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Effect of reduction of aggregate size on the priming effect in a Mollisol under different soil managements

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Summary

The priming effect (PE) is influenced by the amount and quality of soil organic carbon (SOC) held in different soil aggregates sizes. We examined the PE in Mollisols managed for >30 years as a grassland, farmland and bare fallow with 51, 32 and 27 g of SOC kg^{-1} soil, respectively, in a 60-day incubation. Grassland soil contained 75% of aggregates in the macro size fractions >0.25 mm compared to <32% in farmland and bare fallow. Farmland and bare fallow soils contained proportionately more micro size fractions. The effect of aggregate size on the PE was assessed by comparing soils with intact aggregates to those where macroaggregates were reduced to microaggregate size fractions. In the grassland soil, cumulative CO_2 mineralization increased by 20% in the reduced aggregate-size treatment with no effect on farmland or bare fallow soils. Substrate additions to examine the PE included ^{13}C-glucose and ^{13}C-alanine (0.4 g C kg^{-1} dry soil), and inorganic N (2 mg N kg^{-1} dry soil). The PE was in the order glucose > alanine > $(NH_4)_2SO_4$ and was most intense at day 3 of the incubation. Aggregate-size reduction did not affect the PE within soil management treatments regardless of substrate addition. Most of the CO_2 produced was derived from SOC rather than substrate addition and peaked at day 3. There was an interaction between microbial biomass C and dissolved organic C on the PE in the grassland soil only. The results suggested that the PE is an intrinsic characteristic of soils that are more affected by available C than the SOC in different aggregate fractions.

Highlights

- The priming effect from added C substrates was examined in relation to aggregate size.
- Reducing macroaggregates to <0.25 mm increased CO_2 production in a grassland but not agricultural Mollisol.
- The positive priming effect was proportionately reduced in soil dominated by macroaggregates.
- The priming effect is an intrinsic characteristic affected by C additions and less so by aggregate protected C.

KEYWORDS
carbon mineralization, grassland, soil organic carbon, substrate addition

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1 | INTRODUCTION

Decomposition of soil organic carbon (SOC) is a major process that affects the composition of the atmosphere. Soil aggregates impart physical and chemical protection to SOC by occlusion, organo–mineral interactions, nutrient depletion and attenuated oxygen conditions, all of which influence whether decomposition or sequestration of SOC occurs (Six, Bossuyt, Degryze, & Denef, 2004; Zhang, Ding, Luo, Bolan, & Yu, 2015). Mineralization of native SOC can increase or decrease with the amount and quality of new C inputs, leading to positive or negative priming effects (PE) (Hamer & Marschner, 2005; Keith, Singh, & Singh, 2011; Kuzyakov, 2010). The effect of aggregate-size distribution and the availability of C protected within aggregates on the PE is less understood (Tian et al., 2015; Tian, Pausch, Yu, Blagodatskaya, & Kuzyakov, 2016).

Typical PE studies often overlook the influence of macro- and microaggregates, making it difficult to identify specific aggregate-size effects on the magnitude and intensity of PE. Tisdall and Oades (1982) suggested the aggregate hierarchy concept or theory describing the spatial dependence of C protected within micro- and macroaggregate formation. They postulated that microaggregates were formed mainly by polysaccharides complexing to minerals, whereas macroaggregates were bound by roots, mucilage and hyphae. Oades (1984) modified the theory, proposing that microaggregates form within macroaggregates. Subsequent observations found that macroaggregates have a greater proportion of labile C than microaggregates, suggesting that microaggregates play a key role in stabilizing SOC (Elliott, 1986; Six & Paustian, 2014). Recent evidence shows that SOC protected within aggregates has a contrasting biochemical composition depending on aggregate size (Bimueller, Kreyling, Koelbl, von Luetzow, & Koegel-Knabner, 2016). Based on these studies, it would be expected that the C within different aggregate sizes could influence PE outcomes in the presence of added labile C substrates.

Tian et al. (2015) reported that reducing aggregate size by ball milling had no effect on cumulative C mineralization, but found that size contributed disproportionately to total C mineralization. Similar results were found between intact and crushed aggregates under continuous corn cropping and grassland systems (Drury, Yang, Reynolds, & Tan, 2004; Elliott, 1986). These studies did not address the influence of aggregate size on the PE, which is likely to be important for understanding C transfer functions among different aggregate-size classes within the hierarchical aggregate conceptual framework.

Soils under different land use often have different SOC contents and aggregate-size distribution, which can influence the PE. Grassland soils are noted for their large SOC content and aggregate size, and as a result generally mineralize more carbon than those converted to agriculture (Blagodatskaya, Blagodatsky, Anderson, & Kuzyakov, 2007; Cambardella & Elliott, 1994; Elliott, 1986). Smaller rates of C mineralization in agriculture and fallow soils are a result of smaller labile C pools, such as the light or particulate fraction, and proportionately larger mineral-bound carbon in smaller aggregates, representing more recalcitrant SOC pools (Cross & Sohi, 2011). Previous studies on aggregate C mineralization focused on land use and tillage systems (Barto, Alt, Oelmann, Wilcke, & Rillig, 2010; Rabbi, Wilson, Lockwood, Daniel, & Young, 2015). The mineralization of SOC within different aggregate fractions has been studied less (Alvarez, Soriano, Landa, & Gomez, 2007; Bimueller et al., 2016).

