our result demonstrated that FA exhibited a distinct optical signature shared by both the 0–15 cm and 15–35 cm soil layers (Figures 2 and 3). Due to different organic materials and their incorporation depths, FA in the 0–15 soil layer was previously characterized by 15–35 cm soil layer materials, because microbial exogenous metabolism and root exudates are relative in the 0–15 cm soil layer, compared to 15–35 cm soil layer. In fact, our results showed that the fulvic–like component contributes more than the protein–like component to the fluorescent FA and HA in the 0–35 cm soil layer. Further evidence of its high reactivity is the large contribution of tyrosine to the fluorescent FA and its disappearance in HA [48]. Following organic material incorporation with subsoil tillage, there are three main processes involved in reducing the proteinaceous contents of HA. Firstly, soil microorganisms may favor the mineralization of HS components similar to tyrosine, such as amino acids, because they almost instantly

utilize aliphatic compounds [49]. Secondly, plants in northeastern China's ecosystems absorb amino acids and oligopeptides directly as a nitrogen sources, resulting in fewer proteinaceous compounds in Mollisol [50]. Lastly, fewer aromatic compounds and protein–like fluorophores were retained in the 15–35 cm soil layer. The release of HMW aromatic fluorophores led to a decrease in the tyrosine-like components of soil fluorescent HA and the relative concentration in aromatic compounds [51].

4.3. Effects of Different Organic Materials and Their Incorporation Depths on Soil Microbial Community Structure

Soil microorganisms play a key role in biogeochemical processes and are closely associated with soil fertility [52]. Typically, soil microorganisms respond strongly to large amounts of straw and organic manure inputs [53]. Chen et al. [54] found that, compared to no organic material incorporation, the bacterial PLFA, fungal PLFA and total PLFA contents were significantly increased by 75%, 56% and 52% under organic material incorporation treatment, respectively; our study found a similar phenomenon, because the organic material incorporation could provide energy and nutrients for soil microbial growth [11]. Additionally, the increase in bacterial PLFA, fungal PLFA and total PLFA contents were greater in the 15–35 cm soil layer under the organic material incorporation with subsoil tillage than those in the 0–15 cm soil layer, because the nutrients in the 15–35 cm soil layer were relatively low, while the subsoil tillage could break the plough pan layer and promote the nutrient and energy flow between different soil layers [55]. So, organic material incorporation with subsoil tillage would be conducive to soil microbial growth.

The F/B ratios can be used to characterize the level of carbon sequestration in soil [56]. The dominance of fungi in the microbial community is considered to be an important factor in promoting SOC accumulation and reducing SOC turnover. Mycelium of fungi facilitates the formation and stabilization of soil aggregates, increases physical protection against SOC [57], and tissues of fungal origin are more resistant to chemical decay than those of a bacterial origin [58]. Liu et al. [18] showed that subsoil tillage combined with straw incorporation significantly increased bacteria and fungi contents in the 0–20 cm soil layer, total PLFAs in the 20–40 cm soil layer, as well as total PLFA contents and the F/B ratios in the 20–40 cm soil layer ($p < 0.05$). In our study, with the organic matter incorporation, fungi are the major decomposers of plant residues and organic manure, and fungi benefit from organic incorporation [59]. Our result indicated that organic material incorporation with subsoil tillage provided abundant substrate for fungi and improved the carbon sequestration capacity of the soil.

Changes in the (G^+/G^-) ratios can reflect variations in the soil microbial habitat, as well as assessing energy-limiting conditions in the soil [60]. The variation of G^+/G^- is related to the quality of SOC, and the growth of G^- under substrate-enriched conditions leads to lower G^+/G^-. Our results indicated that the organic material input provided sufficient plant carbon source for the growth of G^- to increase the G^- contents, and as straw decomposes, the reduction of unstable carbon favors the growth of G^+, which usually contains high levels of N-acetylglucosamine, a relatively tolerant SOC precursor, and can effectively promote the accumulation of SOC [61]. Moreover, for maize–soybean rotation, G^- is associated with soybean rhizobia. Therefore, the soybean plant system had a lower G^+/G^-. From the perspective of G^+/G^-, it indicates that the organic material incorporation with subsoil tillage can promote SOC accumulation and thus restore soil fertility, and G^+/G^- increases with the decrease of available carbon after soil profile or stable substrate depletion [24]. The G^+/G^- ratios in the 15–35 cm soil layer under the organic material incorporation was greater than that in the 0–15 cm soil layer (Figure 5), because the available carbon and nutrients in the 15–35 cm soil layer were less, and therefore, G^+/G^- was lower in the 15–35 cm soil layer. So, the organic material incorporation with subsoil tillage is a great measure to improve microbial community structure.

