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3 1 **Initial soil organic matter content influences the storage and turnover of litter,**
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6 2 **root and soil carbon in grasslands**

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20 **Author contributions**

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

For Peer Review

37 **Abstract**

38 Grassland degradation is a worldwide problem that often leads to substantial loss of soil organic
39 matter (SOM). To estimate the **potential for carbon (C) accumulation** in degraded grassland soils,
40 we first need to understand how SOM content influences the **transformation of plant C** and its
41 stabilization within the soil matrix. We conducted a greenhouse experiment using C₃ soils with
42 six levels of SOM content; we planted the C₄ grass *Cleistogenes squarrosa* or added its litter to
43 the soils to investigate how SOM content regulates the storage of new soil C derived from litter
44 and roots, the decomposition of extant soil C, and the formation of soil aggregates. **We found**
45 **that with the increase in SOM content, microbial biomass carbon (MBC) and the mineralization**
46 **of litter C increased.** Both the litter addition and planted treatments increased the amount of new
47 C inputs to soil. However, the mineralization of extant soil C was significantly accelerated by the
48 presence of living roots but was not affected by litter addition. Accordingly, the soil C content
49 was significantly higher in the litter addition treatments but was not affected by the planted
50 treatments by the end of the experiment. The soil macroaggregate fraction increased with SOM
51 content and was positively related to MBC. Our experiment suggests that as SOM content
52 increases, plant growth and soil microbial activity increase, which allows microbes to process
53 more plant-derived C and promote new soil C formation. Although long-term field experiments
54 are needed to test the robustness of our findings, our greenhouse experiment suggests that the
55 interactions between SOM content and plant C inputs should be considered when evaluating soil
56 C turnover in degraded grasslands.

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

60 **1 Introduction**

61 Soil organic matter content is a key indicator of soil health, which determines plant
62 productivity and microbial activity (Magdoff and Weil 2004). Grasslands cover 40.5% of the
10
11 63 world's land area (Gibson 2009) and many of them are subjected to disturbance from
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13 64 environmental and land use changes, such as over-grazing (Reid and others 2004) and
14
15 65 conversion from grassland to cropland (Wright and Wimberly 2013). These disturbances result
16
17 66 in large losses of soil organic matter (SOM) and can cause desertification in many grassland
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19 67 ecosystems (Lal 2003; Wang and others 2011; McSherry and Ritchie 2013). Degraded
20
21 68 grasslands are often subjected to extensive erosion, which selectively removes lighter organic
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23 69 matter and finer particles such as clay and silt and leaving heavier sand particles (Li and others
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25 70 2005; Zhou and others 2008). Soil fertility, soil water-holding capacity, and therefore plant
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27 71 productivity, decrease with the severity of soil degradation (Lal 2001). Although there is much
28
29 72 debate on the potential of grasslands to act as a sink for carbon (C), it is commonly agreed that
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31 73 better management of degraded grasslands could reduce soil C loss and enhance the C
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33 74 sequestration capacity of grasslands (Smith 2014). However, this requires a better understanding
34
35 75 of the feedbacks between extant SOM content, plant productivity, microbial activity, and
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37 76 aggregate formation, which all contribute to the storage and stabilization of C in grassland soils.
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43 77 Soils with greater SOM content are often more fertile and have higher water-holding capacity,
44
45 78 which supports higher plant productivity (Saxton and Rawls 2006; Six and Paustian 2014). In
46
47 79 turn, C inputs from plants, including aboveground litter and root litter and root exudates, play a
48
49 80 key role in regulating the soil C balance (Kuzyakov and Domanski 2000; Santos and others
50
51 81 2016). Root inputs are considered as particularly important for soil C storage in grassland
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53 82 ecosystems, whereas aboveground litter contributes less to the formation of SOM (Rasse and
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3 83 others 2005; Bird and Torn 2006; Prescott 2010; Schmidt and others 2011). Recent research
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5 84 suggests that microbes can incorporate labile C into soil more efficiently than recalcitrant C
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8 85 (Cotrufo and others 2013). Consequently, a decline in labile C sources, such as aboveground leaf
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10 86 litter and root exudates, could have a much greater impact on stable SOM formation than
11
12 87 previously thought (Hatton and others 2015; Haddix and others 2016).

14 88 Soil organic matter accumulation is affected not only by C inputs from plants but also by
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16
17 89 microbial activity, which regulates the transformation of plant C to SOM (Cotrufo and others
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19 90 2013). Soil organic matter is the most important energy and nutrient source for soil microbes
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21 91 (Fontaine and others 2011) and changes in SOM content can alter microbial community
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23 92 composition and C use efficiency (CUE; Manzoni and others 2012; Sinsabaugh and others 2016).
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26 93 Losses of SOM associated with soil degradation could therefore reduce the size and activity of
27
28 94 the microbial population (Pascual and others 2000; Bastida and others 2006) and alter the
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30 95 capacity of soil microbial communities to decompose and transform plant-derived C into stable
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32 96 microbial products.

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34
35 97 Finally, the capacity of soil to sequester C also depends on the stability of SOM (Jastrow and
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37 98 others 2007). In this context, soil aggregate formation is one of the key processes for increasing
38
39 99 the residence time of SOM (Six and others 2002b) because C associated with soil
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41 100 microaggregates is physically protected from microbial attack by occlusion within
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43 101 macroaggregates, which slows its decomposition (Denef and others 2007; Six and Paustian
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45 102 2014). The soil macroaggregate fraction often increases with SOM content (Blanco-Canqui and
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47 103 Lal 2004; Bronick and Lal 2005) and polysaccharides derived from aboveground litter and fine
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49 104 roots can improve soil aggregation by acting as binding agents (Six and others 2002b). Hence,
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51 105 SOM content could influence the mean residence time of soil C directly by promoting
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3 106 macroaggregate formation and indirectly by altering plant aboveground litter inputs and root
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8 108 Despite the potential importance of feedbacks between extant SOM content and additional
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10 109 soil C sequestration, few experimental studies have investigated how SOM loss due to soil
11
12 110 degradation affects the stabilization of plant-derived C inputs and therefore the future C
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14 111 sequestration potential of the soil (Tan and others 2014; Castellano and others 2015). Importantly,
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16 112 feedbacks between inputs and storage of new C and the mineralization and release of extant C all
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18 113 contribute to net changes in soil C (Lange and others 2015). Hence, measurements of total soil C
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20 114 change or CO₂ efflux alone cannot elucidate the mechanisms underlying SOM storage. For
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22 115 example, increased inputs of plant-derived C can stimulate the release of older stored soil C via
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24 116 'priming effects', with no net change in soil C content (Kuzyakov and others 2000). Although
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26 117 isotope studies have advanced our knowledge of SOM turnover under new C inputs (e.g., Cheng
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28 118 2009; Blagodatskaya and others 2014), we have yet to determine how the extant SOM content
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30 119 influences the various processes involved in the storage of additional soil C.
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35 120 In this study, we created a SOM gradient to simulate different levels of soil degradation in
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37 121 grasslands. We planted C₄ grass and added C₄ grass leaf litter into C₃ soils to simulate root and
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39 122 aboveground litter inputs, respectively. We used the natural difference in the ¹³C isotope value
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41 123 between the C₄ plants and C₃ soil to track new soil C formation and the decomposition of extant
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43 124 C in soils with different SOM contents. We hypothesized that greater extant SOM results in
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45 125 greater C sequestration of new C inputs because 1) higher SOM content supports greater
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47 126 microbial biomass, which enhances the mineralization of litter-derived C and results in increased
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49 127 formation of new soil C derived from aboveground litter; 2) higher SOM content supports
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51 128 greater root biomass, thereby increasing root-derived new soil C; and 3) higher SOM content
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3 129 improves soil aggregation, resulting in greater physical protection of soil C. However, as
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5 130 increased new C inputs from plants could also stimulate the decomposition of extant SOM via
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8 131 priming effects (Kuzyakov and others 2000), we also tested an alternative hypothesis: 4)
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10 132 increased root C inputs with greater extant SOM content will promote the mineralization and
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12 133 release of stored soil C, resulting in little or no increase in C sequestration.
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17 134 **2 Materials and Methods**