We examined the effect of the reduction of soil aggregate size on the PE in a Mollisol with different management histories implemented >30 years ago with grassland, farmland and bare fallow soils containing 51, 32 and 27 g kg^{-1} SOC, respectively. We specifically sought to challenge the concept that PE is controlled mainly by total SOC content, but instead propose that the quality of the SOC being protected in different aggregate-size fractions has an influence. We hypothesized that an increase in PE occurs when larger aggregates are reduced in size and release a pool of labile SOC compared to soils with smaller aggregate-size distributions. A larger microbial biomass in soils with larger aggregates probably underpins the processes affecting PE outcomes. We conducted an incubation experiment using ^{13}C-labelled and N substrates to identify sources of C mineralization in soils with intact aggregates compared to those with reduced aggregate-size distributions, to examine the effects of land use on the PE.

2 | MATERIALS AND METHODS

2.1 | Site description

Soil samples were collected from the Mollisol area in northeast China at the State Key Experimental Station of Agroecology, Chinese Academy of Sciences, Hailun, Heilongjiang province (47°26′N, 126°38′E). Soils are classified as clay loam Pachic Haploborolls according to the USDA Taxonomy (Soil Survey Staff, 2010). The parent material is loess underlain by glacial outwash. The climate is characterized by a typical temperate continental monsoon, with cold winters and warm summers; the mean annual temperature is 2.2 °C. Total annual precipitation averages 550 mm, with about 358 mm occurring from June to August.

The site was in agricultural use for more than 60 years before it was divided into three long-term trials in 1985:

(i) restored grassland, which was left undisturbed and vegetated naturally without any fertilizer inputs or tillage, and dominated by *Leymus chinesis* species; (ii) bare fallow, maintained by eliminating plant growth by hand hoeing; and (iii) farmland, maintained in a continuous 3-year crop rotation of maize (*Zea mays* L.)–soya bean (*Glycine max* (L.) Merrill.)–wheat (*Triticum aestivum* L.). The farmland treatment consists of an experimental design comprising an N-rate trial, with each N level replicated three times (Song, Han, & Tang, 2007). For the present study, the control farmland treatment plot without N fertilizer input was used. Plot sizes were 360 m^2 for grassland, 180 m^2 for bare fallow and 60 m^2 for each farmland replicate plot. The grassland and bare fallow were not replicated. The SOC atom % ^{13}C was 1.085, 1.085 and 1.084 for bare fallow, farmland and grassland, respectively. The basic soil properties are shown in Table 1, in which available N in soil was measured using the alkaline diffusion method (Bremner & Mulvaney, 1982), inorganic N determined by KCl extraction (Doane & Horwath, 2003), extractable P determined with $NaHCO_3$ extraction (Olsen, Cole, Watanabe, & Dean, 1954) and extractable K with NH_4OAc (Jackson, 1973). Soil pH was measured with deionized water (1:2.5).

2.2 | Soil sampling and preparation

Soil samples were taken from the 0 to 10-cm depth with a probe sampler in August 2016. Ten randomized soil cores from grassland and bare fallow plots were mixed into a composite sample to represent each treatment. For the farmland, the same sampling procedure was performed on each of the three replicate control plots and then mixed into a single sample. The soil samples were transported to the laboratory and stored at 4 °C on the same day. All samples were kept in their intact soil ped structure before being broken into small peds along natural cracks by hand during the air-drying process. All visible organic debris was removed by hand picking before additional soil processing. Soil samples from each land use were separated into two treatments consisting of undisturbed aggregate-size distribution and reduced aggregate-size distribution for incubation. The undisturbed samples consisted of intact aggregates, sieved to <6 mm. In the reduced aggregate-size treatment, aggregate size was reduced by hand rolling a glass cylinder (diameter, 8.0 cm) over undisturbed soil to pass a 1.0-mm sieve.

TABLE 1 Initial characteristics of soils used in the incubations

Properties of treatments	Bare fallow	Farmland	Grassland
Soil organic C/g kg^{-1}	27.2	32.3	50.9
Total N/g kg^{-1}	2.1	2.5	4.0
Dissolved organic C/mg kg^{-1}	187.7	244.7	359.4
Microbial biomass C/mg kg^{-1}	218.0	283.0	506.0
pH/H_2O	5.9	6.3	7.1
Inorganic N/mg kg^{-1}	41.2	34.4	20.6
Potential mineralizable N/mg kg^{-1}	269.0	307.0	450.0
Potentially available P/mg kg^{-1}	22.5	17.6	44.8
Potentially available K/mg kg^{-1}	134.0	152.0	228.0

