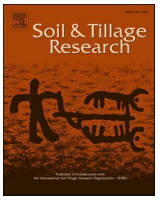
Soil & Tillage Research 218 (2022) 105307

Contents lists available at ScienceDirect

Soil & Tillage Research

journal homepage: www.elsevier.com/locate/still



Effect of long-term tillage and cropping system on portion of fungal and bacterial necromass carbon in soil organic carbon

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ARTICLE INFO

Keywords:
No-tillage
Microbial necromass
Carbon storage
Cropping system

ABSTRACT

The microbial necromass carbon (M-C) is considered to be relatively stable and important part of SOC. However, knowledge about the contribution of M-C storage in increasing SOC storage and the influences of tillage and cropping systems on fungal and bacterial necromass carbon (F-C and B-C) is lacking, especially in the long-term. Here, a subset of treatments in a long-term 16-year study was used to evaluate these changes. The treatments selected for present study were: (1) NTCS: no-tillage with two year corn-soybean (CS) rotation (*Zea mays* L. – *Glycine max* Merr.); (2) MPCS: moldboard plowing with two year corn-soybean rotation; (3) NTCC: no-tillage with continuous corn (CC); (4) MPCC: moldboard plowing with continuous corn; (5) CTCC: conventional tillage with continuous corn and no residue return (traditional tillage practice in China). Amino sugars were measured to calculate the M-C, F-C and B-C and their storage. Three-way ANOVA showed that tillage, depth and their interactions had significant effect on all amino sugars. However, two-way ANOVA in separate layers showed the effects of tillage on all amino sugars mostly occurred in 0–5 cm layer. A decaying exponential model showed the relationship between the M-C and SOC ($R^2 = 0.87$). Tillage showed great effects on the amount of M-C, F-C and B-C storage but had no influence on the proportion of their distribution in SOC storage (%). More than half of the increase in SOC storage existed as M-C storage under CC cropping due to returned residue, which was higher than CS. F-C storage % was not affected by agriculture management (residue return, tillage and cropping), whereas B-C storage % was affected by both quantity and quality of residue. The results suggested that CC cropping system was better for M-C sequestration and that bacteria were relatively more sensitive to agriculture management than fungi.

1. Introduction

Soil organic carbon (SOC) is still receiving enormous attention even though it has been studied for more than a century (Ni et al., 2020; Chen, 2021); globally, soil retains more carbon (C) in SOC than plants and the atmosphere combined (Batjes, 2014; Lehmann and Kleber, 2015). In the traditional view, plant detritus was considered as the main C source in SOC formation while the microbes only act as the decomposers, not contributors (Kogel-Knabner, 2017; Wang et al., 2020). Recently, it has been suggested that microbial necromass plays a far greater role in long-term SOC stabilization than traditionally believed (Simpson et al., 2007; Liang and Balser, 2010; Miltner et al., 2012; Kallenbach et al., 2016; Fernandez et al., 2019). Compared with living microbial biomass which has a fast turnover and constitutes a tiny fraction of SOC, microbial necromass (M-necromass) is considered to be relatively stable and accrues in the soil with iterative microbial community turnover (Ma et al., 2018).

The M-necromass C (M-C) can be evaluated by measuring biomarker amino sugars (AS) (Ni et al., 2020). AS measurements are some of the most important tools for investigating the presence of M-necromass in soils (Amelung et al., 2008; van Groenigen et al., 2010) because their quantities are insignificant in plant residues, making it easy to distinguish M-necromass C from plant residue C; they are mainly derived from

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https://doi.org/10.1016/j.still.2021.105307
Received 1 August 2021; Received in revised form 25 November 2021; Accepted 19 December 2021
Available online 28 December 2021

Nomenclature

M-C	Microbial necromass carbon
B-C	Bacterial necromass carbon
F-C	Fungal necromass carbon
M-C	Storage %: microbial necromass carbon storage / SOC storage * 100%
B-C	Storage %: bacterial necromass carbon storage / SOC storage * 100%
F-C	Storage %: fungal necromass carbon storage / SOC storage * 100%
Δ X storage	X storage – X storage in CTCC (X could be SOC, M-C, B-C or F-C)
Δ X storage %	= Δ X storage / Δ SOC storage * 100% (X could be M-C, B-C or F-C)

fungi and bacteria cell walls and exhibit recalcitrance after cell death (He et al., 2006; Liang and Balser, 2010). Although many types of AS exist in soil, the most important ones are glucosamine (GluN), muramic acid (MurA), mannosamine (ManN) and galactosamine (GalN) (Zhang and Amelung, 1996). The chitin of fungal cell walls is the major component of GluN, but bacterial cell walls and the exoskeletons of soil invertebrates can also make some contribution to the soil GluN pool (Chantigny et al., 1997). MurA is one of the most discriminating biomarkers, as it occurs solely in bacterial cell walls, especially in the murein skeleton of Gram-positive species (Appuhn and Joergensen, 2006). GalN is another significant component of the total amino sugar pool (Glaser et al., 2004), however, the origin of galactosamine is less clear and is typically considered to be nonspecific, as actinomycetes, bacteria and fungi all contain considerable amounts of galactosamine (He et al., 2006). ManN is considered as a cumulative index of amino sugar, as the percentage to which it owes its origin to bacteria or fungi is still unclear (Liang et al., 2007). Separation of ManN is inadequate in most soil hydrolysates, which has resulted in erratic values in measurements and has usually been neglected in previous publications (Appuhn and Joergensen, 2006; Engelking et al., 2007; Indorf et al., 2011). Due to their special characteristics, GluN and MurA give important information on the contribution of fungi and bacteria to M-necromass (Guggenberger et al., 1999) and can be used to indicate relative bacterial versus fungal contributions to SOC (Joergensen and Wichern, 2008).

In agricultural system, plant residue is the primary C input (Ogle et al., 2012) and the quantity and quality of residues strongly affect the SOC formation (De Clercq et al., 2015). Long-term tillage system influenced the composition of SOC (Gao et al., 2018, 2019; Li et al., 2020). Microbial and its derived carbon components are critical for C sequestration under no-tillage (Liang et al., 2018; Lu et al., 2021; Li et al., 2021). A previous study demonstrated that no-tillage (NT) lead to a prominent advantage in the M-C accumulation over conventional tillage (CT) and posed an important question: Will the M-necromass reach an equilibrium state after long-term no-tillage? (Ding et al., 2011). To address this question, it is necessary to clarify the underlying mechanism of SOC stabilization in the conservation tillage system; such clarification could be derived from a long-term study with different tillage systems. Meanwhile, the contribution of M-C could be an important part in the increased SOC storage caused by residue return; there was also lack of study in this area. In the current study, we hypothesize that the M-necromass may reach a steady status after long-term residue return and the M-C could be an important part in the increasing SOC storage resulting from returned residue. Hence, we obtained the soil samples from a long-term arable experiment site with different tillage and cropping system treatments with the following objectives: 1) to test the relationship between M-C and SOC; 2) to assess the distribution of M-C storage within the increased SOC storage and 3) to illustrate the effects of tillage and cropping on the change of fungal necromass C (F-C) storage and bacterial necromass C (B-C) storage.