18 19 20 135 **2.1 Soil, leaf litter, seed sampling and pretreatments**

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23 136 Mineral soil (0-30 cm depth) was taken from the Duolun Restoration Ecology Research
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25 137 Station (42°2'N, 116°17'E), Inner Mongolia, China, in October 2012. The site was an overgrazed
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28 138 steppe, but was fenced to limit access by cattle in 2000. The soil is sandy and classified as a
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30 139 Haplic Calcisol according to the FAO soil classification. Bulk density is 1.31 Mg m^{-3} , mean soil
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32 140 pH is 7.7, and the concentrations of organic carbon (C) and nitrogen (N) are $1.40 \pm 0.02\%$ (n=3)
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34 141 and $0.14 \pm 0.00\%$ (n=3), respectively. The soil was homogenized, passed through a 2-mm mesh
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37 142 sieve to remove coarse fragments, and visible plant residues were removed using tweezers. The
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39 143 soil was divided into two parts: one part was air-dried and the other part was combusted in a
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41 144 muffle furnace at 550°C for three hours to remove the native SOC (German and others 2011).
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44 145 Six experimental levels of SOM were obtained by mixing the air-dried and combusted soil
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46 146 according to different mass percentages as follows: 100% combusted soil (S0), 80% combusted
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48 147 plus 20% air-dried soil (S20), 60% combusted plus 40% air-dried soil (S40), 40% combusted
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50 148 plus 60% air-dried soil (S60), 20% combusted plus 80% air-dried soil (S80), and 100% air-dried
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53 149 soil (S100). Hence, the six soil treatments (hereafter “soil types”) represent a gradient of
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55 150 increasing SOM content (hereafter “SOM levels”; Table 1).
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3 151 The combustion treatment not only removes SOM, but can also alter soil properties, such as
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5 152 texture, nutrient content, mineralogy, and water holding capacity (Tan and others 1986; Certini
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7 153 2005). We therefore analyzed these soil physiochemical properties for each SOM level. Soil
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10 154 texture, was measured using a particle size analyser (Malvern Masterizer 2000, Malvern,
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12 155 Worcestershire, UK) after removal of organic matter and carbonates. Soil samples were digested
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14 156 in a microwave oven (MA-1870, Haier, Qingdao, China), and the concentrations of mineral
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16 157 elements, including calcium (Ca), potassium (K), magnesium (Mg), sodium (Na) and phosphorus
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18 158 (P), were determined by an ICP-ES (ICP; Thermo Scientific, West Palm Beach, USA). Soil
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20 159 mineralogy was identified by X-ray diffraction spectroscopy (Ultima IV; Rigaku Corporation,
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22 160 Tokyo, Japan). Water holding capacity (WHC) was determined by the methods described in
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24 161 Hanson and others (2002). Briefly, air-dried soil samples (20 g) were placed in a funnel lined
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26 162 with pre-weighed filter paper, water was added to the soil until saturation, the soil was allowed to
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28 163 drain for 3 h, and then the soil and filter paper were weighed. We calculated WHC from the
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30 164 difference between the dry and drained net weights of the soil samples. We also analyzed soil C
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32 165 concentration by a CHNOS elemental analyzer (Vario EL III; Elementar Analysensysteme
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34 166 GmbH, Hanau, Germany) to assess the efficiency of the combustion treatment for removing
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36 167 SOM.
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42 168 Leaf litter and seeds of *Cleistogenes squarrosa* were collected from the Duolun steppe in
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44 169 September 2012. *C. squarrosa* is a C₄ grass that is widely distributed in the Inner Mongolian
45
46 170 temperate steppe; its leaf litter has a C concentration of 44.0 ± 0.01% and a δ¹³C of -14.1 ± 0.1‰
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48 171 and its root material has a δ¹³C of -15.7 ± 1.2‰, which are distinguished from the δ¹³C of extant
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50 172 soil C (δ¹³C of -23.8 ± 0.02‰) and allowed us to use such a discrepancy in δ¹³C value to partition
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3 173 litter- or root-derived C from the extant soil C. Leaf litter was oven-dried at 40°C and chopped
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5 174 into 2.5 cm segments. The seeds were air-dried and stored in a dry place until February 2013.
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9 175 **2.2 Greenhouse experiment design**

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11 176 We conducted a greenhouse experiment using plastic pots (100 mm height, 120 mm upper
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13 177 diameter, and 100 mm lower diameter). The air temperature of the greenhouse was maintained at
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15 178 20–30°C and air humidity was kept at 50–60%. We filled 12 pots, each with 746 g of air-dried
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17 179 soil from one of the six soil types, and randomly assigned the pots of each soil type to four
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19 180 treatments: 1) “planted” with *C. squarrosa* seeds; 2) “litter addition” with *C. squarrosa* leaf litter
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21 181 mixed into the soil; 3) planted plus litter addition; and 4) controls without plants or litter inputs.
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23 182 Hence, the experiment comprised three replicate pots of four treatments for each of the six soil
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25 183 types, making a total of 72 pots. For the planted treatment, the *C. squarrosa* seeds were
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27 184 immersed in warm water (40–60 °C) for 4 hours to improve seed germination and then 20–25
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29 185 seeds were planted per pot on February 5, 2013. After germination, 12–13 seedlings were kept in
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31 186 the pots, mimicking the natural plant density at the study site (957 ± 325 plants m⁻²), and the
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33 187 remaining seedlings were removed. For the litter addition treatments, we used 1.07 g of litter per
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35 188 100 g of soil, which was the ratio of litter to soil described for the incubation experiments by
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37 189 Cheng and others (2012). Thus, we mixed 8 g of *C. squarrosa* leaf litter (C = 43.9%, N = 1.2%,
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39 190 C:N = 36.6) into 746 g of soil in each pot. It should be noted that the litter addition rate in this
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41 191 study was approximately two-fold greater per unit area than litter inputs observed in the field. All
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43 192 pots were watered with 150 ml of distilled water every week to avoid plant water stress.
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52 193 **2.3 Variable measurements at the end of the experiment**