2.3 | Incubation design

The undisturbed and reduced aggregate-size soils were preincubated at 22 °C for 7 days at 40% water-holding capacity (WHC) after applying deionized water uniformly with a spray bottle. The preincubated soils (100 g dry weight) were weighed into 120-ml specimen cups and placed into 750-ml Mason jars for a total of 288 jars. A 2 × 4 × 4 factorial experiment was used, corresponding to two aggregate-size treatments, four substrate treatments and four destructive sampling dates. Substrate additions included glucose, alanine and inorganic N as typical components of root exudates and fresh residue inputs and a type of chemical fertilizer application, respectively. A control with no substrate addition was included in the design. Each treatment had three replicates. The added glucose and alanine (0.4 μg C g^{-1} soil) were prepared from 99 atom % uniformly ^{13}C-labelled glucose and alanine diluted with unlabelled glucose or alanine to a final enrichment of 6.0 atom %. The inorganic N treatment received $(NH_4)_2SO_4$ (2 μg N g^{-1} soil), approximately equivalent to three times the ambient KCl-extractable N for each soil. The substrate treatment solutions were added to the preincubated soils and distributed uniformly using a syringe or needle to a final soil moisture content of 60% WHC. The jar lids were modified to include rubber septa for headspace gas sampling. The lids were placed loosely on the jars to allow exchange of headspace when no sampling occurred. The jars were incubated at 22 °C. Soil moisture was checked weekly and deionized water added to maintain 60% WHC as needed. During gas sampling events, lids were removed, jars flushed with CO_2 free air and resealed tightly, and headspace sampled after 24 hours. Headspace samples for CO_2 were taken at 1, 2, 3, 5, 7, 10, 15, 20, 25, 30, 35, 40, 45, 50 and 60 days. At 3, 15, 35 and 60 days of incubation, the headspace samples were also used for ^{13}C-CO_2 analysis. During sampling CO_2 concentrations did not exceed 1% CO_2. On the same days of ^{13}C-CO_2 sampling events, 18 jars representing all treatments and associated replicates were destructively sampled for microbial biomass C (MBC) and dissolved organic C (DOC).

2.4 | Analyses

Water-stable aggregates in both undisturbed and reduced aggregate-size soils were determined by wet-sieving as described by Li, Han, Wang, Qiao, and Xing (2007). Wetted soils were separated into >2-mm large macroaggregates, 2.0–0.25-mm small macroaggregates, 0.25–0.053-mm microaggregates and 0.053-mm silt and clay particles. All fractions were oven-dried at 50 °C and weighed. The C content of each aggregate fraction was determined on a VarioEL CHN elemental analyser (Heraeus Elementar Vario EL, Hanau, Germany).

The headspace CO_2 was determined on a gas chromatograph (Shimadzu GC 2010, Shimane, Japan), and its ^{13}C abundance on an isotope ratio mass spectrometer (Elementar, Isoprime-100, trace Gas-IRMS, Frankfurt, Germany). Soil MBC was determined by chloroform fumigation extraction (Vance, Brookes, & Jenkinson, 1987). The non-fumigated and fumigated soil extracts were passed through a 0.45-μm membrane filter and the DOC concentration of the extracts measured with a TOC analyser (Elementar, Liquid II, Frankfurt, Germany). Microbial biomass C was calculated as the difference between the C content of the fumigated and non-fumigated soil extracts and corrected using a k_{EC} factor of 0.45. Fresh soils were extracted with 2 M KCl solution (60 min of agitation at 20 °C, 1:5 soil:solution ratio) to determine inorganic N concentration colorimetrically with a continuous-flow autoanalyser (San++System, Skalar Analytical B.V., Breda, the Netherlands) (Mulvaney, 1996). Total SOC content and total N were analysed with a VarioEL CHN elemental analyser (Heraeus Elementar Vario EL, Hanau, Germany) in finely ground subsamples of the air-dried field samples.

2.5 | Statistical analysis

The proportion of C-CO_2 derived from SOC and the labelled substrate input was calculated using the following equation:

$$F_S = (C_T - C_C)/(C_S - C_C),$$

where F_S is the proportion of substrate-derived CO_2, C_T is the atom % of ^{13}C of the total CO_2 produced in the treatment incubation, C_C is the atom % of C of the CO_2 produced in the control incubation and C_S is the atom % of C of the substrate added (6 atom %) to the treatment incubation. We calculated the amount of C derived from substrate decomposition by multiplying Fs by the total amount of CO_2 produced during the incubation (both expressed in μg C-CO_2 g^{-1} soil). Similarly, the amount of C derived from the decomposition of SOC was calculated by multiplying the total amount of CO_2 produced during the incubation by (1–F_S). The PE was calculated as the difference between the soil-derived C-CO_2 in the control incubations and in the amended incubations (Bernal et al., 2016; Tian et al., 2016) at days 3, 15, 35 and 60 during the incubation.

The data were analysed using a three-way split-plot analysis of variance (ANOVA) with R 3.4.3 (R Development Core Team, 2010) to compare the effect of aggregate-size reduction, substrate addition and incubation time on CO_2 production, PE, DOC and MBC at days 3, 15, 35 and 60, respectively (Webster, 2007; Webster & Lark, 2018). Before the three-way ANOVA, the residuals of each analysis were checked for normality by the Kolmogorov–Smirnov test and for homoscedastity by the Bartlett test. There was only one composite soil sample taken from each of the three types of soil management; therefore, there was no true replication. The soils were managed for over 30 years under the stated management, representing a long-term management outcome. Aggregate reduction was the main plot and grassland, farmland and bare fallow were replicates (three blocks), and substrate addition and incubation time were subplots. This means that it was impossible to compare the effects of the different types of land management within the ANOVA because of the lack of replication of the original soil samples. Any comparison of results across the three land managements must be considered indicative only. Principal component analysis (PCA) was carried out on the correlation matrix with data from soil respiration and the soil properties. The results were used to determine the effects of substrate addition on CO_2 respiration and soil properties (DOC and MBC). The eigenvalues represented the proportion of the total variance explained for each principal axis. The first few accounted for a large proportion of the variance. The principal components were linear combinations of the original attributes. The PCA was performed using SPSS 19.0.