2. Materials and methods

2.1. Site description

A long-term tillage and crop rotation field experiment was established at the Experimental Station (44°12′N, 125°33′E) of Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, in Dehui County, Jilin Province, China in 2001. The station is located in North Temperate Zone and has a continental monsoon climate. The mean annual temperature and mean annual precipitation are 4.4 °C and 520 mm. The clay loam soil with average 36% clay, 24% silt and 40% sand is classified as black soil (Typic Hapludoll, USDA Soil Taxonomy). Prior to the start of the field experiment, the site had been under conventional tillage with continuous corn (*Zea mays* L.) for more than 15 years. Other details about the site and soil characteristics are provided by Liang et al. (2016).

2.2. Experiment design

The present experiment was conducted on a subset of treatments in the ongoing long-term tillage and crop rotation experiment. The parent field trial was established in a split-plot randomized complete block design with two factors, tillage and cropping system, and four replications. The subset of treatments selected for present study were: (1) NTCS: no-tillage with two year corn-soybean (CS) rotation (*Glycine max* Merr.); (2) MPCS: moldboard plowing with two year corn-soybean rotation; (3) NTCC: no-tillage with continuous corn (CC); (4) MPCC: moldboard plowing with continuous corn; (5) CTCC: conventional tillage with continuous corn (this is the conventional farming practice in Northeast China). In the NTCS and NTCC plots, no soil disturbance occurred except for planting using a no-till planter. To suppress water and wind erosion, 30–35 cm stubble was left standing and un-chopped corn stalks were left on the soil surface interspersed among the stubble in both NT treatments. Soybean residues were directly returned to the soil after harvest. The MPCS, MPCC and CTCC tillage included one moldboard plowing (about 20 cm deep) after corn harvest, spring discing and cultivation and a ridge building in the following June with a modified lister. In the MPCC and MPCS treatments residue was removed prior to fall plowing, and then manually replaced after plowing, in CTCC treatment residue was removed for use as fuel and animal feed and was not returned. Hence, the only difference between MPCC and CTCC was the different residue management, i.e. residue was returned in MPCC, but not in CTCC. All the surface residues were cut into about 30 cm pieces with a heavily ballasted disc before sowing in the following spring. Each treatment received the same amount of commercial preblended inorganic fertilizer, but the fertilizer strategy was different for corn and soybean phase. For corn, 100 kg ha^{-1} N, 45.5 kg ha^{-1} P and 78 kg ha^{-1} K was applied as starter fertilizers by banding with the planter, and an additional 50 kg ha^{-1} N was applied as top dressing at the V-6 growth stage. For the soybean, only starter fertilizer was applied at planting 40 kg ha^{-1} N, 60 kg ha^{-1} P and 80 kg ha^{-1} K.

2.3. Soil sampling and analysis

Seven soil samples were collected from each plot in the corn phase of the rotations in 2017 from three layers (0–5, 5–10, 10–20 cm). Samples were taken using a hand auger with 2.54 cm internal diameter, which allowed the separation of each soil core into two depth segments without compaction. Seven samples were thoroughly mixed to form a composite sample for each depth and each plot. The mixed soil samples were gently broken and air-dried at room temperature. Stubble, stones and visible crop residues were removed and the air-dried soil samples

were sieved to pass 2 mm sieve before further analysis. Total carbon (C) and total nitrogen (N) were determined using a Flash EA1112 Elemental Analyzer (Thermo-Finnigan, Milan, Italy). Since the soil was free of carbonates, SOC was assumed to equal the total C (Liang et al., 2016).

The analysis of AS was conducted according to the method described by Indorf et al. (2011). Three AS, muramic acid (MurA), galactosamine (GalN) and glucosamine (GluN) were determined by high-performance liquid chromatography (HPLC) with fluorescence detection. Briefly, 400–500 mg soil samples were hydrolyzed with 5 mL of 6 M HCl at 105 °C for 8 h under N_2. The solution was filtered through a glass microfibre filter (Whatman GF/F 55 mm diameter). A 60 mL aliquot of hydrolysate was evaporated on a rotary evaporator at 45 °C to remove the HCl and then re-dissolved with ultra-pure H_2O and evaporated to dryness again. The remaining sample was re-dissolved in 300 mL of ultra-pure H_2O and derivatised while mixing using 500 uL of ortho-phthaldialdehyde derivatisation reagent (Sigma Aldrich, USA) to produce indole derivatives. The resulting sample was filtered through a 0.45 μm PTFE membrane mounted on a glass syringe into a 2 mL glass vial. After 120 ± 5 s derivatisation reaction time, 15 μL of the sample was injected into the HPLC. Chromatographic separations were performed on a Shimadzu HPLC system (Shimadzu, Japan) using a Hyperclone C18 column (Phenomenex, Germany; 125 × 4 mm, 5 mm particle size, 12 nm pore size) with a C18 Security Guard™ cartridge (Phenomenex, Germany; 4 × 2 mm) at 35 °C with the fluorescence detector set at 445 nm emission and 330 nm excitation wavelengths.

The MurA, GalN and GluN were used as biomarkers for microbial residues and AS was estimated as the sum of these three amino sugars. F-C and B-C was calculated according to Engelking et al. (2007) which has been widely used in field studies (Joergensen et al., 2010; Sradnick et al., 2014; Shao et al., 2019; Ye et al., 2019; Ni et al., 2020; Zhu et al., 2020). F-C (F-C mg kg^{-1}) was calculated as an index for fungal residues by subtracting bacterial GluN from total GluN, assuming that MurA and GluN exist at a 1–2 M ratio in bacterial cells (Eq. (1)):

$$\text{F-C} = (\text{mmol GluN} - 2 \times \text{mmol MurA}) \times 179.17 \times 9 \quad (1)$$

where 179.17 is the molecular weight of GluN, 9 is the conversion value of fungal GluN to F-C (Joergensen and Wichern, 2008).

We assumed all MurA was derived from bacteria; B-C (mg kg^{-1}) was calculated as Eq. (2):

$$\text{B-C} = \text{MurA} \times 45 \quad (2)$$

where 45 is the conversion value of MurA to bacterial residue (Appuhn and Joergensen, 2006).

The SOC storage was also calculated with the equivalent soil mass down to 20 cm depth (sum of 0–5 cm, 5–10 cm and 10–20 cm) based on the method of Ellert and Bettany (1995) and expressed as C storage in Mg ha^{-1}. The M-C storage (Mg C ha^{-1}) was calculated as: (M-C / SOC) * SOC storage. The M-C storage % = M-C storage / SOC storage * 100%.

2.4. Statistical analysis

In this study, the differences among five treatments for all indices were examined using one-way ANOVA, followed by a Duncan's Multiple Range test at $P < 0.05$. Two-way ANOVA was conducted on a subset of treatments including NTMS, MPMS, NTMM and MPMM to examine the effects of tillage, and cropping system on AS, M-C storage and its distribution in SOC storage (%). Statistical analyses were performed using SPSS 16.0 for windows (SPSS Inc., Chicago, IL USA). A decaying exponential regression model (Ver 9.2, SAS Institute Inc., Cary, NC, USA 2002) was calculated between M-C and SOC. To quantify the relative contribution of tillage and cropping to necromass C storage, variation partitioning analysis (VPA) was performed using the 'varpart' function in the Vegan library (Oksanen et al., 2012) of the R software (R 3.4.0, R Development Core Team 2017).