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3 194 The greenhouse experiment lasted for 191 days when the plants started to senesce. At the end
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5 195 of the experiment, the aboveground biomass in the planted pots was harvested, oven-dried at
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7 196 55°C for 48 h, and then weighed to determine the aboveground biomass. Plant roots were
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9 197 separated from the soil with tweezers, cleaned with deionized water in a 53 µm sieve, oven-dried
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11 198 at 55°C for 48 h, and weighed to determine the belowground biomass. For the litter addition
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13 199 treatments, the remaining litter was manually separated from the soil with tweezers and the large
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15 200 soil particles attached to the litter were removed. The retrieved litter was then air-dried and
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17 201 weighed to estimate remaining mass and to measure the C concentration. The soil from each pot
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19 202 was separated into two subsamples: one was air-dried for aggregate partitioning, total C
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21 203 concentration, and soil ¹³C isotope measurements; the other subsample was sieved through a 2-
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23 204 mm mesh sieve and stored at 4°C for approximately 10 hours for analysis of microbial biomass
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25 205 carbon (MBC).
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31 206 To determine the soil aggregate size fractions, all samples were pre-sieved through 8 mm
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33 207 sieves prior to wet-sieving to remove coarse organic matter and to homogenize the soil samples.
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35 208 Water-stable aggregates (WSA) from each sample were separated into four size classes (>2000,
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37 209 250-2000, 53-250 and <53 µm diameter) using a wet-sieving apparatus with sieve “nests” of
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39 210 corresponding mesh sizes (Six and others 1998, 2000). The four buckets were filled with distilled
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41 211 water so that the water level was below the top sieve. A 50-g air-dried soil sample was placed on
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43 212 the top sieve of each nest, submerged in water for 10 minutes and then the apparatus was shaken
44
45 213 vertically (4 cm) 30 times per minute for 10 minutes. The soil retained in the three largest sieves
46
47 214 was transferred to an aluminium tube, oven-dried at 60°C to limit drying effects on soil organic
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49 215 C, and then weighed. To separate the <53 µm soil fraction from the distilled water, the buckets
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51 216 were left undisturbed for 4 h, allowing the <53 µm soil fraction to settle at the bottom of the
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3 217 buckets; the soil was transferred to aluminium cups, oven-dried and weighed. Here,
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5 218 macroaggregates are defined as the sum of the 250-2000 μm and $>2000 \mu\text{m}$ aggregate fractions,
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7 219 and microaggregates are defined as the sum of $<53 \mu\text{m}$ and $53\text{-}250 \mu\text{m}$ aggregate fractions, given
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9 220 in mass percentages.

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11
12 221 Microbial biomass carbon (MBC) was determined on a 15-g subsample of fresh soil from
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14 222 each pot using the chloroform fumigation-extraction method (Vance and others 1987). Soil C
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16 223 was extracted from 7.5-g subsamples of fumigated and unfumigated soils in 75 ml 0.5-M K_2SO_4
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18 224 solution and was analyzed using a TOC analyzer (High TOC, Elementar Analysensysteme,
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20 225 Hanau, Germany). Microbial biomass carbon was calculated from the difference in extractable C
21
22 226 concentrations between the fumigated and the unfumigated samples, using a conversion factor of
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24 227 0.45.

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28 228 To determine total soil C concentration, samples were air-dried and sieved through a 2-mm
29
30 229 mesh sieve to remove coarse organic matter. The soil was then ground using a ball mill (Retsch
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32 230 MM400, Haan, Germany) and total C was analyzed by a CHNOS elemental analyzer (Vario EL
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34 231 III; Elementar Analysensysteme GmbH, Hanau, Germany). To determine the C isotope ratio
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36 232 ($\delta^{13}\text{C}$, ‰) of the soil, litter and leaf and root samples harvested from live plants, all plant
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38 233 materials were oven-dried at 55°C for 48 h and soil samples were air-dried and sieved (2-mm
39
40 234 mesh). All samples were then ground using a ball mill (Retsch MM400, Haan, Germany) and the
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42 235 $\delta^{13}\text{C}$ ($\pm 0.1\text{‰}$) was analyzed using Combustion Module-Cavity Ring Down Spectroscopy (CM-
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44 236 Automate-CRDS, Picarro, Inc, USA).

50 237 **2.4 Calculations of soil C turnover**

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53 238 *New C inputs*

239 We defined C from rhizosphere deposition or plant litter as “new C” and organic C in the soil
 240 at the start of the experiment as “extant C.” As it is very difficult to measure rhizosphere
 241 deposition, root biomass was used to estimate the quantity of new C inputs from roots (Nguyen
 242 2003). The amount of the mineralized litter C was estimated by multiplying the decomposed
 243 litter mass by the relative proportion of C in the litter. The decomposition constant k (y^{-1}) was
 244 calculated as

$$245 \quad k = \ln(L_0/L_t)/t, \quad (1),$$

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

248 *New C stored in soil*

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

$$252 \quad C_n = C_t \frac{\delta_t - \delta_s}{\delta_p - \delta_s} \quad (2),$$

253 where C_n is the amount of new soil C derived from litter or root inputs, C_t is the total soil C pool
 254 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
 255 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
 256 root material.

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

260 *Net changes in soil C and mineralized extant soil C*

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3 261 The net change in the soil C pool was calculated for each treatment and soil type from the
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5 262 differences between the initial and final soil C concentrations. For the control treatment without
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8 263 plants or litter inputs in each SOM level, the amount of extant soil C mineralized during the
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10 264 experiment was calculated from the difference between the initial soil C concentration and the
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12 265 final soil C concentration; for the treatments with plant C inputs, the mineralized extant soil C
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15 266 was calculated as the difference between new C stored in the soil and the net change in soil C.
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18 267 **2.5 Statistical analysis**

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20
21 268 Three-way factorial analyses of variance (ANOVA) were used to examine the effects of SOM
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23 269 level, plant presence, litter addition, and their interactions on the soil aggregate fractions, MBC,
24
25 270 soil C concentration, mineralization of extant soil C, and net change in soil C. Two-way factorial
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28 271 ANOVAs were used to examine the effects of the SOM level, litter addition and their
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30 272 interactions on root biomass, as well as the effects of the SOM level and plant presence on litter
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32 273 decomposition. When the effects of SOM were significant, the means at given SOM levels were
33
34 274 calculated by averaging across the planted and litter addition treatments, and Tukey's post-hoc
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37 275 tests were conducted to compare these means across the SOM levels.
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39 276 Linear regression was used to explore the relationships between MBC, litter decomposition
40
41 277 rates, macro-aggregate and micro-aggregate fractions, and the mineralization of old soil C vs.
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44 278 new C stored in soil. The differences among the slopes of the linear regressions were tested using
45
46 279 the R package *smatr* (Warton and others 2012). All statistical analyses were performed using R
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48 280 version 3.1.0 (R Core Team 2014) and the results are reported as significant at $P < 0.05$.
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52 53 281 **3 Results**

282 3.1 Initial soil properties under different SOM levels

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

291 3.2 Effects of SOM levels on C inputs and MBC

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

302 3.3 Effects of SOM levels on soil aggregate fractions

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3 303 The mass percentage and absolute mass of >2000 μm aggregate fraction increased with
4
5 304 increasing initial SOM levels in all treatments ($P<0.01$, Fig 3a, Fig. S1a). In the planted
6
7 305 treatments, the >2000 μm aggregate fraction was higher than unplanted treatments at S40, S80
8
9 306 and S100, but not at other SOM levels (SOM \times plant interaction: $P<0.01$; Table S3; Fig. 3a),
10
11 307 whereas in the litter addition treatments, the >2000 μm soil aggregate fraction was significantly
12
13 308 higher ($P<0.01$) than in treatments without litter.