3 | RESULTS

3.1 | Characterization of soil aggregates before incubation

Grassland soil contained the largest SOC content, 87 and 58% more than that of bare fallow and farmland soils, respectively (Table 1). The same trend was seen for DOC and MBC: grassland > farmland > bare fallow. The grassland soil had the largest macroaggregate fraction ($WSA_{>0.25\text{-mm}}$), representing 74% of the total soil mass and 76% of SOC (Table 2). All of the $WSA_{>2\text{-mm}}$ disappeared after aggregate-size reduction, with aggregate sizes mainly in the $WSA_{0.053–0.25\text{-mm}}$ and $WSA_{<0.053\text{-mm}}$ fractions. The C to N ratio of all soils and aggregates ranged from 12 to 15. The C to N ratio of the grassland aggregates was smaller than for the other soils.

TABLE 2 Organic C concentrations of bulk soil (SOC), mass, C, N and ratio of C to N proportion of water-stable aggregates before incubation

Treatments		Soil mass of water-stable aggregates /g 100 g^{-1} soil				OC of water-stable aggregates /g kg^{-1} soil				N of water-stable aggregates /g kg^{-1} soil				C:N of water-stable aggregates			
		>2 mm	0.25–2 mm	0.053–0.25 mm	<0.053 mm	>2 mm	0.25–2 mm	0.053–0.25 mm	<0.053 mm	>2 mm	0.25–2 mm	0.053–0.25 mm	<0.053 mm	>2 mm	0.25–2 mm	0.053–0.25 mm	<0.053 mm
Bare fallow	UA	0.0	15.2	46.6	37.9	0.0	5.1	14.5	7.5	0.0	0.4	1.1	0.7	0.0	14.1	13.5	11.3
	RA	0.0	7.9	45.1	47.0	0.0	2.6	14.0	9.3	0.0	0.2	1.0	0.8	0.0	13.7	13.8	12.2
Farmland	UA	6.3	25.6	38.1	30.0	2.3	9.6	13.3	6.6	0.2	0.7	0.9	0.6	14.4	14.5	14.2	10.3
	RA	0.0	19.9	43.3	36.8	0.0	7.5	15.6	8.4	0.0	0.5	1.1	0.7	0.0	15.6	14.3	11.9
Grassland	UA	23.5	51.4	15.2	10.1	10.3	27.8	9.3	3.1	0.8	2.1	0.7	0.3	12.9	13.6	13.5	11.2
	RA	0.0	48.9	26.0	25.0	0.0	21.6	11.0	6.0	0.0	1.9	0.9	0.6	0.0	11.7	12.0	10.4

UA: undisturbed aggregate size; RA: reduced aggregate size.

3.2 | Effects of soil aggregate size and addition of substrate C on CO_2 production

Cumulative CO_2 production in undisturbed grassland soil produced 128–210% more CO_2 than undisturbed farmland or bare fallow soils without substrate addition (Figure 1). In the grassland soil, cumulative CO_2 mineralization increased 20% in the reduced aggregate-size treatment. The effect of aggregate-size reduction on CO_2 production was observed in grassland soil only (Figure 1). Compared with no substrate addition, the increase in CO_2 produced from glucose and alanine additions in the grassland, bare fallow and farmland soils, was 41 and 48%, 102 and 107%, and 139 and 162%, respectively, more than the control treatments. The total CO_2 produced from the added C substrates was 1.6–2.1-fold larger in grassland than in the other soils regardless of aggregate treatment (Figure 1). These results are indicative only because the comparison between land managements could not be tested statistically because of the lack of replication.

Glucose and alanine additions stimulated CO_2 production mainly in the first 15 days of the incubation (Figure 1). The maximum rate of CO_2 production from the addition of alanine occurred in the first 2 days and was 1.9 and 1.8 times larger in grassland than farmland and bare fallow soils, respectively. All combinations of C substrate addition by soil peaked by 5 to 7 days. The rate of CO_2 production from grassland soil was five times greater than for bare fallow and farmland soils at the end of the incubation regardless of C substrate treatment. These results are indicative only as the comparison between land managements could not be tested statistically because of the lack of replication.

The addition of glucose and alanine produced significantly more CO_2 than the addition of $(NH_4)_2SO_4$ ($P = 0.002$). For the addition of glucose, it was 140–160% more for bare fallow, 102–107% for farmland and 43–48% for grassland soils compared to no addition. The addition of alanine increased CO_2 production by 140–160% for bare fallow, 102–106% for farmland and 41–45% for grassland soils compared to the control. The addition of $(NH_4)_2SO_4$ produced less CO_2; 29–34% for bare fallow, 17–19% for farmland and 3–9% for grassland soil compared to no addition. The comparisons of results across the three land managements are indicative only. In the reduced aggregate treatment of grassland soil, glucose (43%), alanine (41%) and $(NH_4)_2SO_4$ (3%) additions produced more CO_2 than the addition of no substrate, although the effect of aggregate reduction was not significant across the three blocks. There were effects of interactions between substrate addition and incubation time on cumulative CO_2 produced for the different soils and treatments (Table S1, Supporting Information).