3. Results

3.1. Original amino sugar concentration

The contribution of each type of amino sugar to the total amino sugar pool followed the order GluN > GlaN > MurA in all treatments and depths as shown by Table 1. Three-way ANOVA showed that tillage, depth and their interactions had significant effect on all amino sugars (Table 2); the different tillage practices also led to the difference among depths. Hence, separate two-way ANOVA's were conducted to test the influences of tillage and cropping system in each layer (Table 3). The GluN was only affected by tillage in all depths where the NT treatments (NTCC and NTCS) had almost 50% higher GluN content than the other tillage treatments (MPCC, MPCS and CTCC) in 0–5 cm layer. However, the MurA was more sensitive to tillage and cropping system where NT had more MurA than MP and CC had more MurA than CS in 0–5 cm and 5–10 cm layers. In the 0–5 cm layer, GlaN was affected by tillage with NT having greater content than MP (Table 1); the 5–10 cm layer was influenced by cropping system with CC having greater GlaN content than CS. The total AS was affected by tillage in all layers where NT was higher than MP in 0–5 cm layer but NTCC still had the highest AS among all treatments in 5–10 cm layer. Comparing CTCC and MPCC showed that residue return had no influence on the amount of GluN and GalN while CTCC had lower MurA than MPCC; however, there was no difference in total AS because MurA comprised only a small portion of total AS.

Table 1
Mean amino sugar content in all treatments.

Depth (cm)	Treatment	MurA (μg g^{-1})	GalN (μg g^{-1})	GluN (μg g^{-1})	Total AS (μg g^{-1})
0–5	NTCS	54.1 ± 1.7B	271.2 ± 8.1 A	1046.6 ± 35.4 A	1372.7 ± 28.5 A
	MPCS	40.9 ± 1.3D	232.2 ± 6.2B	642.8 ± 20.6B	916.4 ± 25.4B
	NTCC	61.5 ± 0.5 A	269.3 ± 9.9 A	1011.7 ± 21.3 A	1342.7 ± 30.9 A
	MPCC	46.5 ± 0.1 C	233.0 ± 4.7B	648.3 ± 9.16B	927.8 ± 11.7B
	CTCC	41.3 ± 1.1D	230.3 ± 2.8B	630.1 ± 11.1B	901.8 ± 12.8B
5–10	NTCS	39.2 ± 1.3 C	229.8 ± 4.5B	658.0 ± 26.1B	927.1 ± 29.5B
	MPCS	40.1 ± 0.8 C	235.3 ± 1.6B	647.1 ± 11.2B	922.6 ± 10.8B
	NTCC	56.9 ± 2.4 A	265.8 ± 9.0 A	800.2 ± 53.1 A	1123.4 ± 58.6 A
	MPCC	45.8 ± 0.3B	236.5 ± 6.9B	659.8 ± 5.6B	942.1 ± 6.1B
	CTCC	39.7 ± 0.5 C	227.4 ± 7.5B	622.5 ± 19.6B	889.7 ± 27.0B
10–20	NTCS	32.0 ± 0.5B	215.9 ± 3.7B	525.9 ± 15.9 BC	773.9 ± 17.7 C
	MPCS	37.3 ± 0.8 A	227.0 ± 5.3 AB	624.5 ± 14.3A	888.8 ± 15.8 A
	NTCC	37.3 ± 1.3 A	234.9 ± 4.3 A	560.2 ± 12.1B	832.5 ± 17.0B
	MPCC	41.1 ± 1.2 A	234.0 ± 5.3AB	631.3 ± 6.52 A	906.6 ± 3.68 A
	CTCC	30.7 ± 1.2B	231.5 ± 5.2AB	506.9 ± 9.98 C	769.1 ± 13.3 C

MurA: muramic acid; GalN: galactosamine; GluN: glucosamine; AS: amino sugars; NTCS: no-tillage with corn-soybean rotation; MPCS: moldboard plowing with corn-soybean rotation; NTCC: no-tillage with continuous corn; MPCC: moldboard plowing with continuous corn; CTCC: conventional tillage with continuous corn. Average value ± relative standard deviation (%), n = 4. Means in the same column and same layer with the same uppercase letter are not significantly different.

Table 2
The results of three-way ANOVA of the effects of tillage, cropping, depth and their interactions on the mean values of amino sugars, n = 4.

Source of variation		MurA (μg g^{-1})	GalN (μg g^{-1})	GluN (μg g^{-1})	AS (μg g^{-1})
Tillage	*F*	35.79	12.58	65.48	72.27
	P	**< 0.001**	**0.001**	**< 0.001**	**< 0.001**
Cropping	*F*	85.18	6.140	3.244	7.230
	P	**< 0.001**	**0.018**	0.080	**0.011**
Depth	*F*	96.43	10.61	89.69	96.84
	P	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Tillage*Cropping	*F*	9.498	3.100	1.584	2.970
	P	**0.004**	0.087	0.216	0.093
Tillage*Depth	*F*	43.36	8.821	79.44	83.27
	P	**< 0.001**	**0.001**	**< 0.001**	**< 0.001**
Cropping*Depth	*F*	6.694	1.845	3.030	3.961
	P	**0.003**	0.173	0.061	**0.028**
Tillage*Cropping*Depth	*F*	4.495	1.696	2.562	3.479
	P	**0.018**	0.198	0.191	**0.042**

MurA: muramic acid; GalN: galactosamine; GluN: glucosamine; AS: amino sugars; The values shown in bold face are significant at the 0.05 level.

Table 3
The *F* ratios and *P* values from a two-way ANOVA of the effects of tillage, cropping, and their interactions on the mean values of amino sugars in three layers, n = 4.

Depth (cm)	Source of variation		MurA (μg g^{-1})	GalN (μg g^{-1})	GluN (μg g^{-1})	AS (μg g^{-1})
0–5	Tillage	*F*	118.7	18.63	198.4	222.2
		P	**< 0.001**	**0.001**	**< 0.001**	**< 0.001**
	Cropping	*F*	24.90	0.004	0.292	0.090
		P	**< 0.001**	0.952	0.599	0.769
	Tillage*Cropping	*F*	0.482	0.022	0.551	0.499
		P	0.501	0.883	0.472	0.494
5–10	Tillage	*F*	9.404	2.780	4.839	5.894
		P	**0.010**	0.121	**0.048**	**0.032**
	Cropping	*F*	48.24	6.767	5.070	7.952
		P	**< 0.001**	**0.023**	**0.044**	**0.015**
	Tillage*Cropping	*F*	12.770	5.927	3.541	5.324
		P	**0.004**	**0.031**	0.084	**0.040**
10–20	Tillage	*F*	13.48	0.854	33.38	30.86
		P	**0.003**	0.374	**< 0.001**	**< 0.001**
	Cropping	*F*	13.46	5.623	1.966	5.037
		P	**0.003**	**0.035**	0.186	**0.044**
	Tillage*Cropping	*F*	0.298	1.188	0.873	1.436
		P	0.595	0.297	0.369	0.254

MurA: muramic acid; GalN: galactosamine; GluN: glucosamine; AS: amino sugars; The values shown in bold face are significant at the 0.05 level.