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17 309 By contrast, the mass percentage and absolute mass of 250-2000 μm aggregate fraction were
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19 310 unaffected by initial SOM level ($P=0.98$; Table S3; Fig. 3b, Fig. S1b) or litter addition ($P=0.63$;
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21 311 Table S3; Fig. 3b) and was lower in the planted compared to the unplanted treatments ($P=0.04$;
22
23 312 Table S3; Fig. 3b). The mass percentage and absolute mass of 53-250 μm and <53 μm soil
24
25 313 aggregate fractions decreased with initial SOM levels ($P<0.01$; Table S3; Fig. 3c-d, Fig. S1c-d)
26
27 314 and the 53-250 μm fraction was also significantly lower in litter addition treatments ($P=0.02$;
28
29 315 Table S3; Fig. 3a, c), but there was no effect of the planted treatments on either fraction, and no
30
31 316 effect of litter addition treatments on the <53 μm fraction ($P=0.84$; $P=0.24$; Table S3; Fig. 3d).

317 **3.4 Relationship between MBC and litter decomposition rate or soil aggregates**

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

324 **3.5 Effects of SOM levels on soil C turnover**

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3 325 Litter addition increased the amount of new C stored in the soil, with the largest increase at
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5 326 S80 (SOM \times litter interaction: $P < 0.01$; Table S3; Fig. 5a). The planted plus litter addition
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7 327 treatment resulted in a greater increase in the amount of new C stored in the soil than the planted-
8
9 328 only treatment, resulting in a significant plant \times litter interaction ($P < 0.01$; Table S3; Fig. 5a).

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11 329 The mineralization of extant soil C differed significantly among the SOM levels ($P < 0.01$) but
12
13 330 there was no clear pattern with increasing SOM level. However, the planted treatments
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15 331 significantly stimulated the mineralization of extant soil C compared to the unplanted treatments
16
17 332 ($P < 0.01$, Table S3, Fig. 5b), whereas litter addition had no such effect ($P = 0.53$; Table S3; Fig.
18
19 333 5b). The mineralization of extant soil C was positively correlated with new C stored in soil for
20
21 334 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
22
23 335 litter addition treatments ($R^2 = 0.71$, $P < 0.01$, $n = 15$, Fig. 5c); the slopes of the regression lines did
24
25 336 not differ from 1 or among treatments for any of these relationships.

26
27 337 By the end of the experiment, the soil C content was significantly higher in the litter addition
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29 338 treatments ($P < 0.01$) but was not affected by the initial SOM levels ($P = 0.81$) or the planted
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31 339 treatments ($P = 0.33$; Table S3; Fig. 5d). Calculations of the litter C storage efficiency for the litter
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33 340 addition treatments at S20 to S100 revealed that the storage of litter-derived C was lowest at S20
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35 341 (Fig. 6).

342 **4 Discussion**

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344 Soil organic matter content greatly affects the quality and quantity of plant C inputs to the
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346 soil by regulating plant productivity. The C from decomposing plant litter is either released as
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348 CO₂ to the atmosphere, leached through the soil as dissolved organic C, or incorporated into the
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350 soil as organic matter (Bird and others 2008; Mambelli and others 2011). Understanding how
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3 347 SOM levels affect the turnover and storage of plant and soil C is critical for the restoration of
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5 348 degraded grasslands. However, the contribution of plant C to SOM formation is currently poorly
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7 349 quantified (Cotrufo and others 2013). We aimed to address this by tracking the fate of plant C in
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9 350 soils with different levels of SOM. We created a wide SOM gradient (with soil C concentrations
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11 351 ranging from 0.02% to 1.40%) by mixing air-dried and combusted soils. The combustion
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13 352 treatment removed soil organic matter but also altered the soil mineralogy and texture (Table 2);
14
15 353 we therefore discuss our findings with due consideration to changes in other soil properties as a
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17 354 result of such methodological artifacts.
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23 355 **4.1 The role of litter inputs in SOC storage and turnover**

24
25 356 Litter decomposition by soil microbes is a critical step for litter-derived C entering the soil
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27 357 matrix (Cotrufo and others 2013). The positive correlation between MBC and SOM levels
28
29 358 observed in our study (Fig. 2) could be because soils with higher SOM levels not only provide
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31 359 more C substrates but also have higher N concentration to support greater microbial biomass
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33 360 (Manzoni and others 2012), and higher water-holding capacity to maintain a suitable
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35 361 environment for microbes (Sylvia and others 2005). **Higher microbial biomass and the favorable**
36
37 362 **moisture and nutrient conditions suggest that microbes could be more active at higher SOM**
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39 363 **levels, which would lead to faster decomposition of plant litter. Indeed, we found that there was**
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41 364 **a positive correlation between MBC and litter decomposition rate (Fig. 4a), and more litter C**
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43 365 **was mineralized at higher SOM levels (Fig. 1a).** Our study suggests that, during a given period,
44
45 366 less litter-derived C would be incorporated into the soil when litter decomposition rates and
46
47 367 MBC are low (Fig. 1a; Fig. 2; Fig. 5a). This supports emerging evidence for a “microbial filter”
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49 368 whereby labile C from litter is efficiently integrated into the mineral soil matrix through
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51 369 microbial activity, resulting in the formation of stable soil organic C (Cotrufo and others 2013).
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3 370 Our study demonstrated that the capacity of the soil to incorporate litter-derived C varied
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5 371 among SOM levels. The lowest SOM levels also had the lowest C storage efficiencies but the
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7 372 intermediate SOM levels had the highest C storage efficiencies (Fig. 6). Given that the
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9
10 373 physicochemical protection for new C inputs can be saturated (Six and others 2002a; Castellano
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12 374 and others 2015), the ability of soils to sequester additional C could become progressively
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14 375 limited as the SOM content increases. In addition to SOM levels, soil mineralogy and surface
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16 376 properties greatly determine the ability of soil to sequester new organic C. Soils with greater
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18 377 surface area have more capacity to adsorb OM on surfaces, and the stronger attraction between
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20 378 SOM and soil minerals at higher charge density increases SOM stability by forming organo-
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22 379 mineral complexes (Feng and others 2014; Wiesmeier and others 2015). Our combustion
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24 380 treatment could have affected C storage efficiency because it altered soil mineralogy (Table S2)
25
26 381 and significantly decreased the concentration of both silt and clay (Table 2). Although the effect
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28 382 of altered soil mineralogy remains to be explored, the decline in silt and clay is likely to affect
29
30 383 the occlusion and adsorption of litter-derived new C within the mineral matrix (Dungait and
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32 384 others 2012; Wiesmeier and others 2015), which would explain why litter C storage efficiency
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34 385 was particularly low at the lowest SOM level in our study (Fig. 6).
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40 386 Priming effects, the mineralization and release of stored soil C by fresh organic C inputs
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42 387 (Kuzyakov and others 2000), have also been given as an explanation for the lack of increased
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44 388 soil C storage in litter-addition studies (Lajtha and others 2014; Bowden and others 2014).
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46 389 Multiple lines of evidence indicate that higher inputs of plant litter can cause priming effects,
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48 390 offsetting soil C accumulation (Fontaine and others 2004; Sayer and others 2011). However, the
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50 391 role of priming effects in natural ecosystems is questionable, because soil C priming is rarely
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52 392 investigated *in situ* (Sayer and others 2011; Xu and others 2013). In our study, the losses of
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3 393 extant soil C were not significant under litter addition alone, but increased significantly when
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5 394 plants were present (Table S3; Fig. 5b). Although aboveground leaf litter and root deposition are
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7 395 both critical sources of labile C input to soil (Cotrufo and others 2013), our findings suggest that
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9 396 root deposition may induce stronger priming effects than litter.
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13 397 **4.2 The role of root C inputs in SOC turnover**

