Initial soil organic matter content influences the storage and turnover of litter, root and soil carbon in grasslands

Authors: SHAN XU[1, 2, 3], PING LI[1, 2], EMMA J SAYER[4,5,6], BEIBEI ZHANG[1, 2], JING WANG[1, 2], CHUNLIAN QIAO[1, 2] , ZIYANG PENG[1, 2], LIWEI DIAO[1], YONGGAN CHI[1], WEIXING LIU[1], LINGLI LIU[1, 2*]

Author affiliations:

[1] State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Xiangshan, Beijing 100093, China

[2] University of Chinese Academy of Sciences, Yuquanlu, Beijing 100049, China

[3] South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China

[4] Lancaster Environment Centre, Lancaster University, Lancaster, LA1 4YQ

[5] Smithsonian Tropical Research Institute, Balboa, Ancon, Republic of Panama

[6] School of Environment, Earth and Ecosystems, The Open University, Walton Hall, Milton Keynes, MK7 6AA, UK

***Corresponding author:**

Lingli Liu

Tel: +86-10-62836160; Fax: +86-10-82596146; Email: lingli.liu@ibcas.ac.cn

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LLL and SX designed the experiment. SX, PL, ZYP, LWD, CLQ, and JW performed the experiment. SX, LLL and EJS analyzed the data and wrote the manuscript. BBZ, YGC and WXL commented on details of the manuscript drafts.

Abstract

Grassland degradation is a worldwide problem that often leads to substantial loss of soil organic matter (SOM). To estimate the potential for carbon (C) accumulation in degraded grassland soils, we first need to understand how SOM content influences the transformation of plant C and its stabilization within the soil matrix. We conducted a greenhouse experiment using C_3 soils with six levels of SOM content; we planted the C_4 grass *Cleistogenes squarrosa* or added its litter to the soils to investigate how SOM content regulates the storage of new soil C derived from litter and roots, the decomposition of extant soil C, and the formation of soil aggregates. We found that with the increase in SOM content, microbial biomass carbon (MBC) and the mineralization of litter C increased. Both the litter addition and planted treatments increased the amount of new C inputs to soil. However, the mineralization of extant soil C was significantly accelerated by the presence of living roots but was not affected by litter addition. Accordingly, the soil C content was significantly higher in the litter addition treatments but was not affected by the planted treatments by the end of the experiment. The soil macroaggregate fraction increased with SOM content and was positively related to MBC. Our experiment suggests that as SOM content increases, plant growth and soil microbial activity increase, which allows microbes to process more plant-derived C and promote new soil C formation. Although long-term field experiments are needed to test the robustness of our findings, our greenhouse experiment suggests that the interactions between SOM content and plant C inputs should be considered when evaluating soil C turnover in degraded grasslands.

Keywords: soil organic matter content, litter decomposition, soil carbon transformation, soil aggregate, grasslands, microbial biomass

1 Introduction

Soil organic matter content is a key indicator of soil health, which determines plant productivity and microbial activity (Magdoff and Weil 2004). Grasslands cover 40.5% of the world's land area (Gibson 2009) and many of them are subjected to disturbance from environmental and land use changes, such as over-grazing (Reid and others 2004) and conversion from grassland to cropland (Wright and Wimberly 2013). These disturbances result in large losses of soil organic matter (SOM) and can cause desertification in many grassland ecosystems (Lal 2003; Wang and others 2011; McSherry and Ritchie 2013). Degraded grasslands are often subjected to extensive erosion, which selectively removes lighter organic matter and finer particles such as clay and silt and leaving heavier sand particles (Li and others 2005; Zhou and others 2008). Soil fertility, soil water-holding capacity, and therefore plant productivity, decrease with the severity of soil degradation (Lal 2001). Although there is much debate on the potential of grasslands to act as a sink for carbon (C), it is commonly agreed that better management of degraded grasslands could reduce soil C loss and enhance the C sequestration capacity of grasslands (Smith 2014). However, this requires a better understanding of the feedbacks between extant SOM content, plant productivity, microbial activity, and aggregate formation, which all contribute to the storage and stabilization of C in grassland soils.

Soils with greater SOM content are often more fertile and have higher water-holding capacity, which supports higher plant productivity (Saxton and Rawls 2006; Six and Paustian 2014). In turn, C inputs from plants, including aboveground litter and root litter and root exudates, play a key role in regulating the soil C balance (Kuzyakov and Domanski 2000; Santos and others 2016). Root inputs are considered as particularly important for soil C storage in grassland ecosystems, whereas aboveground litter contributes less to the formation of SOM (Rasse and

others 2005; Bird and Torn 2006; Prescott 2010; Schmidt and others 2011). Recent research suggests that microbes can incorporate labile C into soil more efficiently than recalcitrant C (Cotrufo and others 2013). Consequently, a decline in labile C sources, such as aboveground leaf litter and root exudates, could have a much greater impact on stable SOM formation than previously thought (Hatton and others 2015; Haddix and others 2016).

Soil organic matter accumulation is affected not only by C inputs from plants but also by microbial activity, which regulates the transformation of plant C to SOM (Cotrufo and others 2013). Soil organic matter is the most important energy and nutrient source for soil microbes (Fontaine and others 2011) and changes in SOM content can alter microbial community composition and C use efficiency (CUE; Manzoni and others 2012; Sinsabaugh and others 2016). Losses of SOM associated with soil degradation could therefore reduce the size and activity of the microbial population (Pascual and others 2000; Bastida and others 2006) and alter the capacity of soil microbial communities to decompose and transform plant-derived C into stable microbial products.

Finally, the capacity of soil to sequester C also depends on the stability of SOM (Jastrow and others 2007). In this context, soil aggregate formation is one of the key processes for increasing the residence time of SOM (Six and others 2002b) because C associated with soil microaggregates is physically protected from microbial attack by occlusion within macroaggregates, which slows its decomposition (Denef and others 2007; Six and Paustian 2014). The soil macroaggregate fraction often increases with SOM content (Blanco-Canqui and Lal 2004; Bronick and Lal 2005) and polysaccharides derived from aboveground litter and fine roots can improve soil aggregation by acting as binding agents (Six and others 2002b). Hence, SOM content could influence the mean residence time of soil C directly by promoting

macroaggregate formation and indirectly by altering plant aboveground litter inputs and root production.

Despite the potential importance of feedbacks between extant SOM content and additional soil C sequestration, few experimental studies have investigated how SOM loss due to soil degradation affects the stabilization of plant-derived C inputs and therefore the future C sequestration potential of the soil (Tan and others 2014; Castellano and others 2015). Importantly, feedbacks between inputs and storage of new C and the mineralization and release of extant C all contribute to net changes in soil C (Lange and others 2015). Hence, measurements of total soil C change or CO_2 efflux alone cannot elucidate the mechanisms underlying SOM storage. For example, increased inputs of plant-derived C can stimulate the release of older stored soil C via 'priming effects', with no net change in soil C content (Kuzyakov and others 2000). Although isotope studies have advanced our knowledge of SOM turnover under new C inputs (e.g., Cheng 2009; Blagodatskaya and others 2014), we have yet to determine how the extant SOM content influences the various processes involved in the storage of additional soil C.