3.2. M-C storage and its distribution in SOC storage

Linear regression, piece-wise linear regression (figures not shown) and decaying exponential regression analysis were all conducted to examine the relationship between M-C and SOC. After comparison and considering the practical meaning in a biological system, the decaying exponential model (Fig. 1) was selected to represent the particular relationship between M-C and SOC (Eq. (3)).

$$\text{M-C} = -6.12 + 27.25 \times (1 - e^{-0.04 \times \text{SOC}}),\ R^2 = 0.87;\ n = 60 \quad (3)$$

The equation showed M-C and SOC fits well in the decaying exponential model with $R^2 = 0.87$. The M-C storage ranged from 14.90 (Mg C ha^{-1}, CTCC) to 20.87 (Mg C ha^{-1}, NTCC). Tillage influenced the M-C storage, F-C storage and B-C storage predominantly (Table 4). Fig. 2B shows the proportion of M-C, F-C and B-C storage in total SOC storage. The proportion of M-C in SOC storage was approximately 40% for all treatments including CTCC and was not affected by tillage. B-C storage proportion was different between CS and CC which led to the difference in M-C storage proportion between cropping systems (Table 4). The proportion of F-C storage accounted for 30% of total SOC storage but showed no difference among the five treatments (Fig. 2B). VPA was used to quantitatively assess the contribution of tillage and cropping to M-C storage and M-C storage % (Fig. 3). In total, 82%, 75% and 93% variation of M-C storage, F-C storage and B-C storage were explained, respectively. For M-C storage%, 41%, 14% and 96% variation of M-C storage %, F-C storage % and B-C storage % were explained, respectively. Tillage contributed more than cropping in the explanation of M-C storage (60%) and F-C storage (69%). However, cropping explained 79% of the variation of B-C storage and 96% of the variation of B-C storage % which was much higher than tillage (Fig. 3).

3.3. M-C storage and its distribution in Δ SOC storage

Returned residue stimulated the growth of microbes as evident from comparison of CTCC with other treatments (Fig. 2A). Hence, we calculated the increase of necromass C storage of NTCS, MPCS, NTCC and MPCC relative to CTCC (Fig. 4); the notation 'Δ' is used to indicate the change in a parameter relative to CTCC. The Δ M-C storage was significantly higher in NT than MP, and the Δ F-C storage showed higher levels in both NTCC and NTCS indicating a tillage effect (Fig. 4A). However, there was no difference in the proportion of Δ F-C storage in Δ SOC storage among the four treatments (Fig. 4B). More than half of the increase in SOC storage existed as M-C in CC cropping system with residue return (Fig. 4B). The Δ F-C storage and its proportion of Δ SOC storage was always higher in CC than CS cropping system for the same tillage system (Fig. 4A, B).

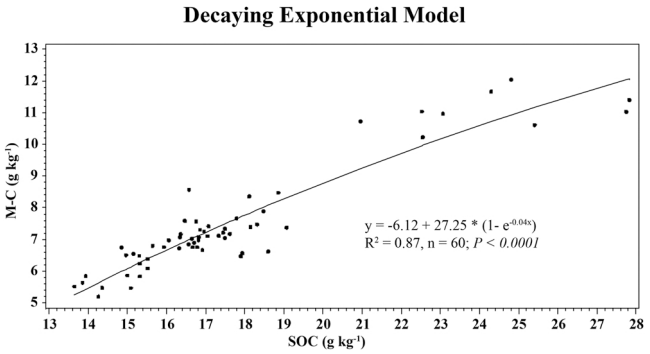

Fig. 1. Decaying exponential model between M-C and SOC, n = 60; SOC: soil organic carbon, M-C: Microbial necromass C.

Table 4
The *F* ratios and *P* values from a two-way ANOVA of the effects of tillage, cropping, and their interactions on the mean values of necromass C storage and distribution in SOC storage (%), n = 4. All C storage data were for the 0–20 cm plow layer.

Source of variation		Necromass C storage (Mg C ha^{-1})			Distribution of necromass C storage (% of SOC storage)		
		Microbial	Fungal	Bacterial	Microbial	Fungal	Bacterial
Tillage	*F*	50.58	37.74	26.83	1.438	2.574	2.000
	P	**< 0.001**	**< 0.001**	**< 0.001**	0.254	0.135	0.183
Cropping	*F*	20.15	4.600	142.2	9.391	1.043	128.0
	P	**< 0.001**	0.053	**< 0.001**	**0.010**	0.327	**< 0.001**
Tillage*Cropping	*F*	5.888	2.645	9.422	0.160	0.191	2.000
	P	**0.032**	0.130	**0.010**	0.696	0.669	0.183

The values shown in bold face are significant at the 0.05 level.

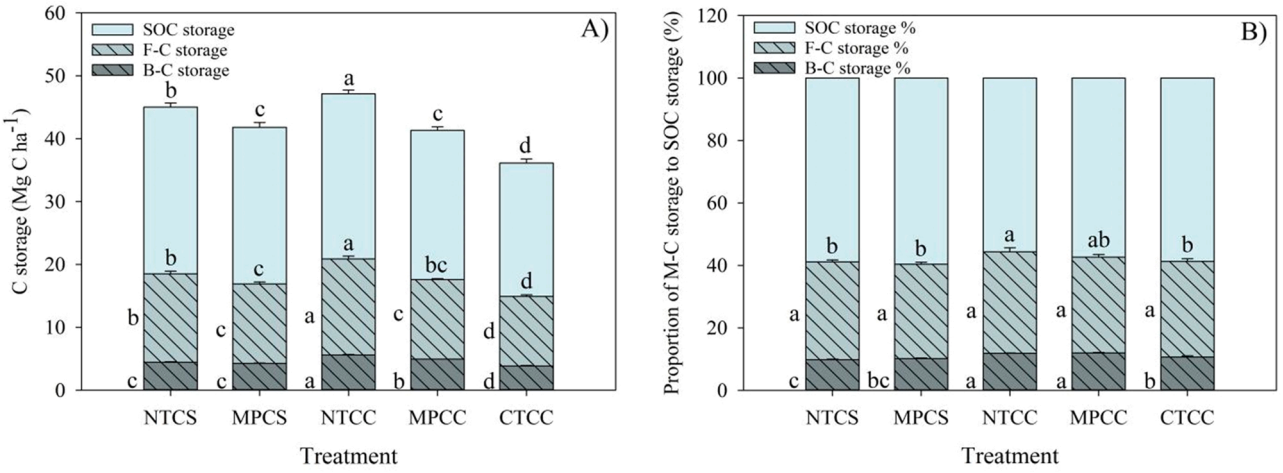

Fig. 2. A) SOC storage and microbial M-C storage (Mg C ha^{-1}); B) Proportion of M-C storage to SOC storage (%) (means, n = 4). Error bars represent standard error. The same lowercase letter left of the bars represent no significant difference among treatments for fungal and bacterial necromass C storage ($P > 0.05$). The same lowercase letter on the top of the bars represent no significant difference among treatments for SOC storage and total M-C storage ($P > 0.05$), bars representing total M-C storage include diagonal lines. The C storage was calculated for the top 20 cm soil layer. SOC: soil organic carbon; F-C: fungal necromass carbon; B-C: bacterial necromass carbon; NTCS: no-tillage with corn-soybean rotation; MPCS: mouldboard plowing with corn-soybean rotation; NTCC: no-tillage with continuous corn; MPCC: mouldboard plowing with continuous corn; CTCC: conventional tillage with continuous corn.