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16 398 Root-derived C, including C from root litter and root exudates, is considered to be the main
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18 399 source of soil C in grassland ecosystems (Kuzyakov and Domanski 2000; Rasse and others 2005;
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20 Schmidt and others 2011). At low SOM contents, the soil is extremely infertile and plants tend to
21 400 invest more C into root production to access nutrients (Dakora and Phillips 2002); in our
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23 401 experiments, this was indicated by the decrease in the root-to-shoot ratio with increasing SOM
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25 402 level (Fig. 1c). The rhizosphere priming effect is closely related to the amount of root C inputs
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27 403 (Cheng 2009) and isotope methods are usually necessary to distinguish between increased soil
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29 404 CO₂ efflux derived from roots and primed soil C (“real positive priming effect”; Kuzyakov and
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31 405 others 2000). We were able to use the changes in the ¹³C values of soil C to calculate the
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33 406 mineralization of extant soil C induced by new root C inputs in the planted treatment and show
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35 407 that root C inputs produced a “real positive priming effect” (Table S3; Fig. 5b). For the planted
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37 408 treatment without litter addition, the regression line for the mineralization of extant soil C vs.
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39 409 new C stored in soil closely followed the 1:1 line (Fig. 5c), suggesting that the storage of new C
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41 410 was offset by the release of extant soil C as CO₂, resulting in no net change in the soil C content.
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43 411 Although our experiment was too short to fully evaluate the effects of root litter on soil C storage,
44
45 412 the results indicate that root C deposition during the growing season made a limited contribution
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47 413 to soil C storage because new C inputs from roots replaced the extant C that was mineralized and
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49 414 released by priming. Such differences in soil C turnover without a net change in soil C stocks
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3 416 would not be detected with soil C content measurements (Kuzyakov and Blagodatskaya 2015),
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5 417 but the replacement of extant soil C with newer and possibly more labile C could affect the
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7 418 overall stability of the soil C pool (Sayer and others 2011). Long-term field investigations are
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9 419 needed to better understand how leaf litter and root processes affect the mineralization of extant
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11 420 soil C via priming effects.
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14 421 We also observed interactive effects of litter addition and plant roots on the accumulation of
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16 422 new C in the soil. There was a non-additive effect of plants and litter addition on the storage of
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18 423 new C, whereby the amount of new soil C in pots with the combined planted + litter addition
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20 424 treatment was less than the sum of new C in the pots with either treatment alone (plant \times litter:
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22 425 Table S3; Fig. 5a). As root biomass was lower when litter was added to the pots (Fig. 1b), we
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24 426 propose that reduced root deposition could account for the smaller relative amount of new C in
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26 427 the combined planted + litter addition treatment.
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31 428 **4.3 The responses of soil aggregate fractions and their roles in soil C stabilization**

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34 429 The mean residence time of soil C is important for soil C sequestration capacity. Apart from
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36 430 organo-mineral complexes (Feng and others 2014), the formation of microaggregates within
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38 431 macroaggregates is an essential mechanism in the physical protection of soil C (Denef and others
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40 432 2007). In our study, the macroaggregate fraction was positively related to MBC in the planted,
41
42 433 litter addition, and planted + litter addition treatments (Fig. 4b), and both also increased with
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44 434 SOM levels (Fig. 3a). These findings suggest that soils with high SOM content support
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46 435 aggregate-binding agents, such as fine roots and microbial hyphae (Tisdall and Oades 1982), and
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48 436 therefore feedbacks between the extant SOM, new organic C inputs and macroaggregate
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50 437 formation are central to the storage and stabilization of soil C. We also found that the
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52 438 microaggregate fraction declined with increasing SOM levels. This is probably because a large
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3 439 proportion of microaggregates were occluded within macroaggregates and the macroaggregate
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5 440 fraction increased with the SOM level (Fig 3a, c, d).
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8 441 Overall, our study indicated that the influence of SOM on the formation of soil
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10 442 macroaggregates could create a positive feedback for SOC accumulation, **because more SOM**
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12 443 **associated with microaggregates is occluded within macroaggregates, which enhances the**
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14 444 **stability of SOC (Fig. S1).** However, it is also possible that the higher proportion of
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16 445 microaggregates under low SOM levels is an artifact of the combustion treatment, whereby
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18 446 macroaggregates were destroyed by heating, releasing the occluded microaggregates. Finally, the
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20 447 soil structure was destroyed by sieving soils before filling the pots at the start of the experiment
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22 448 and the soil structure developed during our experimental period may not be representative of
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24 449 undisturbed soils in the field. Nonetheless, our study indicates potential mechanisms and
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26 450 feedbacks between extant SOM levels and the stabilization of new C inputs, which can be tested
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28 451 in field studies.
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35 452 **5 Conclusions**