In this study, we created a SOM gradient to simulate different levels of soil degradation in grasslands. We planted C_4 grass and added C_4 grass leaf litter into C_3 soils to simulate root and aboveground litter inputs, respectively. We used the natural difference in the ^{13}C isotope value between the C_4 plants and C_3 soil to track new soil C formation and the decomposition of extant C in soils with different SOM contents. We hypothesized that greater extant SOM results in greater C sequestration of new C inputs because 1) higher SOM content supports greater microbial biomass, which enhances the mineralization of litter-derived C and results in increased formation of new soil C derived from aboveground litter; 2) higher SOM content supports greater root biomass, thereby increasing root-derived new soil C; and 3) higher SOM content

improves soil aggregation, resulting in greater physical protection of soil C. However, as increased new C inputs from plants could also stimulate the decomposition of extant SOM via priming effects (Kuzyakov and others 2000), we also tested an alternative hypothesis: 4) increased root C inputs with greater extant SOM content will promote the mineralization and release of stored soil C, resulting in little or no increase in C sequestration.

2 Materials and Methods

2.1 Soil, leaf litter, seed sampling and pretreatments

Mineral soil (0-30 cm depth) was taken from the Duolun Restoration Ecology Research Station (42°2′N, 116°17′E), Inner Mongolia, China, in October 2012. The site was an overgrazed steppe, but was fenced to limit access by cattle in 2000. The soil is sandy and classified as a Haplic Calcisol according to the FAO soil classification. Bulk density is 1.31 Mg m^{-3}, mean soil pH is 7.7, and the concentrations of organic carbon (C) and nitrogen (N) are 1.40 ±0.02% (n=3) and 0.14 ±0.00% (n=3), respectively. The soil was homogenized, passed through a 2-mm mesh sieve to remove coarse fragments, and visible plant residues were removed using tweezers. The soil was divided into two parts: one part was air-dried and the other part was combusted in a muffle furnace at 550°C for three hours to remove the native SOC (German and others 2011). Six experimental levels of SOM were obtained by mixing the air-dried and combusted soil according to different mass percentages as follows: 100% combusted soil (S0), 80% combusted plus 20% air-dried soil (S20), 60% combusted plus 40% air-dried soil (S40), 40% combusted plus 60% air-dried soil (S60), 20% combusted plus 80% air-dried soil (S80), and 100% air-dried soil (S100). Hence, the six soil treatments (hereafter “soil types”) represent a gradient of increasing SOM content (hereafter “SOM levels”; Table 1).

The combustion treatment not only removes SOM, but can also alter soil properties, such as texture, nutrient content, mineralogy, and water holding capacity (Tan and others 1986; Certini 2005). We therefore analyzed these soil physiochemical properties for each SOM level. Soil texture, was measured using a particle size analyser (Malvern Masterizer 2000, Malvern, Worcestershire, UK) after removal of organic matter and carbonates. Soil samples were digested in a microwave oven (MA-1870, Haier, Qingdao, China), and the concentrations of mineral elements, including calcium (Ca), potassium (K), magnesium (Mg), sodium (Na) and phosphorus (P), were determined by an ICP-ES (ICP; Thermo Scientific, West Palm Beach, USA). Soil mineralogy was identified by X-ray diffraction spectroscopy (Ultima IV; Rigaku Corporation, Tokyo, Japan). Water holding capacity (WHC) was determined by the methods described in Hanson and others (2002). Briefly, air-dried soil samples (20 g) were placed in a funnel lined with pre-weighed filter paper, water was added to the soil until saturation, the soil was allowed to drain for 3 h, and then the soil and filter paper were weighed. We calculated WHC from the difference between the dry and drained net weights of the soil samples. We also analyzed soil C concentration by a CHNOS elemental analyzer (Vario EL III; Elementar Analysensysteme GmbH, Hanau, Germany) to assess the efficiency of the combustion treatment for removing SOM.

Leaf litter and seeds of *Cleistogenes squarrosa* were collected from the Duolun steppe in September 2012. *C. squarrosa* is a C_4 grass that is widely distributed in the Inner Mongolian temperate steppe; its leaf litter has a C concentration of 44.0 ±0.01% and a $\delta^{13}C$ of -14.1 ±0.1‰ and its root material has a $\delta^{13}C$ of -15.7 ±1.2‰, which are distinguished from the δ^{13} C of extant soil C ($\delta^{13}C$ of -23.8 ±0.02‰) and allowed us to use such a discrepancy in $\delta^{13}C$ value to partition

litter- or root-derived C from the extant soil C. Leaf litter was oven-dried at 40°C and chopped into 2.5 cm segments. The seeds were air-dried and stored in a dry place until February 2013.

2.2 Greenhouse experiment design

We conducted a greenhouse experiment using plastic pots (100 mm height, 120 mm upper diameter, and 100 mm lower diameter). The air temperature of the greenhouse was maintained at 20–30°C and air humidity was kept at 50–60%. We filled 12 pots, each with 746 g of air-dried soil from one of the six soil types, and randomly assigned the pots of each soil type to four treatments: 1) "planted" with *C. squarrosa* seeds; 2) "litter addition" with *C. squarrosa* leaf litter mixed into the soil; 3) planted plus litter addition; and 4) controls without plants or litter inputs. Hence, the experiment comprised three replicate pots of four treatments for each of the six soil types, making a total of 72 pots. For the planted treatment, the *C. squarrosa* seeds were immersed in warm water (40-60 °C) for 4 hours to improve seed germination and then 20-25 seeds were planted per pot on February 5, 2013. After germination, 12-13 seedlings were kept in the pots, mimicking the natural plant density at the study site (957 $\pm$325 plants m^{-2}), and the remaining seedlings were removed. For the litter addition treatments, we used 1.07 g of litter per 100 g of soil, which was the ratio of litter to soil described for the incubation experiments by Cheng and others (2012). Thus, we mixed 8 g of *C. squarrosa* leaf litter (C = 43.9%, N = 1.2%, C:N = 36.6) into 746 g of soil in each pot. It should be noted that the litter addition rate in this study was approximately two-fold greater per unit area than litter inputs observed in the field. All pots were watered with 150 ml of distilled water every week to avoid plant water stress.