4. Discussion

4.1. Change in amino sugars among treatments and depths

Previous research confirmed that tillage, cropping system and depth had strong effects on SOC (Gao et al., 2017; Wang et al., 2019). In same present experiment site, previous research (Zhang et al., 2018) also reported the influences of tillage cropping system and depth on SOC in the long-term study, hence, in this paper we focussed on the amino sugars and did not discuss the SOC again. The decrease of plant residue changed the microbial community structure with depth, especially fungi and bacteria; these two microbial groups accounted for more than 95% of the total microbial biomass (Sradnick et al., 2014). Zhang et al. (2014) found that long-term NT enhanced the GluN in the surface layer (0–5 cm) compared to MP which was consistent with our findings, indicating that NT stimulated the fungal necromass due to less soil disturbance, residue return on the soil surface, and wetter and cooler soils (Zhang et al., 2012). The MurA also showed an increasing trend under NT compared to MP in both cropping systems which was also demonstrated by Ding et al. (2011). Meanwhile, MurA was also influenced by cropping system where CC had higher MurA content than CS which suggested that corn and soybean residue had different affects on bacterial necromass due to their different qualities and quantities. Our results showed that MurA was affected by both tillage and cropping system in all three layers. This may be a result of faster turnover rate of bacterial residue than fungal residue (Amelung et al., 2002). The GalN, which was considered to be derived from both bacteria and fungi (Engelking et al., 2007), showed the same change pattern as GluN and MurA in the 0–5 cm layer. Comparison of CTCC and MPCC, showed that only MurA had significant change which mirrored that the bacteria were also sensitive to the quantity of residue returned, i.e. no residue returned in CTCC, and all corn residue returned in MPCC.

It has been generally shown that NT has higher AS than MP in surface layer because of abundant residue input and no soil disturbance (Simpson et al., 2004; Zhang et al., 2014). NT resulted in a stratification of total AS due to the depletion of plant residue with increasing depth (Sradnick et al., 2014), and GluN contributed the most to this trend. Tillage redistributes the residue input throughout the plow layer (0–20 cm), hence the AS in MP treatment showed no stratification (Bai et al., 2013). Cropping system had no effect on the AS in our study; this was in contrast with previous studies (Liang et al., 2007; Ding et al., 2011) which showed corn accumulated more AS than soybean. These previous reports were from an incubation study (Liang et al., 2007) or short-term field study (Ding et al., 2011). Our results from the long-term field experiment showed no difference in 0–5 cm layer between cropping systems.

4.2. Effect of tillage, cropping and residue returned on microbial necromass C storage

Long-term agriculture field experiments are particularly valuable for providing crucial resources for the evaluation of management-induced changes in M-C and their contribution to SOC (Glaser et al., 2006). Previous study (West and Post, 2002) concluded that SOC could reach a

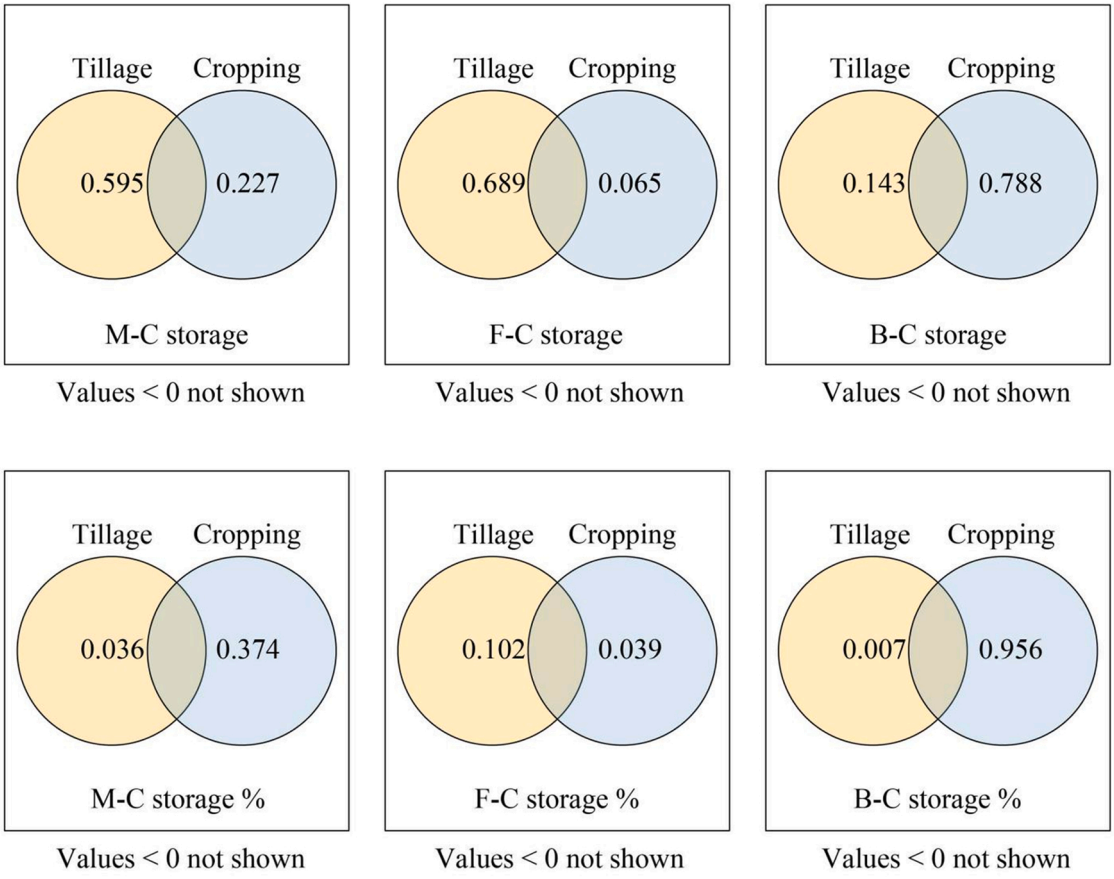

Fig. 3. Variation partitioning of tillage and cropping in M-C storage (%), F-C storage (%) and B-C storage (%).