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38 453 Soil organic matter content is one of the most important indices of soil health but
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40 454 anthropogenic disturbance and climate changes can affect the accumulation and stability of SOM.
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42 455 **Our study highlights** the important role of initial SOM content for regulating soil C formation
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44 456 and stability through direct and indirect effects on the turnover of aboveground litter, root C
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46 457 inputs, and microbial processes. The initial SOM content affected not only plant litter and root C
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48 458 inputs but also the capacity of the soil to incorporate litter- and root-derived C. Feedbacks
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50 459 between SOM, plant litter and plant root inputs are complex, but we show that they interact to
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52 460 influence the accumulation of new C and the mineralization of extant C in the soil. In addition,
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3 461 initial SOM content also influenced the formation of soil aggregates and therefore further
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5 462 enhanced the stability of soil organic C. These results merit further investigation to help us better
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7 463 understand how C is stored and stabilized in the soil. Caution is needed when extrapolating our
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10 464 findings to natural ecosystems because the use of combusted soil and the method of mixing litter
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12 465 into soils potentially introduced methodological artifacts. Furthermore, our single-species, short-
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14 466 term greenhouse experiment may not represent the complex processes in the field. Nevertheless,
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17 467 our results open up several new lines of scientific enquiry, which need to be tested under field
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19 468 conditions. When evaluating the potential of soil C sequestration under future global changes,
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21 469 field and model studies need to consider how initial SOM content could affect the fate of plant-
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24 470 derived C.
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3 **Table 1.** Initial soil carbon concentrations (C%), soil nitrogen concentrations (N%), soil C-to-N
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5 ratio (C:N) (means \pm SE for $n = 3$) and soil $\delta^{13}\text{C}$ values for six experimental levels of soil
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7 organic matter (SOM) content: S0: 100% combusted soil; S20: 80% combusted soil plus 20%
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9 native soil; S40: 60% combusted soil plus 40% native soil; S60: 40% combusted soil plus 60%
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11 native soil; S80: 20% combusted soil plus 80% native soil; S100: 100% native soil. Different
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13 lowercase letters indicate significant differences among SOM levels at $P < 0.05$, where NS
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15 indicates “non-significant.”
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	C (%)	N (%)	C:N	$\delta^{13}\text{C}$ (‰)
S0	0.02 \pm 0.00 ^a	0.02 \pm 0.00 ^a	1.30 \pm 0.12 ^a	--
S20	0.25 \pm 0.05 ^b	0.04 \pm 0.00 ^b	5.98 \pm 0.27 ^b	-24.0
S40	0.58 \pm 0.01 ^c	0.07 \pm 0.00 ^c	7.96 \pm 0.16 ^c	-23.8
S60	0.87 \pm 0.02 ^d	0.10 \pm 0.00 ^d	9.00 \pm 0.25 ^d	-23.8
S80	1.15 \pm 0.01 ^e	0.12 \pm 0.00 ^e	9.38 \pm 0.57 ^d	-24.0
S100	1.40 \pm 0.02 ^f	0.14 \pm 0.00 ^f	9.81 \pm 0.07 ^d	-23.8
<i>P</i> values	<0.01	<0.01	<0.01	NS

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639 **Table 2.** Initial soil mineralogy given as mass percentages, including soil particle size
640 distributions (sand, silt and clay contents) and water holding capacity (WHC); values shown are
641 means \pm SE for $n = 3$ for each of six experimental soil organic matter (SOM) levels: S0: 100%
642 combusted soil; S20: 80% combusted soil plus 20% native soil; S40: 60% combusted soil plus
643 40% native soil; S60: 40% combusted soil plus 60% native soil; S80: 20% combusted soil plus
644 80% native soil; S100: 100% native soil. Different lowercase letters indicate significant
645 differences among SOM levels at $P < 0.05$.

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

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3 **648 Figure legends**
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5 **649 Figure 1.** Final values of **a)** mineralized litter carbon (C), **b)** root biomass C inputs and **c)** root-
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8 **650** to-shoot ratios in soils with different experimental soil organic matter (SOM) levels after 191
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10 **651** days in a greenhouse study; S is SOM level where S0 is 100% combusted soil; S20 is 80%
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12 **652** combusted soil plus 20% native soil; S40 is 60% combusted soil plus 40% native soil; S60 is 40%
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14 **653** combusted soil plus 60% native soil; S80 is 20% combusted soil plus 80% native soil; S100 is
15
16 **654** 100% native soil. **SOM: soil organic matter level. Different lowercase letters indicate significant**
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18 **655** **differences among SOM levels across all treatments ($P < 0.05$).**
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22 **656 Figure 2.** Microbial biomass carbon (MBC) in soils with different experimental soil organic
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24 **657** matter (SOM) levels after 191 days in a greenhouse study. The abbreviations follow the legend
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26 **658** in Figure 1. Different lowercase letters indicate significant differences among SOM levels across
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28 **659** all treatments ($P < 0.05$).
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31 **660 Figure 3.** Mass percentages of soil aggregate fractions in soils with different experimental soil
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33 **661** organic matter (SOM) levels and carbon inputs; **a)** $>2000 \mu\text{m}$, **b)** $250\text{-}2000 \mu\text{m}$, **c)** $53\text{-}250 \mu\text{m}$, **d)**
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35 **662** $<53 \mu\text{m}$ aggregate fraction. Abbreviations follow the legend in Figure 1. Different lowercase
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37 **663** letters indicate significant differences among SOM levels across all treatments ($P < 0.05$).
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40 **664 Figure 4.** The relationship between microbial biomass carbon and **a)** the litter decomposition
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42 **665** rate and **b)** the macroaggregate fraction under different experimental treatments. The
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44 **666** macroaggregate fraction is the sum of the $>2000 \mu\text{m}$ and $250\text{-}2000 \mu\text{m}$ aggregate fractions (in
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46 **667** mass percentage). The abbreviations follow the legends in Figures 1 and 2.
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49 **668 Figure 5.** Changes in carbon (C) pools in soils with different experimental soil organic matter
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51 **669** (SOM) levels and C inputs: **a)** new C stored in soil, **b)** mineralization of extant soil C, **c)** the
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53 **670** relationship between new C stored in soil and mineralization of extant soil C, and **d)** net soil C
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3 671 change; abbreviations follow the legend in Figure 1. Different lowercase letters indicate
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5 672 significant differences among SOM levels across all treatments ($P < 0.05$).
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8 673 **Figure 6.** Litter carbon (C) storage efficiency (the ratio of litter-derived new soil C to
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10 674 mineralized litter C) in soils with different experimental initial soil organic matter (SOM) levels;
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12 675 abbreviations follow the legend in Figure 1. Different lowercase letters indicate significant
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14 676 differences among the means for SOM levels ($P < 0.05$).
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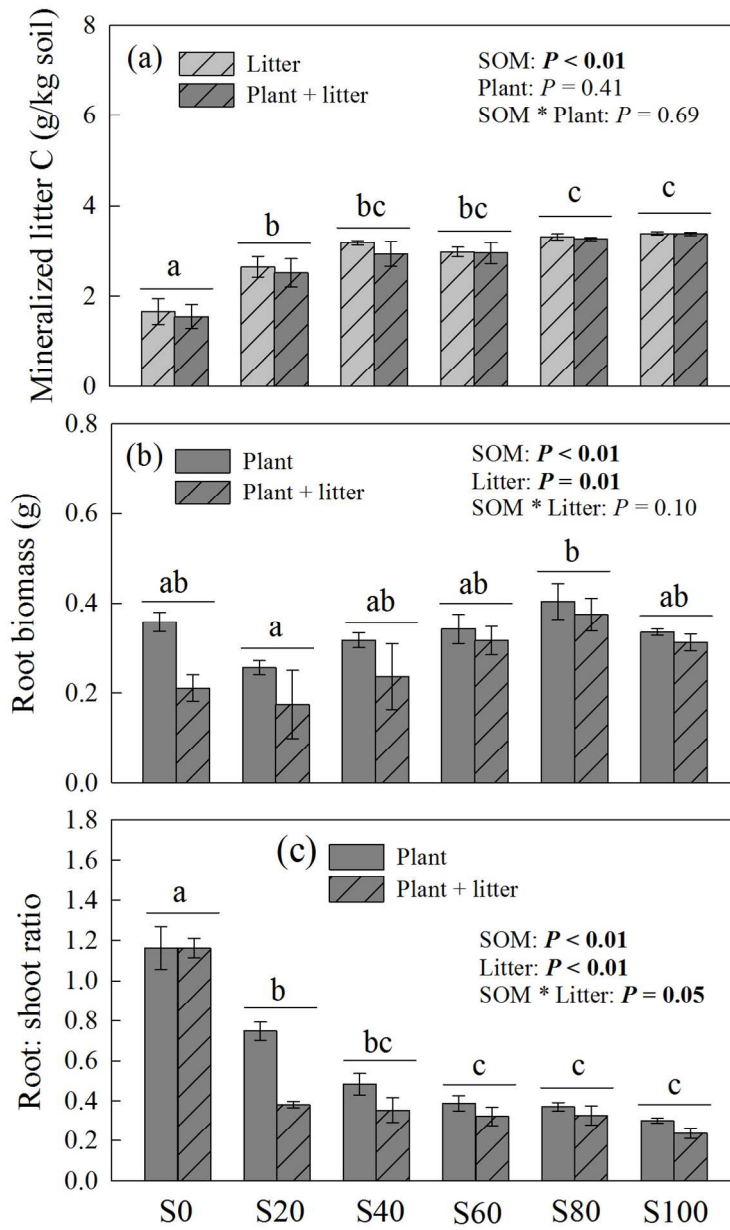
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Figure 1