3.3 | Changes in DOC and microbial biomass C

Grassland soil had more initial DOC than bare fallow (46%) and farmland (32%) soils before the incubation (Table 1). The average DOC for all treatments decreased gradually over incubation time. After 60 days, the DOC of the soils decreased to 26–52% of their initial values (Figure 2).

The effect of adding glucose and alanine on MBC was pronounced in all soils ($P = 0.033$). The grassland soil had more MBC than bare fallow (133%) and farmland (79%) soils at the beginning of incubation (Table 1). For the first 15 days, MBC peaked for all treatments with substrate addition, and then gradually declined. Over the 60-day incubation, MBC decreased to 42–68% of initial values for the soils (Figure 3). The largest MBC was observed in treatments with alanine addition.

3.4 | Carbon source partitioning from soil and substrate addition

The amount of mineralized C derived from additions of glucose and alanine peaked at day 3 and then gradually decreased (Figure 4). Most of the CO_2 produced was derived from SOC and peaked at day 3. The CO_2-C produced from alanine was 24–35% more than that from glucose for soils at day 3 of the incubation. The CO_2-C originating from SOC in the alanine treatment was less than for glucose in all soils.

3.5 | Priming effect from substrate additions

The PE was mainly positive for all soils and treatments. The largest PE was for soils with the addition of glucose at day 3 (Figure 5). Compared with glucose and alanine, $(NH_4)_2SO_4$ induced the smallest or even negative PE in all soils. There was no significant difference in the PE between undisturbed and reduced aggregate-size treatments across all soils (Table S2, Supporting Information). Most of the changes in PE between undisturbed and reduced aggregate-size treatments were positive for soils with glucose addition, although the effect of aggregate reduction was not significant. The PE declined regardless of whether or not it was positive or negative for all treatments after day 3. The PCA analysis of selected soil properties explained 61.5 and 29.2% of the total variance for PC1 and PC2, respectively (Figure 6). The separation between individuals on PC1 was controlled by DOC, whereas that on PC2 was associated with MBC.

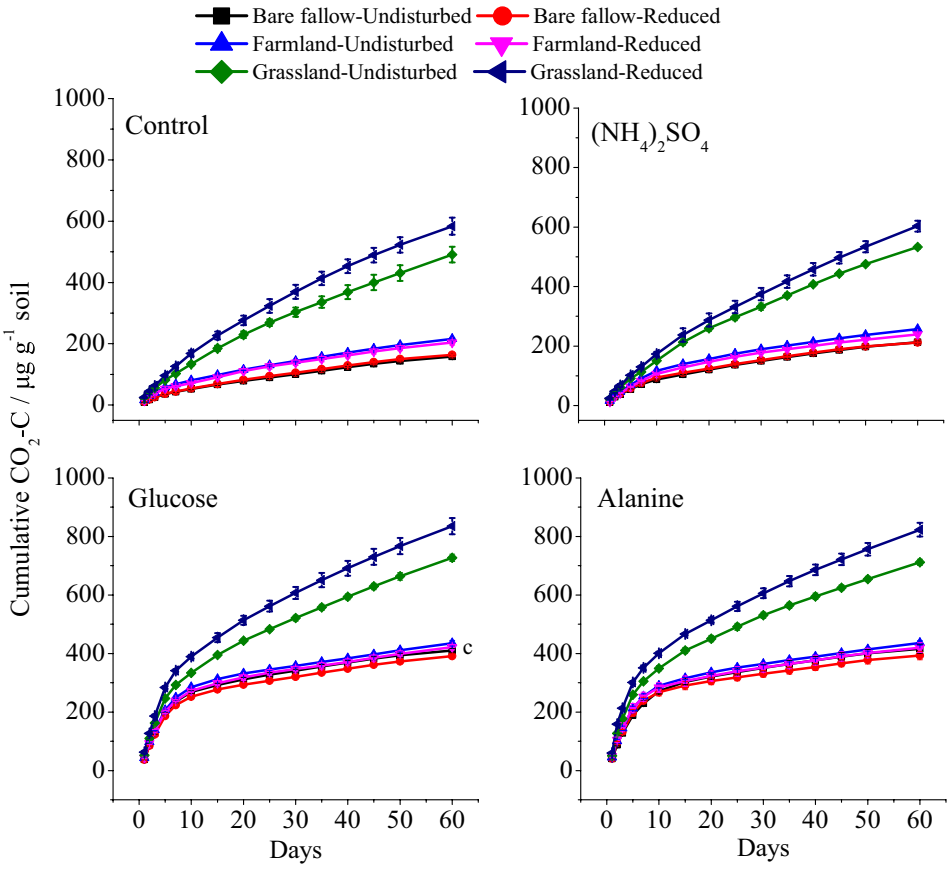

FIGURE 1 Cumulative CO_2-C production over 60-day incubation for bare fallow, farmland and grassland with or without $(NH_4)_2SO_4$, glucose and alanine additions. Means ($n = 3$) and standard errors are shown

4 | DISCUSSION

4.1 | Effect of soil aggregate-size treatment on C mineralization

The abundance and size distribution of soil aggregates affect SOC content and its stability. Bimueller et al. (2016) showed that fine aggregates (<2 mm) contained more SOC than coarse aggregate (2–6.3 mm), with the largest C concentration in aggregate sizes of 0.25–2 mm. The C distribution within aggregates in our study followed this pattern. Tisdall and Oades (1982) suggested that microaggregates <0.25 mm are stabilized at the macroaggregate level by labile SOC sources such as microbial exocellular polysaccharides.