4.4. Effects of Different Organic Materials and Their Incorporation Depths on HS Structure and Soil Microbial Community Structure Characteristics

In this study, a PLS–PM was used to analyze the contribution of each indicator to SOC under different organic materials and their incorporation depths. Two factors mainly affect soil properties, HS structure and soil microbial communities. On one side, SOC–rich soils usually have more available C, which may be beneficial for microbial growth [62]. This was evidenced by the positive correlation between the six indicators (FA–C, HA–C, FAFI, FAHIX, HAFI and HAHIX) and SOC contents in our study (Figure 7). On the other side, microbial communities along the PC1 axis varied with different organic material incorporation management (Figure 6). Taken together with the significant differences in bacterial PLFA contents in different organic material incorporation systems, we found that different crops provide unique percentages of unstable organic matter to microbial decomposer communities [63]. In maize–soybean planting systems, the relatively large proportion of unstable carbon sources is more favorable to the growth of the most competitive fungi [64,65]. PLS–PM results indicated that fungi communities have a significant direct effect on soil HS structure. It is suggested that fungi took a more positive role in nutrient cycling via biochemical reactions in the 15–35 cm soil layer under different soil management. In conclusion, we found that the HS structure and soil microbial communities could be distinguished under different organic materials and their incorporation depths, based on PLFA analysis and HS structure. Microbial community indicators, FAHIX and HA–C, were most closely associated with HS structure.

5. Conclusions

This study showed that organic material incorporation with subsoil tillage had positive effects on soil properties, HS structure and microbial community structure characteristics. Compared to conventional tillage with no organic material incorporation, organic material incorporation with subsoil tillage significantly increased bacterial and fungi and total PLFA contents in the 0–35 cm soil layer. PLS–PM indicated that fungi played a dominant role in organic material degradation of the 0–35 cm soil layer. Thus, the effect of different organic materials and their incorporation depths on soil microbial community characteristics could be amplified under organic material incorporation with subsoil tillage practice, which could provide energy and nutrients for microbes. We concluded that straw and organic manure incorporation with subsoil tillage practice could be a strong potential to improve HS structure and microbial community characteristics of the 0–35 cm soil layer by increasing SOC and nutrient contents distinctly, and, ultimately, promote soil fertility in northeast China. In this study, we only studied the chemical properties; the physical properties of the soil are just as important. Our next study will explore the effects of different organic materials and their incorporation depths on the physical composition of SOC.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy13082169/s1, Figure S1: Relative percentage of HA fluorescence components under different organic materials and their incorporation depths. Figure S2: Relative percentage of HA fluorescence components under different organic materials and their incorporation depths.

Author Contributions: W.Z. and X.L. conceived and designed the experiments. J.G. conducted the experiments and statistics. X.H., J.Y. and X.C. revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Strategic Priority Research Program of Chinese Academy of Sciences (XDA28070100), the National Key R&D Program of China (YFD20221500100) and China Agriculture Research System of MOF and MARA (CARS04).

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

Conflicts of Interest: The authors declare that they have no conflict of interest.