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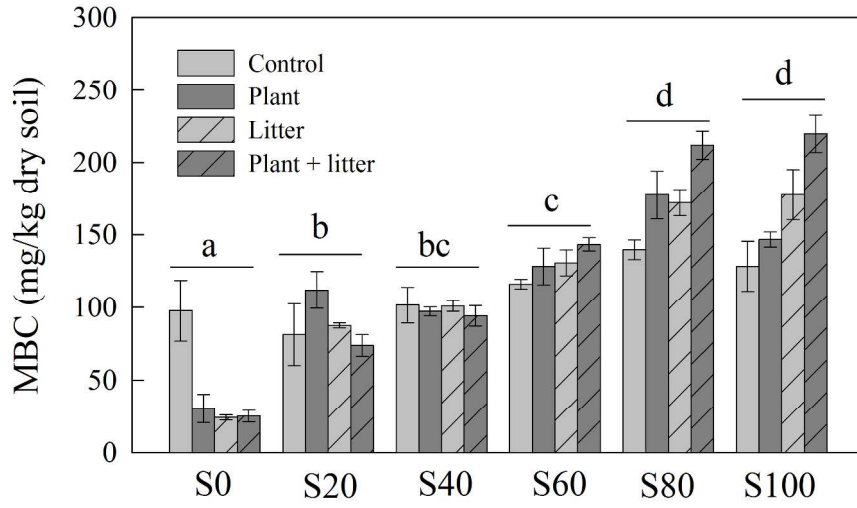
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Figure 2



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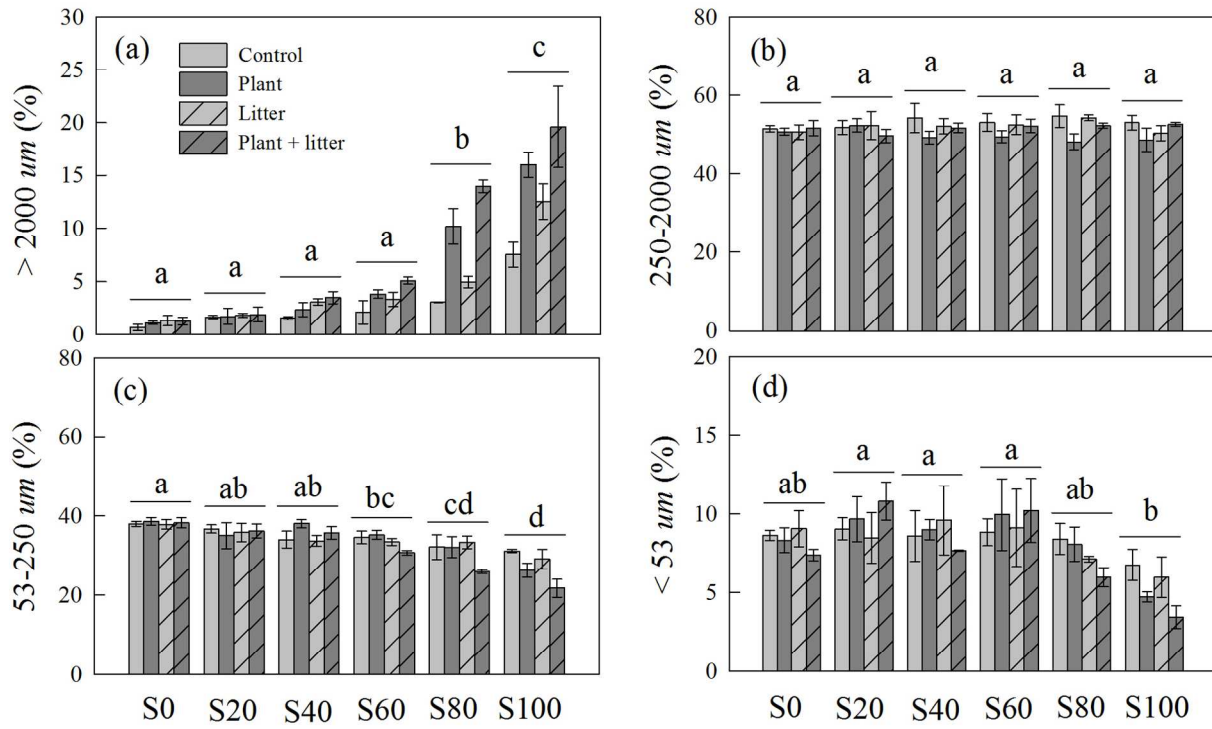
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Figure 3