2.3 Variable measurements at the end of the experiment

The greenhouse experiment lasted for 191 days when the plants started to senesce. At the end of the experiment, the aboveground biomass in the planted pots was harvested, oven-dried at 55°C for 48 h, and then weighed to determine the aboveground biomass. Plant roots were separated from the soil with tweezers, cleaned with deionized water in a 53 μm sieve, oven-dried at 55°C for 48 h, and weighed to determine the belowground biomass. For the litter addition treatments, the remaining litter was manually separated from the soil with tweezers and the large soil particles attached to the litter were removed. The retrieved litter was then air-dried and weighed to estimate remaining mass and to measure the C concentration. The soil from each pot was separated into two subsamples: one was air-dried for aggregate partitioning, total C concentration, and soil ^{13}C isotope measurements; the other subsample was sieved through a 2-mm mesh sieve and stored at 4°C for approximately 10 hours for analysis of microbial biomass carbon (MBC).

To determine the soil aggregate size fractions, all samples were pre-sieved through 8 mm sieves prior to wet-sieving to remove coarse organic matter and to homogenize the soil samples. Water-stable aggregates (WSA) from each sample were separated into four size classes (>2000, 250-2000, 53-250 and <53 μm diameter) using a wet-sieving apparatus with sieve "nests" of corresponding mesh sizes (Six and others 1998, 2000). The four buckets were filled with distilled water so that the water level was below the top sieve. A 50-g air-dried soil sample was placed on the top sieve of each nest, submerged in water for 10 minutes and then the apparatus was shaken vertically (4 cm) 30 times per minute for 10 minutes. The soil retained in the three largest sieves was transferred to an aluminium tube, oven-dried at 60℃ to limit drying effects on soil organic C, and then weighed. To separate the <53 μm soil fraction from the distilled water, the buckets were left undisturbed for 4 h, allowing the <53 μm soil fraction to settle at the bottom of the

buckets; the soil was transferred to aluminium cups, oven-dried and weighed. Here, macroaggregates are defined as the sum of the 250-2000 μm and >2000 μm aggregate fractions, and microaggregates are defined as the sum of <53 μm and 53-250 μm aggregate fractions, given in mass percentages.

Microbial biomass carbon (MBC) was determined on a 15-g subsample of fresh soil from each pot using the chloroform fumigation-extraction method (Vance and others 1987). Soil C was extracted from 7.5-g subsamples of fumigated and unfumigated soils in 75 ml 0.5-M K_2SO_4 solution and was analyzed using a TOC analyzer (High TOC, Elementar Analysensysteme, Hanau, Germany). Microbial biomass carbon was calculated from the difference in extractable C concentrations between the fumigated and the unfumigated samples, using a conversion factor of 0.45.

To determine total soil C concentration, samples were air-dried and sieved through a 2-mm mesh sieve to remove coarse organic matter. The soil was then ground using a ball mill (Retsch MM400, Haan, Germany) and total C was analyzed by a CHNOS elemental analyzer (Vario EL III; Elementar Analysensysteme GmbH, Hanau, Germany). To determine the C isotope ratio ($\delta^{13}C$, ‰) of the soil, litter and leaf and root samples harvested from live plants, all plant materials were oven-dried at 55ºC for 48 h and soil samples were air-dried and sieved (2-mm mesh). All samples were then ground using a ball mill (Retsch MM400, Haan, Germany) and the $\delta^{13}C$ (±0.1‰) was analyzed using Combustion Module-Cavity Ring Down Spectroscopy (CM-Automate-CRDS, Picarro, Inc, USA).

2.4 Calculations of soil C turnover

New C inputs

We defined C from rhizosphere deposition or plant litter as "new C" and organic C in the soil at the start of the experiment as "extant C." As it is very difficult to measure rhizosphere deposition, root biomass was used to estimate the quantity of new C inputs from roots (Nguyen 2003). The amount of the mineralized litter C was estimated by multiplying the decomposed litter mass by the relative proportion of C in the litter. The decomposition constant k (y^{-1}) was calculated as

$$k = \ln(L_0/L_t)/t, \quad (1),$$

where L_0 is the litter mass at the beginning of the experiment (8 g dry weight), L_t is the remaining mass of litter at the end of the experiment, and t is the duration of the experiment in years (0.52).

New C stored in soil

Here, we defined new C stored in soil as the C derived from roots or litter during the experiment. The amount of new C was calculated from the distinct $\delta^{13}C$ values of plant inputs and soil C using the following equation to partition the different sources of C (Cheng 1996):

$$C_n = C_t \frac{\delta_t - \delta_s}{\delta_p - \delta_s} \quad (2),$$

where C_n is the amount of new soil C derived from litter or root inputs, C_t is the total soil C pool at the end of the experiment, δ_t is the $\delta^{13}C$ value of the total soil C pool (C_t) at the end of the experiment, δ_s is the $\delta^{13}C$ value of the initial soil, and δ_p is the $\delta^{13}C$ value of *C. squarrosa* litter or root material.

Litter C storage efficiency was calculated as the ratio of litter-derived new soil C to mineralized litter C (Stewart and others 2007), where high values of litter C storage efficiency indicate that more litter-derived C is stored in the soil instead of being released as CO_2.

Net changes in soil C and mineralized extant soil C

The net change in the soil C pool was calculated for each treatment and soil type from the differences between the initial and final soil C concentrations. For the control treatment without plants or litter inputs in each SOM level, the amount of extant soil C mineralized during the experiment was calculated from the difference between the initial soil C concentration and the final soil C concentration; for the treatments with plant C inputs, the mineralized extant soil C was calculated as the difference between new C stored in the soil and the net change in soil C.

2.5 Statistical analysis

Three-way factorial analyses of variance (ANOVA) were used to examine the effects of SOM level, plant presence, litter addition, and their interactions on the soil aggregate fractions, MBC, soil C concentration, mineralization of extant soil C, and net change in soil C. Two-way factorial ANOVAs were used to examine the effects of the SOM level, litter addition and their interactions on root biomass, as well as the effects of the SOM level and plant presence on litter decomposition. When the effects of SOM were significant, the means at given SOM levels were calculated by averaging across the planted and litter addition treatments, and Tukey's post-hoc tests were conducted to compare these means across the SOM levels.

Linear regression was used to explore the relationships between MBC, litter decomposition rates, macro-aggregate and micro-aggregate fractions, and the mineralization of old soil C vs. new C stored in soil. The differences among the slopes of the linear regressions were tested using the R package smatr (Warton and others 2012). All statistical analyses were performed using R version 3.1.0 (R Core Team 2014) and the results are reported as significant at $P < 0.05$.

3 Results

3.1 Initial soil properties under different SOM levels

The concentrations of mineral elements in soils, including Ca, K, Mg, Na and P, were similar among SOM levels (Table S1), but total soil C, total soil N and the soil C:N ratio increased with SOM level (Table 1). Regarding soil mineralogy, the mass percentages of hydromica, amphibole, kaolinite and chlorite were also similar among SOM levels (Table S2), whereas the mass percentage of quartz increased and feldspar decreased with SOM levels (Table 2). Soil texture differed with SOM level, whereby the mass percentage of silt and clay increased and sand decreased with SOM level (Table 2). In addition, the water-holding capacity of the soil increased with SOM level (Table 2).