References

1. Schlesinger, W.H.; Andrews, J.A. Soil respiration and the global carbon cycle. *Biogeochemistry* **2000**, *48*, 7–20. [CrossRef]
2. Gerke, J. Concepts and Misconceptions of Humic Substances as the Stable Part of Soil Organic Matter: A Review. *Agronomy* **2018**, *8*, 76. [CrossRef]
3. Senesi, N. Humic Substances as Natural Nanoparticles Ubiquitous in the Environment. In *Molecular Environmental Soil Science at the Interfaces in the Earths Critical Zone*; Xu, J., Huang, P.M., Eds.; Springer: Berlin/Heidelberg, Germany, 2010; pp. 249–250. [CrossRef]
4. Olk, D.C.; Bloom, P.R.; Perdue, E.M.; Mcknight, D.M.; Chen, Y.; Farenhorst, A.; Senesi, N.; Chin, Y.P.; Schmitt-Kopplin, P.; Hertkorn, N. Environmental and Agricultural Relevance of Humic Fractions Extracted by Alkali from Soils and Natural Waters. *J. Environ. Qual.* **2019**, *48*, 217–232. [CrossRef] [PubMed]
5. Nie, X.; Li, Z.; Huang, J.; Huang, J.Q.; Liu, L.; Xiao, H.B.; Liu, C.; Zeng, G.M. Thermal stability of organic carbon in soil aggregates as affected by soil erosion and deposition. *Soil Tillage Res.* **2018**, *175*, 82–90. [CrossRef]
6. Coble, P.G. Characterization of marine and terrestrial DOM in seawater using excitation emission matrix spectroscopy. *Mar. Chem.* **1996**, *51*, 325–346. [CrossRef]
7. Li, W.T.; Chen, S.Y.; Xu, Z.X.; Li, Y.; Shuang, C.D.; Li, A.M. Characterization of dissolved organic matter in municipal wastewater using fluorescence PARAFAC analysis and chromatography multi-excitation/emission scan: A comparative study. *Environ. Sci. Technol.* **2014**, *48*, 2603–2609. [CrossRef]
8. Yang, L.; Tan, L.; Zhang, F.; Gale, W.J.; Cheng, Z.; Sang, W. Duration of continuous cropping with straw return affects the composition and structure of soil bacterial communities in cotton fields. *Can. J. Microbiol.* **2018**, *64*, 167–181. [CrossRef]
9. Senesi, N.; Miano, T.M.; Provenzano, M.R.; Brunetti, G. Characterization, differentiation, and classification of humic substances by fluorescence spectroscopy. *Soil Sci.* **1991**, *152*, 259–271. [CrossRef]
10. Stedmon, C.A.; Bro, R. Characterizing dissolved organic matter fluorescence with parallel factor analysis: A tutorial. *Limnol. Oceanogr. Methods* **2008**, *6*, 572–579. [CrossRef]
11. Breulmann, M.; Masyutenko, N.P.; Kogut, B.M.; Schroll, R.; Dörfler, U.; Buscot, F.; Schulz, E. Short-term bioavailability of carbon in soil organic matter fractions of different particle sizes and densities in grassland ecosystems. *Sci. Total Environ.* **2014**, *497*, 29–37. [CrossRef]
12. Bonner, M.T.L.; Shoo, L.P.; Brackin, R.; Schmidt, S. Relationship between microbial composition and substrate use efficiency in tropical soil. *Geoderma* **2018**, *315*, 96–103. [CrossRef]
13. Shao, P.; Liang, C.; Lynch, L.; Xie, H.; Bao, X. Reforestation accelerates soil organic carbon accumulation: Evidence from microbial biomarkers. *Soil Biol. Biochem.* **2019**, *131*, 182–190. [CrossRef]
14. Ai, C.; Liang, G.; Sun, J.; He, P.; Tang, S.; Zou, W.; Wang, X. The alleviation of acid soil stress in rice by inorganic or organic ameliorants is associated with changes in soil enzyme activity and microbial community composition. *Biol. Fertil. Soils* **2015**, *51*, 465–477. [CrossRef]
15. Dai, X.; Zhou, W.; Liu, G.; Liang, G.; He, P.; Liu, Z. Soil C/N and pH together as a comprehensive indicator for evaluating the effects of organic substitution management in subtropical paddy fields after application of high-quality amendments. *Geoderma* **2019**, *337*, 1116–1125. [CrossRef]
16. Xu, M.; Cardenas, L.M.; Horrocks, C.; Lopez-Aizpun, M.; Zhang, J.; Zhang, F.; Dungait, J. The effect of tillage management on microbial functions in a maize crop at different slope positions. *Geoderma* **2021**, *401*, 115171. [CrossRef]
17. Deng, J.; Deng, Y.; Sun, Z.; Wang, G.; Cao, L.; Yuan, H.; Huang, D.; Jia, H. Tillage and residue management affect growing-season soil respiration in paddy fields. *Soil Tillage Res.* **2022**, *218*, 105315. [CrossRef]
18. Liu, X.; Batande, S.N.; Zhang, X.W.; Dou, S. Effects of artificially altered soil structure on 15N absorption and utilization for maize (*Zea mays* L.) at the seedling stage. *Appl. Ecol. Environ. Res.* **2022**, *20*, 1873–1885. [CrossRef]
19. Li, L.J.; Han, X.Z. Changes of soil properties and carbon fractions after long-term application of organic amendments in Mollisols. *Catena* **2016**, *143*, 140–144. [CrossRef]
20. Yan, X.; Cai, Z.; Wang, S.; Smith, P. Direct measurement of soil organic carbon content change in the croplands of China. *Glob. Chang. Biol.* **2011**, *17*, 1487–1496. [CrossRef]
21. Zhang, J.; Wei, Y.; Liu, J.; Yuan, J.; Liang, Y.; Ren, J.; Cai, H. Effects of maize straw and its biochar application on organic and humic carbon in water-stable aggregates of a Mollisol in Northeast China: A five-year field experiment. *Soil Tillage Res.* **2019**, *190*, 1–9. [CrossRef]
22. Schmidt, R.; Gravuer, K.; Bossange, A.V.; Mitchell, J.; Scow, K. Long-term use of cover crops and no-till shift soil microbial community life strategies in agricultural soil. *PLoS ONE* **2018**, *13*, e0192953. [CrossRef] [PubMed]
23. Bertolino, A.V.F.A.; Fernandes, N.F.; Miranda, J.P.L.; Souza, A.P.; Lopes, M.R.S.; Palmieri, F. Effects of plough pan development on surface hydrology and on soil physical properties in Southeastern Brazilian plateau. *J. Hydrol.* **2010**, *393*, 94–104. [CrossRef]
24. Liu, X.; Peng, C.; Zhang, W.; Li, S.; An, T.; Xu, Y.; Ge, Z.; Xie, N.; Wang, J. Subsoiling tillage with straw incorporation improves soil microbial community characteristics in the whole cultivated layers: A one-year study. *Soil Tillage Res.* **2022**, *215*, 105188. [CrossRef]
25. Chang, L.; Meng, L.; Jun, C.; Bo, L.; Fang, C. Effects of straw carbon input on carbon dynamics in agricultural soils: A meta-analysis. *Glob. Chang. Biol.* **2014**, *20*, 1366–1381. [CrossRef]