Studies on altering the aggregate-size distribution by comparing crushed with undisturbed aggregates indicated that larger aggregates contain protected labile C (Cambardella & Elliott, 1994). This labile SOC can be mineralized rapidly after the disruption of macroaggregates into smaller aggregates, for example caused by tillage events or soil preparation for incubations. A larger MBC is often associated with soils with larger aggregate-size distributions, as noted in our study. The increase in total CO_2 production following macroaggregate-size reduction also includes C mineralized from the microaggregates, which can also contain smaller amounts of decomposable C (Six & Paustian, 2014). Six, Elliott, and Paustian (2000) reported that the concentration of inter-microaggregate C was two-fold greater in intact macroaggregates than when they were reduced to microaggregates. This is consistent with greater CO_2 production observed in macroaggregates crushed into microaggregates (Elliott, 1986). The protection of this labile C within large macroaggregates demonstrates the spatial protection of SOC pools that affect total C mineralization.

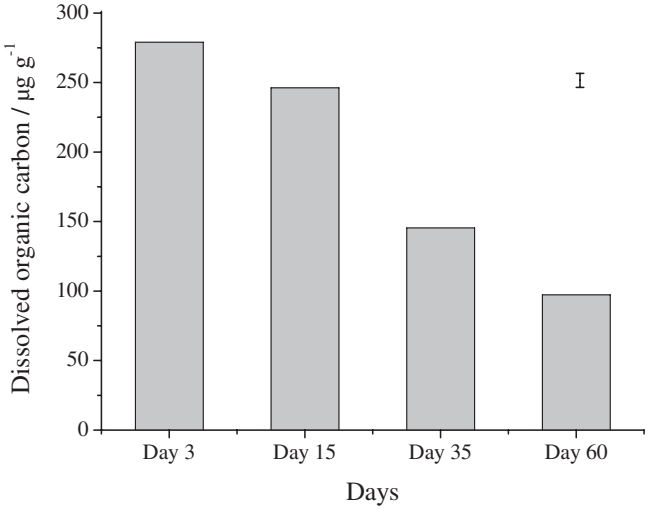

FIGURE 2 Dynamics of dissolved organic carbon over the incubation time. Means were calculated based on $n = 9$, and standard error was determined by the incubation time

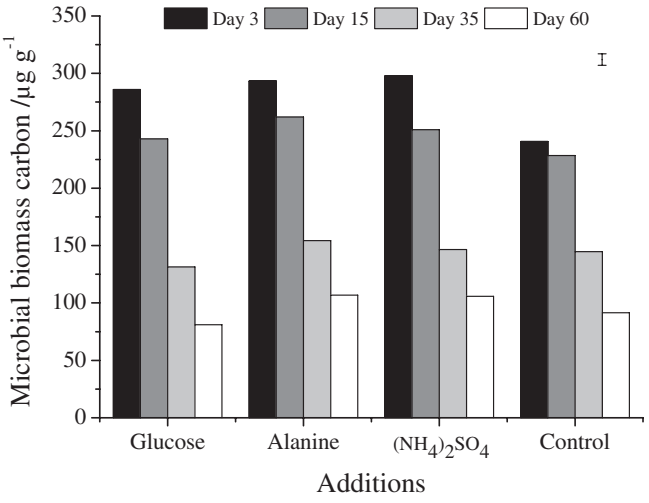

FIGURE 3 Dynamics of microbial biomass carbon over the incubation time. Means were calculated based on $n = 9$, and standard error was determined from the interaction of substrate addition and incubation time

The results in our study were similar where cumulative CO_2 production was larger in the reduced aggregate-size treatment for grassland soil, which had the largest fraction of macroaggregates compared to the other soils. The C to N ratio in the >2.0 mm undisturbed grassland soil was 12.9, which was less than the farmland soil and might have contributed to greater CO_2 production following aggregate-size reduction. There were small differences in total CO_2 produced in bare fallow and farmland soils regardless of aggregate treatment. This can probably be explained by the

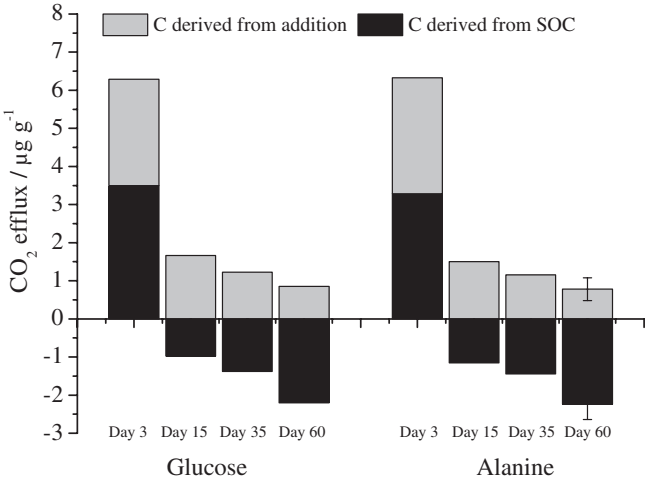

FIGURE 4 Contribution of soil and of addition of C source to the priming effects in CO_2 efflux at days 3, 15, 35 and 60. Treatments included Glu (glucose) and Ala (alanine). CO_2 efflux values were transformed to the natural logarithm. Means were calculated based on $n = 9$, and standard errors were determined from the interaction of substrate addition and incubation time