3.2 Effects of SOM levels on C inputs and MBC

Our experimental SOM levels influenced litter decomposition and root biomass, whereby the proportion of mineralized litter C increased with initial SOM level (Fig. 1a) and root biomass differed among treatments (Fig. 1b). Although there was no clear relationship between root biomass and SOM levels (Fig. 1b), the root: shoot ratio decreased with increasing SOM level (Fig. 1c). Soils with higher initial SOM levels also had higher MBC (P<0.01; Fig. 2; Table S3) but MBC in the planted treatments did not differ from the unplanted treatments at any SOM level except for S80, where higher MBC in the planted treatment resulted in a significant SOM level × plant interaction (P<0.01; Fig. 2; Table S3). Litter addition did not influence MBC at low SOM levels, but MBC increased significantly with litter addition at S80 and S100, resulting in a significant SOM × litter interaction (P<0.01; Fig. 2; Table S3).

3.3 Effects of SOM levels on soil aggregate fractions

The mass percentage and absolute mass of >2000 μm aggregate fraction increased with increasing initial SOM levels in all treatments (P<0.01, Fig 3a, Fig. S1a). In the planted treatments, the >2000 μm aggregate fraction was higher than unplanted treatments at S40, S80 and S100, but not at other SOM levels (SOM × plant interaction: P<0.01; Table S3; Fig. 3a), whereas in the litter addition treatments, the >2000 μm soil aggregate fraction was significantly higher (P<0.01) than in treatments without litter.

By contrast, the mass percentage and absolute mass of 250-2000 μm aggregate fraction were unaffected by initial SOM level (P=0.98; Table S3; Fig. 3b, Fig. S1b) or litter addition (P=0.63; Table S3; Fig. 3b) and was lower in the planted compared to the unplanted treatments (P=0.04; Table S3; Fig. 3b). The mass percentage and absolute mass of 53-250 μm and <53 μm soil aggregate fractions decreased with initial SOM levels (P<0.01; Table S3; Fig. 3c-d, Fig. S1c-d) and the 53-250 μm fraction was also significantly lower in litter addition treatments (P=0.02; Table S3; Fig. 3a, c), but there was no effect of the planted treatments on either fraction, and no effect of litter addition treatments on the <53 μm fraction (P=0.84; P=0.24; Table S3; Fig. 3d).

3.4 Relationship between MBC and litter decomposition rate or soil aggregates

The litter decomposition rate (k) was positively related to MBC in the litter addition treatments both with and without plants (R^2=0.56, P<0.01, n=17 and R^2=0.64, P<0.01, n=17, respectively; Fig. 4a). The macroaggregate fraction was positively related to MBC in the planted (R^2=0.39, P=0.01, n=18), litter addition (R^2=0.33, P=0.04, n=17) and planted plus litter addition treatments (R^2=0.69, P<0.01, n=18; Fig. 4b). The slopes did not differ among treatments for any of these relationships (Fig 4a-b).

3.5 Effects of SOM levels on soil C turnover

Litter addition increased the amount of new C stored in the soil, with the largest increase at S80 (SOM × litter interaction: $P<0.01$; Table S3; Fig. 5a). The planted plus litter addition treatment resulted in a greater increase in the amount of new C stored in the soil than the planted-only treatment, resulting in a significant plant × litter interaction ($P<0.01$; Table S3; Fig. 5a).

The mineralization of extant soil C differed significantly among the SOM levels ($P<0.01$) but there was no clear pattern with increasing SOM level. However, the planted treatments significantly stimulated the mineralization of extant soil C compared to the unplanted treatments ($P<0.01$, Table S3, Fig. 5b), whereas litter addition had no such effect ($P=0.53$; Table S3; Fig. 5b). The mineralization of extant soil C was positively correlated with new C stored in soil for the planted ($R^2=0.66$, $P<0.01$, $n=13$), litter addition ($R^2=0.27$, $P=0.05$, $n=15$), and planted plus litter addition treatments ($R^2=0.71$, $P<0.01$, $n=15$, Fig. 5c); the slopes of the regression lines did not differ from 1 or among treatments for any of these relationships.

By the end of the experiment, the soil C content was significantly higher in the litter addition treatments ($P<0.01$) but was not affected by the initial SOM levels ($P=0.81$) or the planted treatments ($P=0.33$; Table S3; Fig. 5d). Calculations of the litter C storage efficiency for the litter addition treatments at S20 to S100 revealed that the storage of litter-derived C was lowest at S20 (Fig. 6).

4 Discussion

Soil organic matter content greatly affects the quality and quantity of plant C inputs to the soil by regulating plant productivity. The C from decomposing plant litter is either released as CO_2 to the atmosphere, leached through the soil as dissolved organic C, or incorporated into the soil as organic matter (Bird and others 2008; Mambelli and others 2011). Understanding how

SOM levels affect the turnover and storage of plant and soil C is critical for the restoration of degraded grasslands. However, the contribution of plant C to SOM formation is currently poorly quantified (Cotrufo and others 2013). We aimed to address this by tracking the fate of plant C in soils with different levels of SOM. We created a wide SOM gradient (with soil C concentrations ranging from 0.02% to 1.40%) by mixing air-dried and combusted soils. The combustion treatment removed soil organic matter but also altered the soil mineralogy and texture (Table 2); we therefore discuss our findings with due consideration to changes in other soil properties as a result of such methodological artifacts.

4.1 The role of litter inputs in SOC storage and turnover

Litter decomposition by soil microbes is a critical step for litter-derived C entering the soil matrix (Cotrufo and others 2013). The positive correlation between MBC and SOM levels observed in our study (Fig. 2) could be because soils with higher SOM levels not only provide more C substrates but also have higher N concentration to support greater microbial biomass (Manzoni and others 2012), and higher water-holding capacity to maintain a suitable environment for microbes (Sylvia and others 2005). Higher microbial biomass and the favorable moisture and nutrient conditions suggest that microbes could be more active at higher SOM levels, which would lead to faster decomposition of plant litter. Indeed, we found that there was a positive correlation between MBC and litter decomposition rate (Fig. 4a), and more litter C was mineralized at higher SOM levels (Fig. 1a). Our study suggests that, during a given period, less litter-derived C would be incorporated into the soil when litter decomposition rates and MBC are low (Fig. 1a; Fig. 2; Fig. 5a). This supports emerging evidence for a “microbial filter” whereby labile C from litter is efficiently integrated into the mineral soil matrix through microbial activity, resulting in the formation of stable soil organic C (Cotrufo and others 2013).