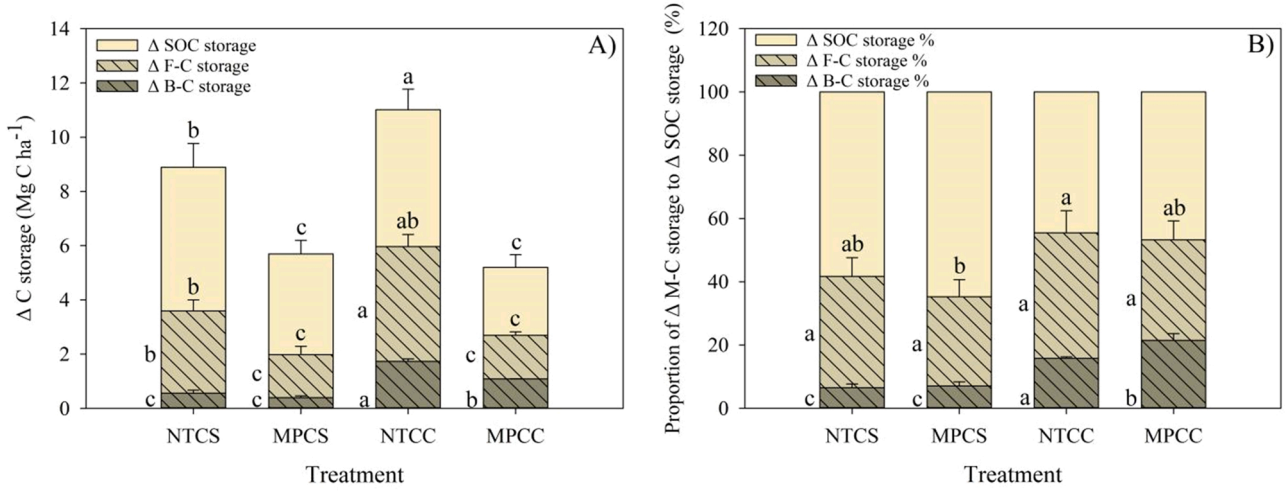

Fig. 4. A) Δ SOC storage and Δ M-C storage (Mg C ha^{-1}); B) Proportion of Δ M-C storage to Δ SOC storage (%) (means, n = 4). Error bars represent standard error. The symbol is used to indicate change in C storage (M-C, B-C, F-C or SOC) relative to conventional tillage continuous corn (CTCC). The same lowercase letter left of the bars represent no significant difference among treatments for fungal and bacterial necromass C storage, respectively ($P > 0.05$). The same lowercase letter on the top of the bars represent no significant difference among treatments for SOC storage and total microbial necromass C storage ($P > 0.05$), bars representing total microbial necromass C storage include diagonal lines. The C storage was calculated for the top 20 cm soil layer. SOC: soil organic carbon; F-C: fungal necromass carbon; B-C: bacterial necromass carbon; NTCS: no-tillage with corn-soybean rotation; MPCS: mouldboard plowing with corn-soybean rotation; NTCC: no-tillage with continuous corn; MPCC: mouldboard plowing with continuous corn.

stable status in 15–20 years following soil tillage change from CT to NT, thus it is crucial to evaluate the M-C after long-term application of the treatments. Previous studies suggested that the relationship between M-C and SOC was linear (Liang et al., 2007; Ding et al., 2017). However, those results were concluded from short term studies. In contrast, Lauer et al. (2011) considered that the M-C would reach a steady state in the process of soil restoration and maybe it was driven by the clay fraction, which contained most of the M-C in soil. In our study, there appears to be

a general flattening of the M-C vs. SOC curve beyond SOC about 21 g kg^{-1}. We fitted the M-C vs. SOC data to linear, piecewise linear and decaying exponential regression models. The piecewise linear model (not shown) showed a flattening of the relationship with an inflection point at SOC of 24.5 g kg^{-1} but unfortunately, there were insufficient data beyond the inflection point to conclusively confirm a flattening of the curve. The data fit well to a decaying exponential equation; this equation is widely used for modeling growth and physical and biological processes where the rate of change is proportional to distance from a fixed value. This model is asymptotic to a steady state value and gives a hint that the relationship may reach a steady state value at high SOC levels; more data at higher levels of SOC are required to conclusively determine if the M-C vs. SOC relationship does indeed reach a steady state level. Hence, a longer term observation to achieve higher SOC levels could be done in the future on data from our site or similar long-term experiments to determine if a steady state condition is reached.

The M-C storage varied greatly among soil types of grassland, forest and agriculture (Ni et al., 2020). The M-C storage of approximately 40% of SOC storage in our study was less than the lower bound of the range of 47–80% reported by Liang (2019). The aforementioned range was generated by a model and it might be different from actual field data (Fan and Liang, 2015). The M-C storage was higher in NT than MP (Fig. 3A) which conferred with conclusions of previous studies (Guggenberger et al., 1999; Ding et al., 2011; Zhang et al., 2014). M-C was influenced by the type of plant material added to soil which resulted in corn residue accumulating more M-C than soybean (Liang et al., 2007) due to its higher C/N ratio (Cai et al., 2018), and possibly greater quantity (Ding et al., 2011; Zhang et al., 2018). Furthermore, understanding the F-C and B-C distribution in SOC can provide insights into how the respective microbes govern C and N cycling in soil (Simpson et al., 2004). Liang et al. (2019) concluded that F-C (> 70%) contributed more to SOC than B-C (26%–28%) which was consistent with our results. Nevertheless, there was no difference in proportion of F-C storage to SOC storage among treatments including CTCC which indicated that residue, tillage and cropping management had no effect on this ratio. In our experiment, residue quantity (compare MPCC to CTCC) and quality (compare CC and CS) had a significant effect on B-necromass (Table 1). Notably, cropping system contributed 78.8% to the explanation of variance of B-C storage to SOC storage proportion which emphasizes the residue quality and quantity effects on B-necromass. Bai et al. (2013) elucidate that compared with bacteria, fungi seem to be less dependent on the quality of the residue from an incubation test. Here, we draw the same conclusion in a long-term field study (16-year): the opposite influences of tillage and crop on fungal and bacterial necromass C storage proportion might be due to the different living behavior of fungi and bacteria; fungal energy channels are considered as slow cycles and bacteria are more sensitive to the soil environment changes (Joergensen et al., 2008).

Residue return has been suggested as an effective way to increase SOC storage in agroecosystems (Liu et al., 2014; Wu et al., 2019). The increase in SOC storage (Δ SOC storage) by residue return was also regulated by tillage and cropping (Zhang et al., 2020) which must result in different distribution of fungal and bacterial necromass C storage. Calculating the ratio of Δ M-C storage to Δ SOC storage is conducive to assessing the role of residue return in SOC sequestration in long-term because necromass C represents the stable component of SOC (Miltner et al., 2012). More than half Δy SOC storage existed as M-C storage under CC cropping which was higher than CS crop rotation suggesting that CC cropping system was better for SOC long-term sequestration. This is in agreement with other study using physical fractionation to assess the influence of cropping system on SOC sequestration (Zhang et al., 2018). The difference of Δ M-C storage proportion among treatments was induced by the difference of Δ B-C storage proportion; there was no difference in the Δ F-C storage proportion among the four treatments.

5. Conclusion

Our investigation showed that the M-C and SOC data fit well to a decaying exponential model which suggested that M-C may reach a steady state in the future at higher SOC. Tillage significantly affected the M-C, F-C, B-C storage but had no influence on their contribution as a percentage of total SOC storage. More than half of the increase in SOC storage due to no-tillage existed as M-C storage under CC cropping which was higher than CS, suggesting that CC cropping system was better for SOC long-term sequestration. F-C storage % was not affected by agriculture management (residue return, tillage and cropping), whereas B-C storage % was affected by both quantity and quality of residue which indicated that bacterial C storage was more sensitive to agriculture management.

Declaration of Competing Interest

None.