26. Li, L.-J.; You, M.-Y.; Shi, H.-A.; Ding, X.-L.; Qiao, Y.-F.; Han, X.-Z. Soil CO_2 emissions from a cultivated Mollisol: Effects of organic amendments, soil temperature, and moisture. *Eur. J. Soil Biol.* **2013**, *55*, 83–90. [CrossRef]
27. Staff, S.S. *Keys to Soil Taxonomy*, 11th ed.; United States Department of Agriculture, Natural Resources Conservation Service: Washington, DC, USA, 2010.
28. Carter, M.R.; Gregorich, E.G. *Soil Sampling and Methods of Analysis*, 2nd ed.; Taylor & Francis: Oxfordshire, UK, 2012.
29. Vance, E.D.; Brookes, P.C.; Jenkinson, D.S. Microbial biomass measurements in forest soils-determination of kc values and tests of hypothese to explatin the failure of the chloroform fumigation incubation method in acid soils. *Soil Biol. Biochem.* **1987**, *19*, 689–696. [CrossRef]
30. Zhang, L.; Xie, Y.; Liu, J.; Zhong, S.; Qian, Y.; Gao, P. An Overlooked Entry Pathway of Microplastics into Agricultural Soils from Application of Sludge-Based Fertilizers. *Environ. Sci. Technol.* **2020**, *54*, 4248–4255. [CrossRef]
31. Bossio, D.A.; Scow, K.M. Impacts of carbon and flooding on soil microbial communities: Phospholipid fatty acid profiles and substrate utilization patterns. *Microb. Ecol.* **1998**, *35*, 265–278. [CrossRef]
32. Frostegard, A.; Baath, E. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biol. Fertil. Soils* **1996**, *22*, 59–65. [CrossRef]
33. Zelles, L. Phospholipid fatty acid profiles in selected members of soil microbial communities. *Chemosphere* **1997**, *35*, 275–294. [CrossRef]
34. Djukic, I.; Zehetner, F.; Mentler, A.; Gerzabek, M.H. Microbial community composition and activity in different Alpine vegetation zones. *Soil Biol. Biochem.* **2010**, *42*, 155–161. [CrossRef]
35. Fanin, N.; Kardol, P.; Farrell, M.; Nilsson, M.C.; Gundale, M.J.; Wardle, D.A. The ratio of Gram-positive to Gram-negative bacterial PLFA markers as an indicator of carbon availability in organic soils. *Soil Biol. Biochem.* **2019**, *128*, 111–114. [CrossRef]
36. Sun, S.; Wu, Y.; Zhang, J.; Wang, G.; Deluca, T.H.; Zhu, W.; Li, A.; Duan, M.; He, L. Soil warming and nitrogen deposition alter soil respiration, microbial community structure and organic carbon composition in a coniferous forest on eastern Tibetan Plateau. *Geoderma* **2019**, *353*, 283–292. [CrossRef]
37. Ai, C.; Zhang, S.; Zhang, X.; Guo, D.; Zhou, W.; Huang, S. Distinct responses of soil bacterial and fungal communities to changes in fertilization regime and crop rotation. *Geoderma* **2018**, *319*, 156–166. [CrossRef]
38. Huang, T.; Yang, N.; Lu, C.; Qin, X.; Siddique, K.H.M. Soil organic carbon, total nitrogen, available nutrients, and yield under different straw returning methods. *Soil Tillage Res.* **2021**, *214*, 105171. [CrossRef]
39. Wang, Y.; Wu, P.; Mei, F.; Ling, Y.; Qiao, Y.; Liu, C.; Leghari, S.J.; Guan, X.; Wang, T. Does continuous straw returning keep China farmland soil organic carbon continued increase? A meta-analysis. *J. Environ. Manag.* **2021**, *288*, 112391. [CrossRef]