Our study demonstrated that the capacity of the soil to incorporate litter-derived C varied among SOM levels. The lowest SOM levels also had the lowest C storage efficiencies but the intermediate SOM levels had the highest C storage efficiencies (Fig. 6). Given that the physicochemical protection for new C inputs can be saturated (Six and others 2002a; Castellano and others 2015), the ability of soils to sequester additional C could become progressively limited as the SOM content increases. In addition to SOM levels, soil mineralogy and surface properties greatly determine the ability of soil to sequester new organic C. Soils with greater surface area have more capacity to adsorb OM on surfaces, and the stronger attraction between SOM and soil minerals at higher charge density increases SOM stability by forming organo-mineral complexes (Feng and others 2014; Wiesmeier and others 2015). Our combustion treatment could have affected C storage efficiency because it altered soil mineralogy (Table S2) and significantly decreased the concentration of both silt and clay (Table 2). Although the effect of altered soil mineralogy remains to be explored, the decline in silt and clay is likely to affect the occlusion and adsorption of litter-derived new C within the mineral matrix (Dungait and others 2012; Wiesmeier and others 2015), which would explain why litter C storage efficiency was particularly low at the lowest SOM level in our study (Fig. 6).

Priming effects, the mineralization and release of stored soil C by fresh organic C inputs (Kuzyakov and others 2000), have also been given as an explanation for the lack of increased soil C storage in litter-addition studies (Lajtha and others 2014; Bowden and others 2014). Multiple lines of evidence indicate that higher inputs of plant litter can cause priming effects, offsetting soil C accumulation (Fontaine and others 2004; Sayer and others 2011). However, the role of priming effects in natural ecosystems is questionable, because soil C priming is rarely investigated *in situ* (Sayer and others 2011; Xu and others 2013). In our study, the losses of

extant soil C were not significant under litter addition alone, but increased significantly when plants were present (Table S3; Fig. 5b). Although aboveground leaf litter and root deposition are both critical sources of labile C input to soil (Cotrufo and others 2013), our findings suggest that root deposition may induce stronger priming effects than litter.

4.2 The role of root C inputs in SOC turnover

Root-derived C, including C from root litter and root exudates, is considered to be the main source of soil C in grassland ecosystems (Kuzyakov and Domanski 2000; Rasse and others 2005; Schmidt and others 2011). At low SOM contents, the soil is extremely infertile and plants tend to invest more C into root production to access nutrients (Dakora and Phillips 2002); in our experiments, this was indicated by the decrease in the root-to-shoot ratio with increasing SOM level (Fig. 1c). The rhizosphere priming effect is closely related to the amount of root C inputs (Cheng 2009) and isotope methods are usually necessary to distinguish between increased soil CO_2 efflux derived from roots and primed soil C ("real positive priming effect"; Kuzyakov and others 2000). We were able to use the changes in the ^{13}C values of soil C to calculate the mineralization of extant soil C induced by new root C inputs in the planted treatment and show that root C inputs produced a "real positive priming effect" (Table S3; Fig. 5b). For the planted treatment without litter addition, the regression line for the mineralization of extant soil C *vs*. new C stored in soil closely followed the 1:1 line (Fig. 5c), suggesting that the storage of new C was offset by the release of extant soil C as CO_2, resulting in no net change in the soil C content. Although our experiment was too short to fully evaluate the effects of root litter on soil C storage, the results indicate that root C deposition during the growing season made a limited contribution to soil C storage because new C inputs from roots replaced the extant C that was mineralized and released by priming. Such differences in soil C turnover without a net change in soil C stocks

would not be detected with soil C content measurements (Kuzyakov and Blagodatskaya 2015), but the replacement of extant soil C with newer and possibly more labile C could affect the overall stability of the soil C pool (Sayer and others 2011). Long-term field investigations are needed to better understand how leaf litter and root processes affect the mineralization of extant soil C via priming effects.

We also observed interactive effects of litter addition and plant roots on the accumulation of new C in the soil. There was a non-additive effect of plants and litter addition on the storage of new C, whereby the amount of new soil C in pots with the combined planted + litter addition treatment was less than the sum of new C in the pots with either treatment alone (plant × litter: Table S3; Fig. 5a). As root biomass was lower when litter was added to the pots (Fig. 1b), we propose that reduced root deposition could account for the smaller relative amount of new C in the combined planted + litter addition treatment.

4.3 The responses of soil aggregate fractions and their roles in soil C stabilization

The mean residence time of soil C is important for soil C sequestration capacity. Apart from organo-mineral complexes (Feng and others 2014), the formation of microaggregates within macroaggregates is an essential mechanism in the physical protection of soil C (Denef and others 2007). In our study, the macroaggregate fraction was positively related to MBC in the planted, litter addition, and planted + litter addition treatments (Fig. 4b), and both also increased with SOM levels (Fig. 3a). These findings suggest that soils with high SOM content support aggregate-binding agents, such as fine roots and microbial hyphae (Tisdall and Oades 1982), and therefore feedbacks between the extant SOM, new organic C inputs and macroaggregate formation are central to the storage and stabilization of soil C. We also found that the microaggregate fraction declined with increasing SOM levels. This is probably because a large

proportion of microaggregates were occluded within macroaggregates and the macroaggregate fraction increased with the SOM level (Fig 3a, c, d).

Overall, our study indicated that the influence of SOM on the formation of soil macroaggregates could create a positive feedback for SOC accumulation, because more SOM associated with microaggregates is occluded within macroaggregates, which enhances the stability of SOC (Fig. S1). However, it is also possible that the higher proportion of microaggregates under low SOM levels is an artifact of the combustion treatment, whereby macroaggregates were destroyed by heating, releasing the occluded microaggregates. Finally, the soil structure was destroyed by sieving soils before filling the pots at the start of the experiment and the soil structure developed during our experimental period may not be representative of undisturbed soils in the field. Nonetheless, our study indicates potential mechanisms and feedbacks between extant SOM levels and the stabilization of new C inputs, which can be tested in field studies.

5 Conclusions

Soil organic matter content is one of the most important indices of soil health but anthropogenic disturbance and climate changes can affect the accumulation and stability of SOM. Our study highlights the important role of initial SOM content for regulating soil C formation and stability through direct and indirect effects on the turnover of aboveground litter, root C inputs, and microbial processes. The initial SOM content affected not only plant litter and root C inputs but also the capacity of the soil to incorporate litter- and root-derived C. Feedbacks between SOM, plant litter and plant root inputs are complex, but we show that they interact to influence the accumulation of new C and the mineralization of extant C in the soil. In addition,

initial SOM content also influenced the formation of soil aggregates and therefore further enhanced the stability of soil organic C. These results merit further investigation to help us better understand how C is stored and stabilized in the soil. Caution is needed when extrapolating our findings to natural ecosystems because the use of combusted soil and the method of mixing litter into soils potentially introduced methodological artifacts. Furthermore, our single-species, short-term greenhouse experiment may not represent the complex processes in the field. Nevertheless, our results open up several new lines of scientific enquiry, which need to be tested under field conditions. When evaluating the potential of soil C sequestration under future global changes, field and model studies need to consider how initial SOM content could affect the fate of plant-derived C.