Acknowledgments

This research was supported by the National Natural Science Foundation of China (42101277, 41877095 and 42077046), Science and Technology Project for Black Soil Granary (XDA28080200), Strategic Priority Research Program of the Chinese Academy of Sciences (XDA2307050103), the Key Research Program of Frontier Sciences, CAS (QYZDB-SSW-DQC035), Innovation Leadership and Team Program in Sciences and Technologies for Young and Middle-aged Scientists of Jilin Province (20200301022RQ), and the Youth Innovation Promotion Association of Chinese Academy of Sciences.

References

Amelung, W., Brodowski, S., Sandhage-Hofmann, A., Bol, R., 2008. Combining biomarker with stable isotope analyses for assessing the transformation and turnover of soil organic matter. Adv. Agron. 100, 155–250.
Amelung, W., Lobe, I., Du Preez, C.C., 2002. Fate of microbial residues in sandy soils of the South African Highveld as influenced by prolonged arable cropping. Eur. J. Soil Sci. 53 (1), 29–35.
Appuhn, A., Joergensen, R.G., 2006. Microbial colonisation of roots as a function of plant species. Soil Biol. Biochem. 38, 1040–1051.
Bai, Z., Bodé, S., Huygens, D., Zhang, X.D., Boeckx, P., 2013. Kinetics of amino sugar formation from organic residues of different quality. Soil Biol. Biochem. 57 (3), 814–821.
Batjes, N.H., 2014. Total carbon and nitrogen in the soils of the world. Eur. J. Soil Sci. 47, 151–163.
Cai, A.D., Liang, G.P., Zhang, X.B., Zhang, W.J., Li, L., Rui, Y.C., Xu, M.G., Luo, Y.Q., 2018. Long-term straw decomposition in agro-ecosystems described by a unified three-exponentiation equation with thermal time. Sci. Total Environ. 636, 699–708.
Chantigny, M.H., Angers, D.A., PreVost, D., Vezina, L.P., Chalifour, F.P., 1997. Soil aggregation and fungal and bacterial biomass under annual and perennial cropping systems. Soil Sci. Soc. Am. J. 61, 262–267.
Chen, J.M., 2021. Carbon neutrality: toward a sustainable future. Innovation 2, 100127.
De Clercq, T., Heiling, M., Dercon, G., Resch, C., Aigner, M., Mayer, L., Mao, Y., Elsen, A., Steier, P., Leifeld, J., 2015. Predicting soil organic matter stability in agricultural fields through carbon and nitrogen stable isotopes. Soil Biol. Biochem. 88, 29–38.
Ding, X.L., Zhang, B., Zhang, X.D., Yang, X.M., Zhang, X.P., 2011. Effects of tillage and crop rotation on soil microbial residues in a rainfed agroecosystem of Northeast China. Soil Till. Res. 114 (1), 43–49.
Ding, X.L., Qiao, Y.F., Filley, T., Wang, H.Y., Lü, X.X., Zhang, B., Wang, J.K., 2017. Long-term changes in land use impact the accumulation of microbial residues in the particle-size fractions of a Mollisol. Biol. Fertil. Soils 53 (3), 281–286.
Engelking, B., Flessa, H., Joergensen, R.G., 2007. Shifts in amino sugar and ergosterol contents after addition of sucrose and cellulose to soil. Soil Biol. Biochem. 39 (8), 2111–2118.
Fan, Z., Liang, C., 2015. Significance of microbial asynchronous anabolism to soil carbon dynamics driven by litter inputs. Sci. Rep. 5, 9575.
Fernandez, C.W., Heckman, K., Kolka, R., Kennedy, P.G., 2019. Melanin mitigates the accelerated decay of mycorrhizal necromass with peatland warming. Ecol. Lett. 22 (3), 498–505.
Gao, L., Becker, E., Liang, G., Houssou, A.A., Wu, H., Wu, X., Cai, D., Degré, A., 2017. Effect of different tillage systems on aggregate structure and inner distribution of organic carbon. Geoderma 288, 97–104.
Gao, L., Wang, B., Li, S., Wu, H., Wu, X., Liang, G., Gong, D.Z., Zhang, X.M., Cai, D.X., Degré, A., 2018. Soil wet aggregate distribution and pore size distribution under

