Aggregate size and their disruption affect ^{14}C -labeled glucose mineralization and priming effect

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ABSTRACT

Soil organic matter (SOM) pools, allocated within various aggregates, are characterized by different degradability and turnover rates that depend on the spatial accessibility of organics and their recalcitrance. Hence, to understand the processes and mechanisms of SOM cycling and stability, the contribution of individual aggregate size classes to the total CO_2 efflux including extra mineralization via priming effect (PE) should be considered. In this study, we determined whether aggregate size classes and their disruption affected the mineralization of SOM and induced PE depending on the primer amount. Soil samples were separated into three aggregate size classes ($>2\text{ mm}$, $2\text{--}0.25\text{ mm}$ macroaggregates and $<0.25\text{ mm}$ microaggregates). Half of the samples within each class were left intact, whereas half were crushed. After the addition of two levels of ^{14}C -labeled glucose, the amount of ^{14}C in CO_2 efflux and microbial biomass were measured several times during the 49-day incubation.

Cumulative SOM-derived CO_2 production from the macroaggregates was 16–21% greater than the CO_2 production from the microaggregates after 49 days. The percentage of glucose mineralized to CO_2 increased with the level of glucose addition, but ^{14}C incorporation into microbial biomass decreased, indicating lower carbon (C) use efficiency at high substrate availability. Aggregate disruption had no effect on the cumulative total and SOM-derived CO_2 production, but it increased glucose mineralization up to 11.2% while the percentage of added glucose incorporated into microbial biomass in macroaggregates decreased. The PE increased with an increased glucose level for the intact aggregates. Aggregate disruption increased the PE in all aggregate sizes under low glucose level. In summary, our findings demonstrate that the aggregate size class has clear effects on C mineralization while their disruption affects the added labile C decomposition and transformation, indicating the relevance of soil structure for SOM cycling in terms of priming and C sequestration.

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1. Introduction

Soil organic matter (SOM) consists of various compounds with varying degradability and turnover rates (Stevenson, 1994; Von Lützow et al., 2007). Hence, to understand the processes and mechanisms of soil carbon (C) cycling and stability, it is necessary to consider the contribution of individual SOM pool to the total CO_2

efflux including extra mineralization via priming effects (PE), i.e., via acceleration (positive priming) or retardation (negative priming) of SOM mineralization by the addition of easily available substrates to the soil (Ohm et al., 2007; Blagodatskaya and Kuzyakov, 2008).

Soil organic matter is protected from microbial decomposition by occlusion within stable microaggregates (Six et al., 1999). Mineralization studies have reported that organic matter associated with macroaggregates ($>0.25\text{ }\mu\text{m}$) was more rapidly mineralized than that associated with microaggregates ($<0.25\text{ mm}$) (Gregorich et al., 1989; Mutuo et al., 2006). This is because the organic matter associated with microaggregates is formed by primary mineral particles coupled together by plant and microbial

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Table 1
Results of analysis of variance on the effect of aggregate size class, glucose level and crushing treatments on Σ total CO₂ and Σ glucose-derived CO₂ production after 3 and 49 days incubation.

Effect	df	$\sum$ total CO ₂ production (mg C g ⁻¹ aggregate)				df	$\sum$ glucose-derived CO ₂ production (% of ¹⁴ C input)			
		1–3 days		1–49 days			1–3 days		1–49 days	
		p value	Contribution(%) ^b	p value	Contribution(%)		p value	Contribution(%)	p value	Contribution(%)
A ^a	2	< 0.0002	2.28	0.0002	23.1	2	< 0.0001	8.01	< 0.0001	15.2
G	2	< 0.0001	91.4	< 0.0001	50.6	1	< 0.0001	70.9	< 0.0001	61.9
C	1	0.0549	0.67	0.442	0.65	1	< 0.0001	12.4	< 0.0001	12.4
A * G	4	0.112	0.82	0.086	9.57	2	0.610	0.05	0.240	0.26
A * C	2	0.301	0.25	0.195	3.69	2	< 0.0001	7.19	< 0.0001	7.74
G * C	2	0.027	0.81	0.161	5.14	1	0.108	0.14	0.034	0.44
A * G * C	4	0.945	0.07	0.247	6.12	2	0.270	0.14	0.920	0.01

Values in bold indicate significant differences.

^a A: aggregate sizes; G: glucose level; C: crushing.

^b The contribution(%) means the percentage of each factor for explaining overall variance.

debris and humified organics which protect SOM against decomposition better than the organic matter associated with macroaggregates (Elliott, 1986; Six et al., 2002; Denef et al., 2007). Labile fractions of SOM are easily affected by PE (Kuzyakov et al., 2000). However, evident PE has also been observed for SOM fractions with low degradability (Hamer and Marschner, 2005). SOM pools are allocated within various aggregates in which they have different degradability and turnover rates. Therefore, we assume that more C will be mineralized and a higher PE will be observed in macroaggregates because the SOM is less recalcitrant and more labile than in microaggregates (Elliott, 1986; Six et al., 2002; Denef et al., 2007), and SOM pools will be involved in PE according to their availability (Blagodatskaya et al., 2011).

The disruption of soil aggregates by management practices release physically protected organic materials, and thus enhances C mineralization (Elliott, 1986; Six et al., 1999). Increases in the amount of readily decomposable C were observed after the disruption of aggregates (Gregorich et al., 1989). By contrast, aggregate crushing did not result in increased CO₂ production from soil under a continuous corn cropping (Drury et al., 2004). Elliott (1986) also did not find evident effects of crushing on CO₂ production from various aggregates under long-term cultivated soils, but they found significant effects in grasslands. These differences may be ascribed to the different characteristics of SOM and aggregates depending on ecosystems and management practices. Generally, little information is available on the effect of the disruption of aggregate size classes on extra C mineralization. Hence, whether aggregate disruption alone increases SOM mineralization and the extent to which the PE will be affected by such disruption remains unknown. We hypothesize that aggregate disruption increases SOM mineralization and the PE because

protected organic matter becomes spatially accessible to micro-organisms.