Acknowledgements

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Table 1. Initial soil carbon concentrations (C%), soil nitrogen concentrations (N%), soil C-to-N ratio (C:N) (means ± SE for n = 3) and soil $\delta^{13}C$ values for six experimental levels of soil organic matter (SOM) content: S0: 100% combusted soil; S20: 80% combusted soil plus 20% native soil; S40: 60% combusted soil plus 40% native soil; S60: 40% combusted soil plus 60% native soil; S80: 20% combusted soil plus 80% native soil; S100: 100% native soil. Different lowercase letters indicate significant differences among SOM levels at $P < 0.05$, where NS indicates "non-significant."

	C (%)	N (%)	C:N	$\delta^{13}C$ (‰)
S0	0.02±0.00^{a}	0.02±0.00^{a}	1.30±0.12^{a}	--
S20	0.25±0.05^{b}	0.04±0.00^{b}	5.98±0.27^{b}	-24.0
S40	0.58±0.01^{c}	0.07±0.00^{c}	7.96±0.16^{c}	-23.8
S60	0.87±0.02^{d}	0.10±0.00^{d}	9.00±0.25^{d}	-23.8
S80	1.15±0.01^{e}	0.12±0.00^{e}	9.38±0.57^{d}	-24.0
S100	1.40±0.02^{f}	0.14±0.00^{f}	9.81±0.07^{d}	-23.8
P values	**<0.01**	**<0.01**	**<0.01**	NS

Table 2. Initial soil mineralogy given as mass percentages, including soil particle size distributions (sand, silt and clay contents) and water holding capacity (WHC); values shown are means ± SE for n = 3 for each of six experimental soil organic matter (SOM) levels: S0: 100% combusted soil; S20: 80% combusted soil plus 20% native soil; S40: 60% combusted soil plus 40% native soil; S60: 40% combusted soil plus 60% native soil; S80: 20% combusted soil plus 80% native soil; S100: 100% native soil. Different lowercase letters indicate significant differences among SOM levels at $P < 0.05$.

	Quartz (%)	Feldspar (%)	Sand (%)	Silt (%)	Clay (%)	WHC (%)
S0	24.00±2.08^{a}	67.67±1.45^{a}	78.43±0.82^{a}	20.72±0.82^{a}	0.85±0.01^{a}	29.66±0.50^{a}
S20	25.93±0.57^{a}	64.85±0.46^{ab}	71.99±0.17^{b}	26.46±0.16^{b}	1.54±0.02^{b}	31.26±0.55^{a}
S40	26.62±0.30^{ab}	63.71±0.56^{bc}	66.57±0.13^{c}	31.22±0.14^{c}	2.21±0.01^{c}	30.86±0.51^{ab}
S60	28.93±0.28^{bc}	61.00±0.81^{cd}	60.26±0.12^{d}	36.76±0.15^{d}	2.98±0.03^{d}	32.49±0.50^{bc}
S80	30.91±0.11^{cd}	59.16±0.34^{d}	54.02±0.28^{e}	42.36±0.27^{e}	3.61±0.00^{e}	33.88±0.30^{c}
S100	32.33±0.33^{d}	56.67±1.20^{e}	48.09±0.56^{f}	47.62±0.59^{f}	4.30±0.10^{f}	36.13±0.68^{d}
P values	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**

Figure legends

Figure 1. Final values of **a)** mineralized litter carbon (C), **b)** root biomass C inputs and **c)** root-to-shoot ratios in soils with different experimental soil organic matter (SOM) levels after 191 days in a greenhouse study; S is SOM level where S0 is 100% combusted soil; S20 is 80% combusted soil plus 20% native soil; S40 is 60% combusted soil plus 40% native soil; S60 is 40% combusted soil plus 60% native soil; S80 is 20% combusted soil plus 80% native soil; S100 is 100% native soil. SOM: soil organic matter level. Different lowercase letters indicate significant differences among SOM levels across all treatments ($P < 0.05$).

Figure 2. Microbial biomass carbon (MBC) in soils with different experimental soil organic matter (SOM) levels after 191 days in a greenhouse study. The abbreviations follow the legend in Figure 1. Different lowercase letters indicate significant differences among SOM levels across all treatments ($P < 0.05$).

Figure 3. Mass percentages of soil aggregate fractions in soils with different experimental soil organic matter (SOM) levels and carbon inputs; **a)** >2000 μm, **b)** 250-2000 μm, **c)** 53-250 μm, **d)** <53 μm aggregate fraction. Abbreviations follow the legend in Figure 1. Different lowercase letters indicate significant differences among SOM levels across all treatments ($P < 0.05$).

Figure 4. The relationship between microbial biomass carbon and **a)** the litter decomposition rate and **b)** the macroaggregate fraction under different experimental treatments. The macroaggregate fraction is the sum of the >2000 μm and 250-2000 μm aggregate fractions (in mass percentage). The abbreviations follow the legends in Figures 1 and 2.

Figure 5. Changes in carbon (C) pools in soils with different experimental soil organic matter (SOM) levels and C inputs: **a)** new C stored in soil, **b)** mineralization of extant soil C, **c)** the relationship between new C stored in soil and mineralization of extant soil C, and **d)** net soil C

change; abbreviations follow the legend in Figure 1. Different lowercase letters indicate significant differences among SOM levels across all treatments ($P < 0.05$).

Figure 6. Litter carbon (C) storage efficiency (the ratio of litter-derived new soil C to mineralized litter C) in soils with different experimental initial soil organic matter (SOM) levels; abbreviations follow the legend in Figure 1. Different lowercase letters indicate significant differences among the means for SOM levels ($P < 0.05$).