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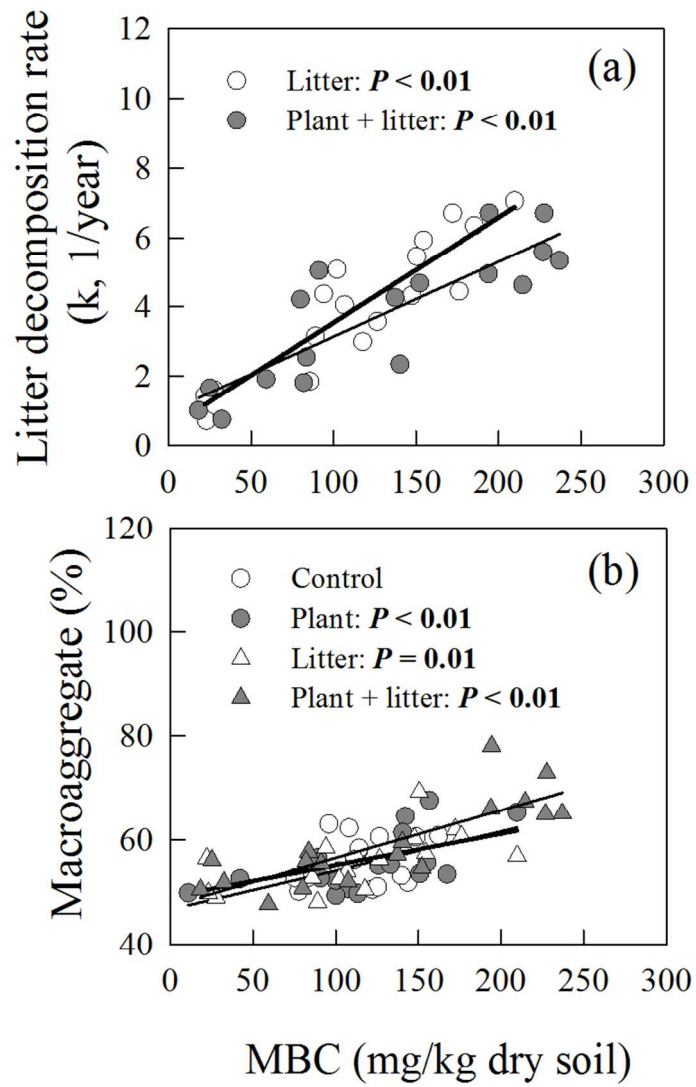
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Figure 4



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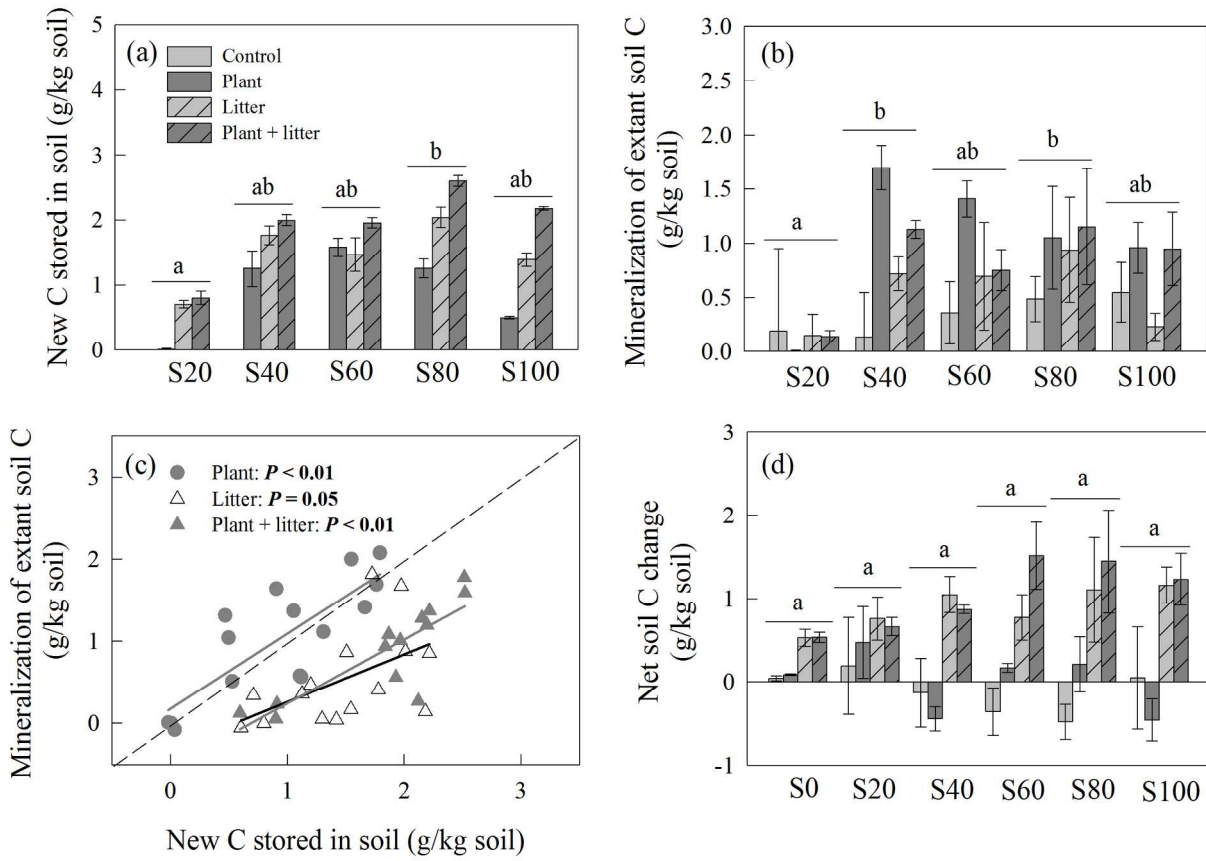
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Figure 5



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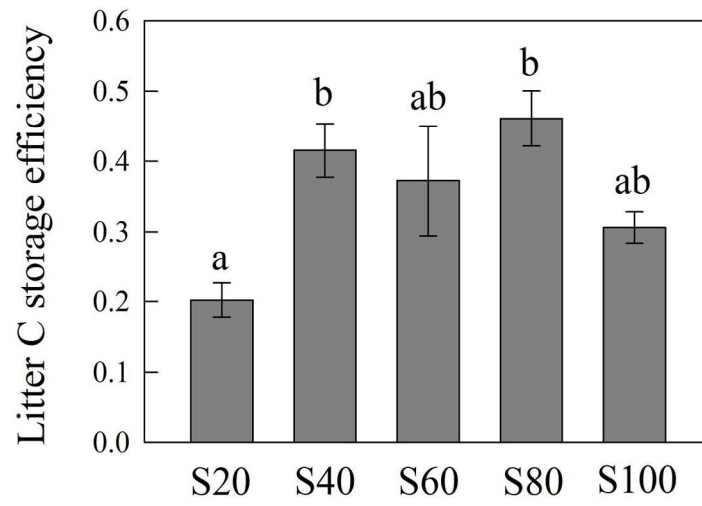
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Figure 6



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