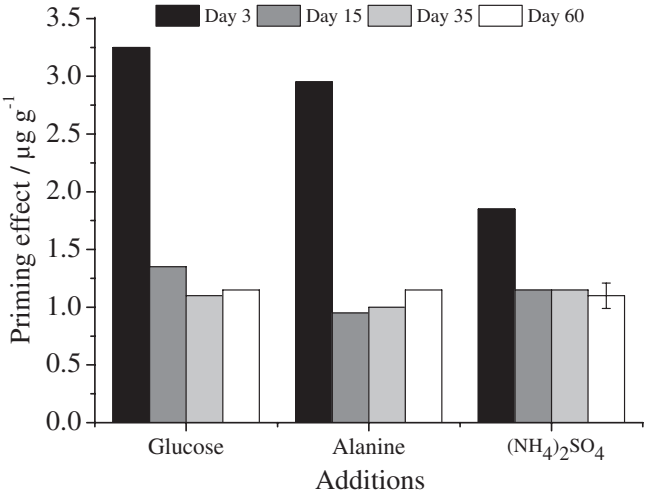

FIGURE 5 Priming effect under substrate addition treatments at days 3, 15, 35 and 60. Priming effect values were transformed to the natural logarithm of the value +3. SE: standard error was determined from the split-plot residual mean squares ($n = 9$)

absence of aggregates >2.0 mm in bare fallow and a smaller amount in farmland soils (20% of grassland soil).

4.2 | Soil microbial biomass and dissolved organic carbon

The mass and quality of C within aggregates of different sizes affects the size of microbial biomass and activity. This results in different rates of C mineralization across aggregate sizes because of the location of labile or bioavailable SOC (Bimueller et al., 2016). For example, Jiang, Wright, Wang, and Li (2011) showed that microbial activity was largest in 1.0–2.0-mm aggregates. The >2.0-mm SOC fraction in the undisturbed grassland soil contained 20% of the total SOC. The grassland soil with a larger macroaggregate fraction had more MBC than farmland and bare fallow soils. Reducing aggregate size had little effect on MBC among soils or aggregate treatments.

As mentioned above, reducing aggregate size in grassland soil increased total C mineralization and is consistent with the release of labile C following the crushing of macroaggregates (Elliott, 1986). The release of labile C might also be partly explained by the turnover of different microbial groups within different sizes of aggregate fractions. Fungi are preferentially located in macroaggregates (Otten et al., 2001) and disrupting them might provide a C source. Bacteria in general have a lower C-use efficiency than fungi (Keiblinger et al., 2010), which might contribute to increased C mineralization as a result of the exposed SOC after aggregate-size reduction. Overall, MBC declined regardless of aggregate treatment in all soils, probably from substrate depletion during the incubation (Moreno-Cornejo, Zornoza, Doane, Faz, & Horwath, 2015).

4.3 | Effects of SOC and substrate additions on CO_2 production

Glucose and alanine are typical components of root exudates and fresh residue inputs that are easily utilized and lead to increased CO_2 production (Blagodatskaya et al., 2007; Kuzyakov & Cheng, 2001; Liu et al., 2017). In our study, the addition 0.4 mg C g^{-1} soil of glucose and alanine increased CO_2 production in all aggregate treatments. Bimueller et al. (2016) found that the addition of 0.0487 and 4.87 mg glucose-C g^{-1} soil with 5% C content significantly increased total C mineralization. Bernal et al. (2016) reported that 0.4 mg glucose-C or alanine-C g^{-1} in soil containing 2.2–3.4% C stimulated soil C mineralization. In contrast, the addition of 0.0204 mg glucose-C g^{-1} soil to soil with 1.2% C content had no effect on total C mineralization (Tian et al., 2015). Our soils had a range of 2.7–5.1% C, with grassland having the largest content. The results suggested that soils with smaller SOC contents responded

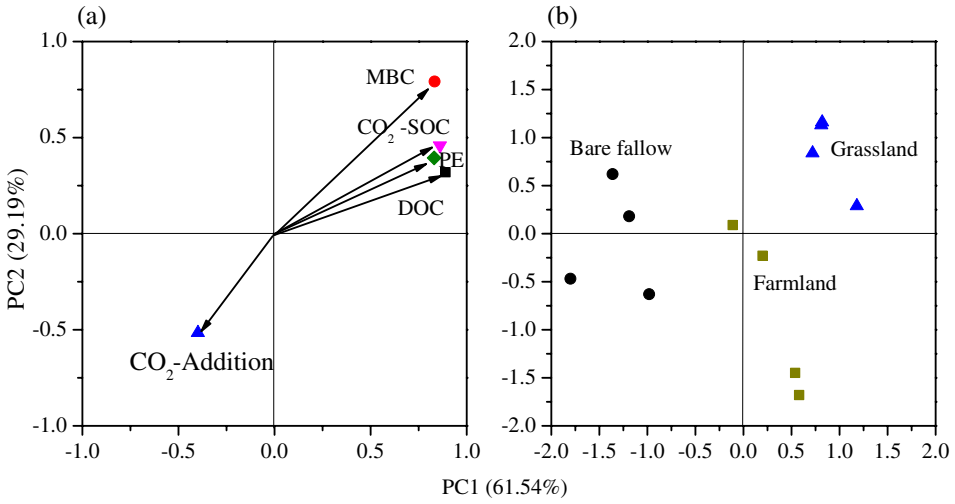

FIGURE 6 Results of the principal component analysis (PCA) on CO_2 produced from soil organic matter (CO_2-SOC), from substrate (CO_2-addition), primed CO_2 (PE), and soil properties DOC (dissolved organic carbon) and MBC (microbial biomass carbon). Plots in the plane of the first two principal components of (a) the eigenvector values and (b) the PC scores of undisturbed and reduced aggregate size for grassland, farmland and bare fallow soils with glucose and alanine addition