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Figure 1

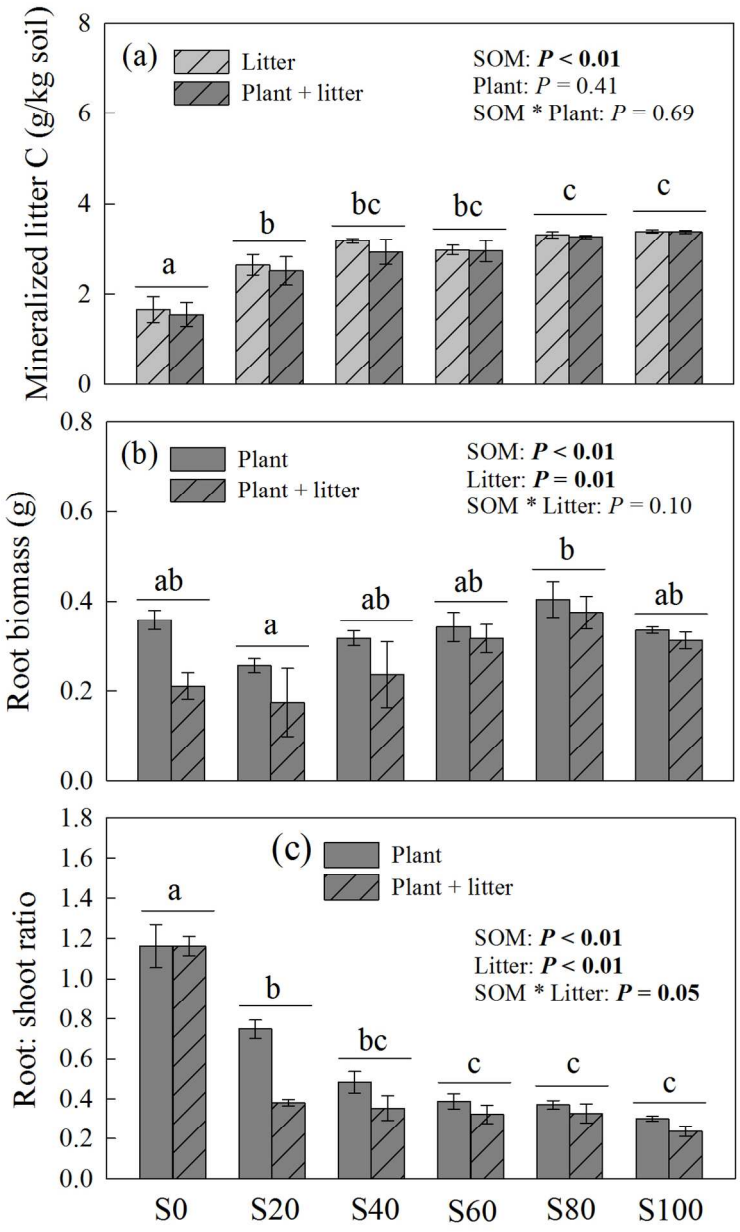

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Figure 2

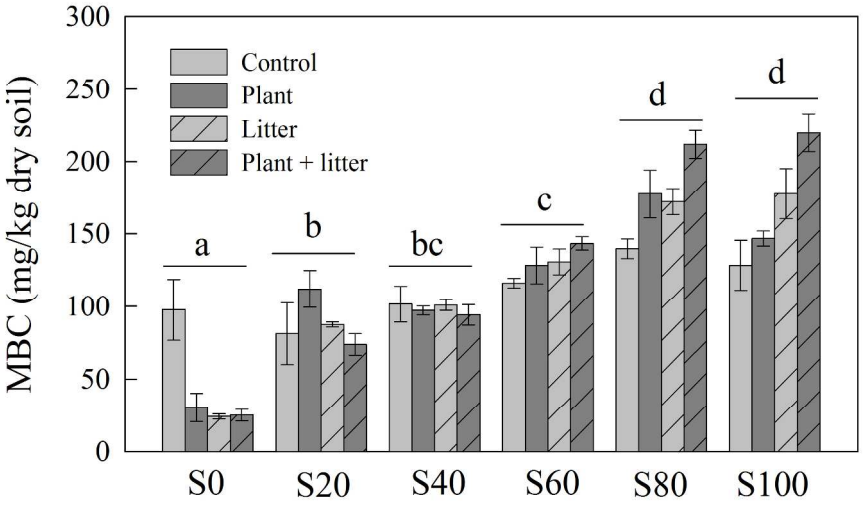

Figure 3

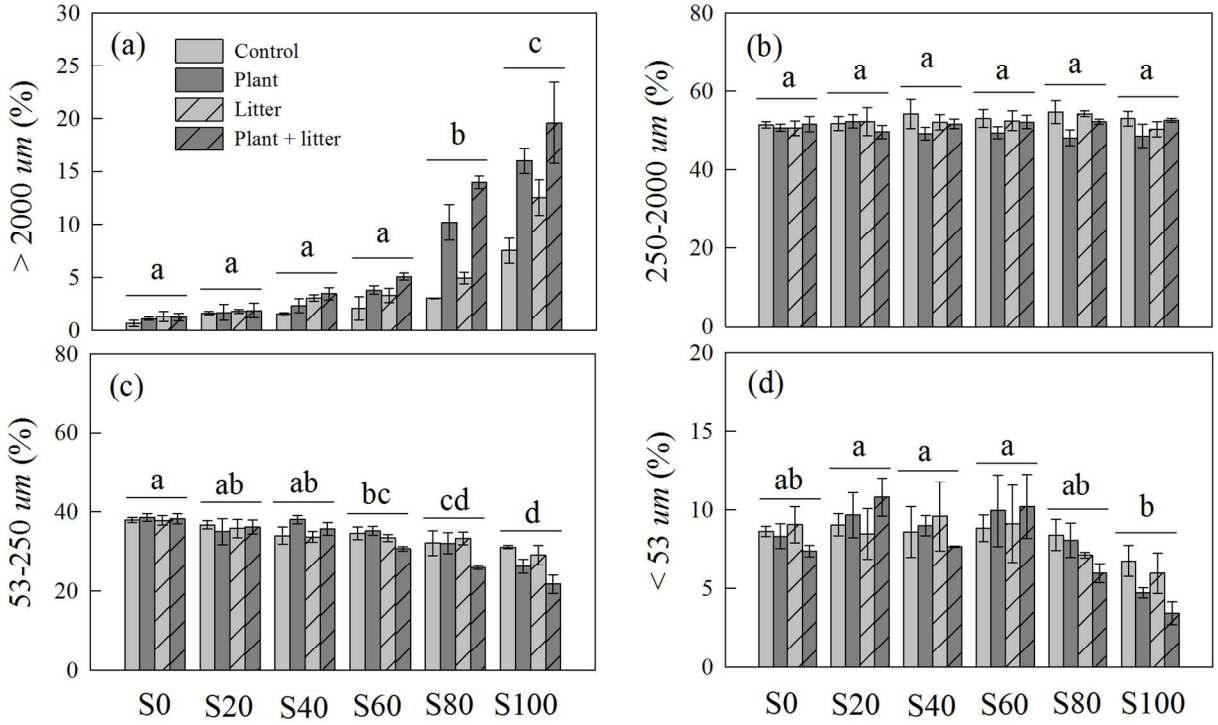

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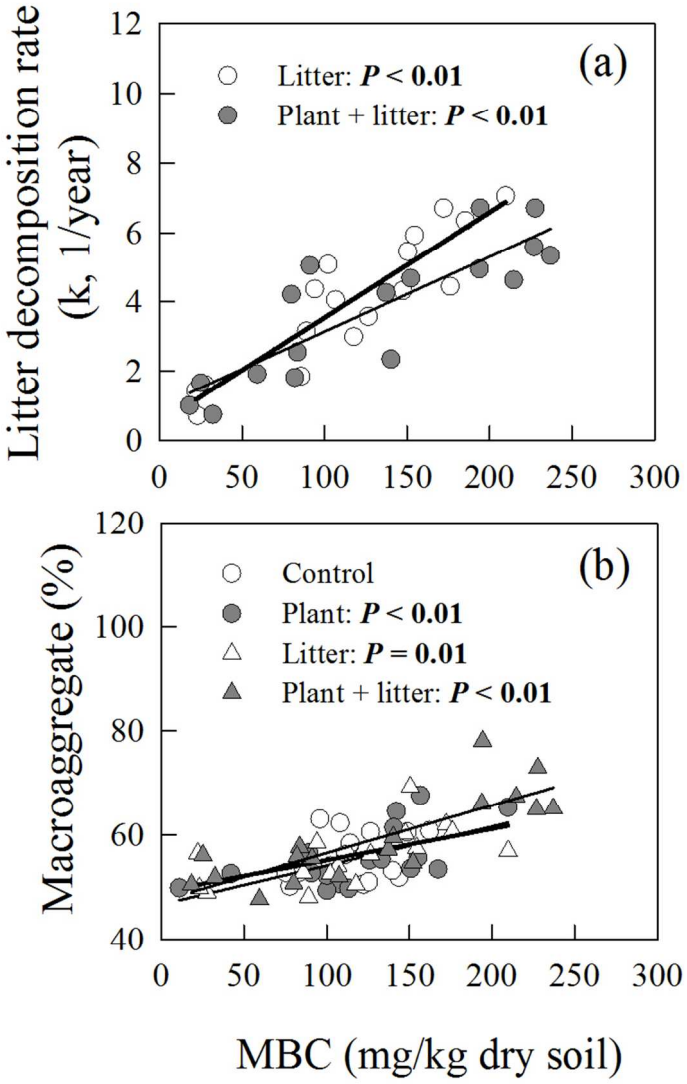