40. Urioste, A.M.; Hevia, G.G.; Hepper, E.N.; Anton, L.E.; Bono, A.A.; Buschiazzo, D.E. Cultivation effects on the distribution of organic carbon, total nitrogen and phosphorus in soils of the semiarid region of Argentinian Pampas. *Geoderma* **2006**, *136*, 621–630. [CrossRef]
41. Mu, X.; Zhao, Y.; Liu, K.; Ji, B.; Guo, H.; Xue, Z.; Li, C. Responses of soil properties, root growth and crop yield to tillage and crop residue management in a wheat-maize cropping system on the North China Plain. *Eur. J. Agron.* **2016**, *78*, 32–43. [CrossRef]
42. Guan, S.; Liu, S.J.; Liu, R.Y.; Zhang, J.J.; Ren, J.; Cai, H.G.; Lin, X.X. Soil organic carbon associated with aggregate-size and density fractions in a Mollisol amended with charred and uncharred maize straw. *J. Integr. Agric.* **2019**, *18*, 1496–1507. [CrossRef]
43. Zhao, H.; Ning, P.; Chen, Y.; Liu, J.; Ghaffar, S.A.; Tian, X.; Shi, J. Effect of straw amendment modes on soil organic carbon, nitrogen sequestration and crop yield on the North-Central Plain of China. *Soil Use Manag.* **2019**, *35*, 511–525. [CrossRef]
44. Zhao, H.; Lv, Y.; Wang, X.; Zhang, H.; Yang, X. Tillage impacts on the fractions and compositions of soil organic carbon. *Geoderma* **2012**, *189*, 397–403. [CrossRef]
45. Cui, T.T.; Dou, S.; Chen, Y.N.; Huang, Y.; Wang, L.L. Effect of deep applied corn stalks on composition of soil humus and structure of humic acid. *Acta Pedol. Sin.* **2014**, *4*, 718–725.
46. Li, W.; Li, X.; Han, C.; Gao, L.; Wu, H.; Li, M. A new view into three-dimensional excitation-emission matrix fluorescence spectroscopy for dissolved organic matter. *Sci. Total Environ.* **2023**, *855*, 158963. [CrossRef]
47. Mcknight, D.M.; Boyer, E.W.; Westerhoff, P.K.; Doran, P.T.; Kulbe, T.; Andersen, D.T. Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. *Limnol. Oceanogr.* **2001**, *46*, 38–48. [CrossRef]
48. Metcalfe, D.B.; Hermans, T.D.G.; Ahlstrand, J.; Becker, M.; Berggren, M.; Bjork, R.G.; Bjorkman, M.P.; Blok, D.; Chaudhary, N.; Chisholm, C.; et al. Patchy field sampling biases understanding of climate change impacts across the Arctic. *Nat. Ecol. Evol.* **2018**, *2*, 1443–1448. [CrossRef]
49. Farrell, M.; Hill, P.W.; Farrar, J.; Deluca, T.H.; Roberts, P.; Kielland, K.; Dahlgren, R.; Murphy, D.V.; Hobbs, P.J.; Bardgett, R.D.; et al. Oligopeptides Represent a Preferred Source of Organic N Uptake: A Global Phenomenon? *Ecosystems* **2013**, *16*, 133–145. [CrossRef]
50. Näsholm, T.; Ekblad, A.; Nordin, A.; Giesler, R.; Hogberg, M.; Hogberg, P. Boreal forest plants take up organic nitrogen. *Nature* **1998**, *392*, 914–916. [CrossRef]
51. Zhang, X.; Hutchings, J.A.; Bianchi, T.S.; Liu, Y.; Arellano, A.R.; Schuur, E.G. Importance of lateral flux and its percolation depth on organic carbon export in Arctic tundra soil: Implications from a soil leaching experiment. *J. Geophys. Res. Biogeosci.* **2017**, *122*, 796–810. [CrossRef]
52. Creamer, C.A.; De Menezes, A.B.; Krull, E.S.; Sanderman, J.; Rosa, N.W.; Farrell, M. Microbial community structure mediates response of soil C decomposition to litter addition and warming. *Soil Biol. Biochem.* **2015**, *80*, 175–188. [CrossRef]