less to inputs of C substrate or that total available C was limiting.

4.4 | Priming effect and partitioning of C sources

The input of an available substrate to soil can either accelerate (positive priming) or retard (negative priming) the decomposition of SOC (Cyle et al., 2016). The same added C substrates resulted in different total CO_2 produced among bare fallow, farmland and grassland soils. Soluble substrates often cause a short and pulsed PE of SOC (Miao et al., 2017). We observed a strong PE from the added C substrates (0.4 mg C g^{-1} soil) within 3 days for all treatments and soils (Figure 4). This result is consistent with a strong PE reported during the first 1 to 10 days of typical laboratory incubations by Hamer and Marschner (2005). Typically, substrate additions cause a rapid increase in CO_2 production within 3 days, followed by the incorporation of substrate C into soil microbial biomass by day 7 (Zhang, Ding, Yu, & He, 2013). They also found that longer periods of incubation following additions of substrate can lead to negative PE results, as noted in our study.

The SOC in macroaggregates has a larger concentration in general, but a proportionately smaller contribution to total SOC than the smaller aggregate size. In our study, macroaggregates (> 0.25 mm) contained 2.3 to 7.5 times more C in farmland and grassland soils than that in bare fallow. Regardless of aggregate treatment, the PE was not affected by the substrate additions. This result indicates that the effect of substrate addition was stronger than aggregate-size reduction and disproved our hypothesis that suggested the release of labile SOC from reducing aggregate size would increase the PE. This is somewhat surprising because the total C mineralization in grassland soil was larger following aggregate-size reduction in the absence of C input.

The different substrate additions showed distinctly different PE results across soils, suggesting different substrate utilization patterns or efficiency (Table S2, Supporting Information). The mineralization of glucose was less than that of alanine in all aggregate treatments and soils and showed a larger PE at day 3. Compared with glucose and alanine, the smallest PE was for all the treatments with inorganic N. The addition of inorganic N increased decomposition of relatively recalcitrant forms of SOC when accompanied by sufficient labile organic C (Bernal et al., 2016; Hobbie, 2005).

Studies on the effect of the addition of labile substrates to soil have indicated that it is often related to the alteration of microbial size, community composition and associated metabolic activities (Derrien et al., 2014). The amounts of MBC and DOC increased with C addition in grassland. The result is supported by a PCA analysis, which showed an effect of the amount of DOC and MBC size on PC1 and PC2 in the grassland soil (Figure 5). The separation on PC1 was controlled by DOC, whereas that on PC2 was associated with MBC. Under conditions with limited available C, MBC is often less in the presence of labile substrates and there is an increase in microbial dependence on SOC (Liu et al., 2017; Tian et al., 2016). In contrast, van der Wal and de Boer (2017) suggest that microorganisms prefer to use SOC rather than labile substrates, but the response varies considerably in relation to soil properties. In our study, the grassland soil with a larger and probably more active and diverse microbial community mineralized less of the labile C inputs than the other soils (Table 1). This is consistent with the concept of preferential substrate utilization, where competition for energy and nutrients occurs between microorganisms specializing in the decomposition of labile inputs compared with those decomposing stable SOC (Fontaine, Mariotti, & Abbadie, 2003). The farmland soil showed an intermediate response that was consistent with its intermediate SOC, DOC and aggregate-size distribution compared to the other soils. The bare fallow soil mineralized less SOC, undoubtedly because it had most of its SOC in the mineral-bound fraction of smaller aggregate-size fractions. Keiluweit et al. (2015) suggested that the magnitude and direction of the priming effects are dictated by antecedent quality and quantity of mineral-bound soil organic matter.

5 | CONCLUSIONS

The grassland soil with a larger >2.0-mm aggregate fraction contained more total SOC and more >0.25-mm aggregates, which resulted in even greater C mineralization following aggregate-size reduction. Despite having a smaller >0.25-mm aggregate fraction than the grassland soil, the C mineralization of farmland soil was similar to that of the bare fallow soil, which contained no aggregates >2.0 mm. All soils produced a positive PE following additions of glucose and alanine substrates. There was no effect of aggregate-size reduction on PE for any soil or treatment. There was an interaction between microbial biomass C and dissolved organic C on the PE in the grassland with substrate additions. Overall, the results indicated that reducing aggregate size, for example through soil preparation, affected total CO_2 mineralization in soils with a large aggregate-size fraction, but had little effect on the PE. This suggests that the aggregate effect on PE is an intrinsic soil property that is affected more by simple C substrates such as root exudates or fresh litter than the SOC contained within the different aggregate-size fractions.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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