Figure 4



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735 **Figure 5**

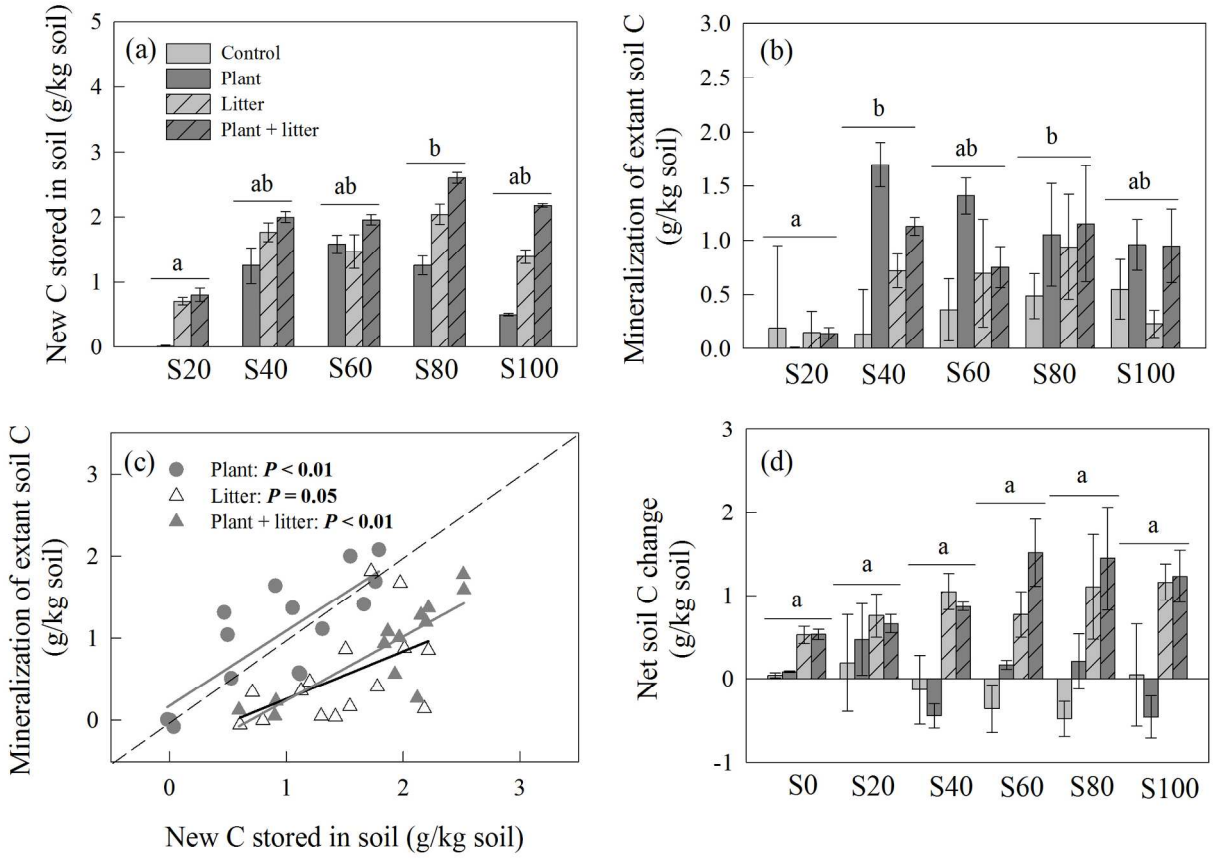

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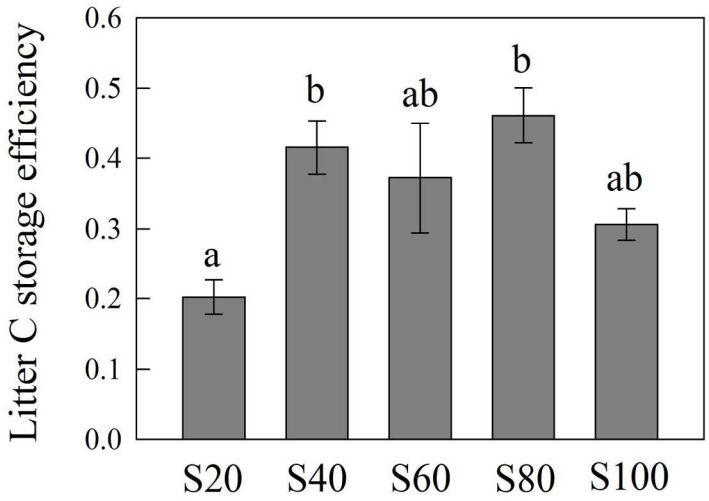
Figure 6



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