53. Zhao, X.; Wang, S.; Xing, G. Nitrification, acidification, and nitrogen leaching from subtropical cropland soils as affected by rice straw-based biochar: Laboratory incubation and column leaching studies. *J. Soils Sediments* **2014**, *14*, 471–482. [CrossRef]
54. Chen, Z.; Wang, H.; Liu, X.; Zhao, X.; Lu, D.; Zhao, J.; Li, C. Changes in soil microbial community and organic carbon fractions under short-term straw return in a rice-wheat cropping system. *Soil Tillage Res.* **2017**, *165*, 121–127. [CrossRef]
55. Bigott, A.F.; Hoy, J.W.; Fultz, L.M. Soil properties, microbial communities, and sugarcane yield in paired fields with short- or long-term sugarcane cultivation histories. *Appl. Soil Ecol.* **2019**, *142*, 166–176. [CrossRef]
56. Ananyeva, N.D.; Castaldi, S.; Stolnikova, E.V.; Kudeyarov, V.N.; Valentini, R. Fungi-to-bacteria ratio in soils of European Russia. *Arch. Agron. Soil Sci.* **2015**, *61*, 427–446. [CrossRef]
57. Peng, S.; Guo, T.; Liu, G. The effects of arbuscular mycorrhizal hyphal networks on soil aggregations of purple soil in southwest China. *Soil Biol. Biochem.* **2013**, *57*, 411–417. [CrossRef]
58. Liang, C.; Schimel, J.P.; Jastrow, J.D. The importance of anabolism in microbial control over soil carbon storage. *Nat. Microbiol.* **2017**, *2*, 17105. [CrossRef]
59. Yu, C.; Li, Y.; Mo, R.; Deng, W.; Zhu, Z.; Liu, D.; Hu, X. Effects of long-term straw retention on soil microorganisms under a rice-wheat cropping system. *Arch. Microbiol.* **2020**, *202*, 1915–1927. [CrossRef]
60. Rankoth, L.M.; Udawatta, R.P.; Gantzer, C.J.; Jose, S.; Veum, K.; Dewanto, H.A. Cover Crops on Temporal and Spatial Variations in Soil Microbial Communities by Phospholipid Fatty Acid Profiling. *Agron. J.* **2019**, *111*, 1693–1703. [CrossRef]
61. Simpson, A.J.; Song, G.; Smith, E.; Lam, B.; Novotny, E.H.; Hayes, M.H.B. Unraveling the structural components of soil humin by use of solution-state nuclear magnetic resonance spectroscopy. *Environ. Sci. Technol.* **2007**, *41*, 876–883. [CrossRef]
62. Acosta-Martinez, V.; Cruz, L.; Sotomayor-Ramirez, D.; Perez-Alegria, L. Enzyme activities as affected by soil properties and land use in a tropical watershed. *Appl. Soil Ecol.* **2007**, *35*, 35–45. [CrossRef]
63. Wardle, D.A.; Bardgett, R.D.; Klironomos, J.N.; Setala, H.; VanDer, P.W.H.; Wall, D.H. Ecological linkages between aboveground and belowground biota. *Science* **2004**, *304*, 1629–1633. [CrossRef]
64. Orwin, K.H.; Buckland, S.M.; Johnson, D.; Turner, B.L.; Smart, S.; Oakley, S.; Bardgett, R.D. Linkages of plant traits to soil properties and the functioning of temperate grassland. *J. Ecol.* **2010**, *98*, 1074–1083. [CrossRef]
65. Thomson, B.C.; Ostle, N.; Mcnamara, N.; Bailey, M.J.; Whiteley, A.S.; Griffiths, R.I. Vegetation Affects the Relative Abundances of Dominant Soil Bacterial Taxa and Soil Respiration Rates in an Upland Grassland Soil. *Microb. Ecol.* **2010**, *59*, 335–343. [CrossRef] [PubMed]