different tillage systems after 16 years in the loess plateau of China. Catena 173, 38–47.
Gao, L., Wang, B., Li, S., Han, Y., Zhang, X.M., Gong, D.Z., Ma, M.C., Liang, G.P., Wu, H. J., Wu, X.P., Cai, D.X., Degré, A., 2019. Effects of different long-term tillage systems on the composition of organic matter by 13C CP/TOSS NMR in physical fractions in the loess plateau of China. Soil Till. Res. 194, 104321.
Glaser, B., Millar, N., Blum, H., 2006. Sequestration and turnover of bacterial- and fungal-derived carbon in a temperate grassland soil under long-term elevated atmospheric pCO_2. Glob. Chang. Biol. 12, 1521–1531.
Glaser, B., Turrión, M.B., Alef, K., 2004. Amino sugars and muramic acid—biomarkers for soil microbial community structure analysis. Soil Biol. Biochem. 36 (3), 399–407.
Guggenberger, G., Frey, S.D., Six, J., Paustian, K., Elliott, E.T., 1999. Bacterial and fungal cell-wall residues in conventional and no-tillage agroecosystems. Soil Sci. Soc. Am. J. 63, 1188–1198.
He, H.B., Xie, H.T., Zhang, X.D., 2006. A novel GUMS technique to assess N-15 and C-13 incorporation into soil amino sugars. Soil Biol. Biochem. 38, 1083–1091.
Indorf, C., Dyckmans, J., Khan, K.S., Joergensen, R.G., 2011. Optimisation of amino sugar quantification by HPLC in soil and plant hydrolysates. Biol. Fertil. Soils 47, 387–396.
Joergensen, R.G., Wichern, F., 2008. Quantitative assessment of the fungal contribution to microbial tissue in soil. Soil Biol. Biochem. 40 (12), 2977–2991.
Joergensen, R.G., Mäder, P., Fließbach, A., 2010. Long-term effects of organic farming on fungal and bacterial residues in relation to microbial energy metabolism. Biol. Fertil. Soils 46, 303–307.
Kallenbach, C.M., Frey, S.D., Grandy, A.S., 2016. Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. Nat. Commun. 7, 13630.
Kogel-Knabner, I., 2017. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter: fourteen years on. Soil Biol. Biochem. 105, A3–A8.
Lauer, F., Kösters, R., Preez, C.C.D., Amelung, W., 2011. Microbial residues as indicators of soil restoration in South African secondary pastures. Soil Biol. Biochem. 43 (4), 787–794.
Lehmann, J., Kleber, M., 2015. The contentious nature of soil organic matter. Nature 528, 60–68.
Li, S., Lu, J., Liang, G., Wu, X., Zhang, M., Plougonven, E., Wang, Y.F., Gao, L.L., Abdelrhman, A.A., Song, X.J., Liu, X.T., Degré, A., 2020. Factors governing soil water repellency under tillage management: the role of pore structure and hydrophobic substances. Land Degrad. Dev. 32, 1046–1059.
Li, Y., Li, Z., Cui, S., Liang, G., Zhang, Q.P., 2021. Microbial-derived carbon components are critical for enhancing soil organic carbon in no-tillage croplands: a global perspective. Soil Till. Res. 205, 104758.
Liang, A.Z., Yang, X.M., Zhang, X.P., Chen, X.W., McLaughlin, N.B., Wei, S.C., Zhang, Y., Jia, S.X., Zhang, S.X., 2016. Changes in soil organic carbon stocks under 10-year conservation tillage on a Black soil in Northeast China. J. Agric. Sci. 154 (08), 1425–1436.
Liang, C., Balser, T.C., 2010. Microbial production of recalcitrant organic matter in global soils: implications for productivity and climate policy. Nat. Rev. Microbiol. 9 (1), 75-75.
Liang, C., Zhang, X.D., Rubert IV, K.F., Balser, T.C., 2007. Effect of plant materials on microbial transformation of amino sugars in three soil microcosms. Biol. Fertil. Soils 43 (6), 631–639.
Liang, C., Amelung, W., Lehmann, J., Kästner, M., 2019. Quantitative assessment of microbial necromass contribution to soil organic matter. Glob. Chang. Biol. 25, 3578–3590.
Liang, G.P., Wu, H.J., Houssou, A.A., Cai, D.X., Wu, X.P., Gao, L.L., Wang, B.S., Li, S.P., 2018. Soil respiration, glomalin content, and enzymatic activity response to straw application in a wheat-maize rotation system. J. Soils Sediments 18, 697–707.
Liu, C., Lu, M., Cui, J., Li, B., Fang, C.M., 2014. Effects of straw carbon input on carbon dynamics in agricultural soils: a meta-analysis. Glob. Chang. Biol. 20, 1366–1381.
Lu, J., Li, S., Wu, X., Liang, G., Gao, C., Li, J.H., Jin, D.S., Wang, B.S., Zhang, M.N., Zheng, F.J., Degré, A., 2021. The dominant microorganisms vary with aggregates sizes in promoting soil carbon accumulation under straw application. Arch. Agron. Soil Sci. 1955354.
Ma, T., Zhu, S.S., Wang, Z.H., Chen, D., Dai, G.H., Feng, B.W., Su, X.Y., Hu, H.F., Li, K.H., Han, W.X., Liang, C., Bai, Y.F., Feng, X.J., 2018. Divergent accumulation of microbial necromass and plant lignin components in grassland soils. Nat. Commun. 9, 3480.
Miltner, A., Bombach, P., Schmidt-Brücken, B., Kästner, M., 2012. SOM genesis: microbial biomass as a significant source. Biogeochemistry 111 (1–3), 41–55.
Ni, X.Y., Liao, S., Tan, S.Y., Wang, D.Y., Peng, Y., Yue, K., Wu, F.Z., Yang, Y.S., 2020. A quantitative assessment of amino sugars in soil profiles. Soil Biol. Biochem. 143, 107762.
Ogle, S.M., Swan, A., Paustian, K., 2012. No-till management impacts on crop productivity, carbon input and soil carbon sequestration. Agric. Ecosyst. Environ. 149, 37–49.
Shao, P.S., Liang, C., Lynch, L., Xie, H.T., Bao, X.L., 2019. Reforestation accelerates soil organic carbon accumulation: evidence from microbial biomarkers. Soil Biol. Biochem. 131, 182–190.
Simpson, A.J., Simpson, M.J., Smith, E., Kelleher, B.P., 2007. Microbially derived inputs to soil organic matter: are current estimates too low? Environ. Sci. Technol. 41 (23), 8070–8076.
Simpson, R.T., Frey, S.D., Six, J., Thiet, R.K., 2004. Preferential accumulation of microbial carbon in aggregate structures of no-tillage soils. Soil Sci. Soc. Am. J. 68 (4), 1249–1255.
Sradnick, A., Oltmanns, M., Raupp, J., Joergensen, R.G., 2014. Microbial residue indices down the soil profile after long-term addition of farmyard manure and mineral fertilizer to a sandy soil. Geoderma 226–227, 79–84.
van Groenigen, K.-J., Bloem, J., Bååth, E., Boeckx, P., Rousk, J., Bodé, S., Forristal, D., Jones, M.B., 2010. Abundance, production and stabilization of microbial biomass under conventional and reduced tillage. Soil Biol. Biochem. 42, 48–55.
Wang, B., Gao, L., Yu, W., Wei, X., Li, J., Li, S., Song, X., Liang, G., Cai, D., Wu, X., 2019. Distribution of soil aggregates and organic carbon in deep soil under long-term conservation tillage with residual retention in dryland. J. Arid Land 11, 241–254.
Wang, X., Wang, C., Cotrufo, M.F., Sun, L.F., Jiang, P., Liu, Z.P., Bai, E., 2020. Elevated temperature increases the accumulation of microbial necromass nitrogen in soil via increasing microbial turnover. Glob. Chang. Biol. 26, 5277–5289.
West, T.O., Post, W.M., 2002. Soil organic carbon sequestration rates by tillage and crop rotation: a global data analysis. Soil Sci. Soc. Am. J. 66, 1930–1946.
Wu, L., Zhang, W., Wei, W., He, Z.L., Kuzyakov, Y., Bold, R., Hu, R.G., 2019. Soil organic matter priming and carbon balance after straw addition is regulated by long-term fertilization. Soil Biol. Biochem. 135, 383–391.
Ye, G.P., Lin, Y.X., Kuzyakov, Y., Liu, D.Y., Luo, J.F., Lindsey, S., Wang, W.J., Fan, J.B., Ding, W.X., 2019. Manure over crop residues increases soil organic matter but decreases microbial necromass relative contribution in upland Ultisols: results of a 27-year field experiment. Soil Biol. Biochem. 134, 15–24.
Zhang, B., He, H., Ding, X., Zhang, X., Zhang, X., Yang, X., Filley, T.R., 2012. Soil microbial community dynamics over a maize (Zea mays L.) growing season under conventional- and no-tillage practices in a rainfed agroecosystem. Soil Till.. Res. 124, 153–160.
Zhang, B., Drury, C.F., Yang, X.M., Reynolds, W.D., Zhang, X.D., 2014. Effects of long-term and recently imposed tillage on the concentration and composition of amino sugars in a clay loam soil in Ontario, Canada. Soil Till. Res. 135, 9–17.
Zhang, X.D., Amelung, W., 1996. Gas chromatographic determination of muramic acid, glucosamine, mannosamine, and galactosamine in soils. Soil Biol. Biochem. 28, 1201–1206.
Zhang, Y., Li, X.J., Gregorich, E.G., McLaughlin, N.B., Zhang, X.P., Guo, Y., Liang, A.Z., Fan, R.Q., Sun, B.J., 2018. No-tillage with continuous corn cropping enhances soil aggregation and organic carbon storage in Northeast China. Geoderma 330, 204–211.
Zhang, Y., Li, X.J., Gregorich, E.G., McLaughlin, N.B., Zhang, X.P., Guo, Y.F., Liang, A.Z., 2020. Tillage and cropping effects on soil organic carbon: biodegradation and storage in density and size fractions. Eur. J. Soil Sci. 71, 1188–1199.
Zhu, X., Jackson, R.D., Delucia, E.H., Tiedje, J.M., Liang, C., 2020. The soil microbial carbon pump: from conceptual insights to empirical assessments. Glob. Chang. Biol. 26, 6032–6039.