Generally, a positive correlation between the amount of added substance and its microbial mineralization has been observed (Bremer and van Kessel, 1990; Mary et al., 1993; Schneckenberger et al., 2008). In addition, the PE increases with the increased addition of substrates (Blagodatskaya and Kuzyakov, 2008; Paterson and Sim, 2013). Therefore, we hypothesize that the PE will increase with the amount of added substrates but that the magnitude of the increase may depend on the aggregate size classes.

To test our hypotheses, two levels of uniformly labeled ¹⁴C-glucose were added to intact and crushed aggregates of different size classes. The productions of total, SOM-derived and glucose-derived CO₂ were monitored during 49-day incubation. We determined whether aggregate size classes and their disruption affect the mineralization of SOM and of the added glucose; and if PE varied with the level of added glucose, aggregate size classes and disruption.

2. Materials and methods

2.1. Soil sampling and aggregate preparation

Soil (loamy Haplic Luvisol originated from loess) was sampled in April 2012 from the upper layer (0–10 cm) of a maize field located northwest of Göttingen, Germany (51°33'N and 9°53'E). The soil had the following characteristics: 1.2% SOC content; 0.13% TN content; soil pH (CaCl₂) was 6.0 and bulk density of 1.4 g cm⁻³ (Pausch and Kuzyakov, 2012).

The soil samples were stored in airtight polypropylene bags, placed in a cooler box after sampling, and transported to the laboratory. Once in the laboratory, plant roots and leaves were carefully removed by hand picking, then the soil samples were stored at 4°C for no longer than one week before aggregate processing. Soil was gently manually crumbled to about 5 mm pieces, transferred to two sieves (2 and 0.25 mm), and shaken for 2 min. Thereafter, the aggregates remaining on top of the sieves were collected. Large macroaggregates (>2 mm) were collected from the 2 mm sieve, small macroaggregates from the 0.25 mm sieve, and microaggregates (<0.25 mm) classified as material that passed through the 0.25 mm sieve. Preliminary tests showed that the sieving duration was sufficient to separate the various aggregates size classes while minimizing aggregate abrasion during the sieving process (Dorodnikov et al., 2009). The soil aggregates were then spread out in a thin layer and air dried. Thereafter, subsamples of each aggregate size class were ground using a ball mill for 1 min, and these samples were defined as crushed aggregates.

Table 2
Results of analysis of variance on the effect of aggregate size class, glucose level and crushing treatments on Σ SOM-derived CO₂ production after 3 and 49 days incubation.

Effect	df	Σ SOM-derived CO ₂ (mg C g ⁻¹ aggregate)			
		1–3 days		1–49 days	
		p value	Contribution(%) ^b	p value	Contribution(%)
A ^a	2	<0.0001	14.7	0.0002	23.8
G	2	<0.0001	52.3	0.0088	11.7
C	1	0.066	1.67	0.408	0.76
A * G	4	0.065	5.00	0.082	9.78
A * C	2	0.541	0.60	0.204	3.57
G * C	2	0.004	6.33	0.099	5.26
A * G * C	4	0.674	1.20	0.233	6.33

Values in bold indicate significant differences.

^a A: aggregate sizes; G: glucose level; C: crushing.

^b The contribution(%) means the percentage of each factor for explaining overall variance.

2.2. Incubation and sampling

Soil samples (20 g, dry weight) of the different uncrushed and crushed aggregate size classes were placed into 250 ml jars. The moisture content was adjusted to 50% of the water holding capacity (WHC), and the soil was then pre-incubated at 22 °C for one week. In total, a $3 \times 2 \times 3$ factorial experiment was established corresponding to three levels of aggregate sizes, two levels of aggregate crushing and three levels of glucose addition. The C content was 1.23% in the >2 mm aggregate size, 1.36% in the 2–0.25 mm aggregates size while 1.15% in the <0.25 mm aggregates size before incubation.

The following levels of glucose additions included: (1) without glucose (G0, addition of water only), (2) low glucose addition (GL, corresponding to $20.4 \mu\text{g C g}^{-1}$ soil), and (3) high glucose addition (GH, corresponding to $204 \mu\text{g C g}^{-1}$ soil). The added glucose was prepared from unlabeled glucose mixed with uniformly labeled ^{14}C -glucose (99530 DPM per jar) and water (G0). The appropriate glucose solutions were then added to the different pre-incubated soils. The soil then reached a final soil moisture content of 70% WHC. The low glucose concentration was approximately 10% of the microbial biomass C of the sieved bulk soil, whereas the high level reached approximately 100%.

Small vials with 3 ml of 1 M NaOH were placed in the incubation jars to trap CO_2 after adding distilled water (G0 treatment) or the glucose solution (GL and GH treatments) to the soil. The jars were immediately sealed air-tight and were incubated for 49 days at 22 °C and at 70% WHC. The NaOH solution was exchanged 6 h, 1, 3, 7, 12, 21, 36 and 49 days after glucose addition. After changing NaOH solution each time, the incubation jars were kept open for 30 min to maintain adequate O_2 level. In addition, three incubation jars containing only the NaOH were used as blanks to correct for the CO_2 trapped from the air inside the vessels. Three replicates of each treatment were destructively sampled at days 3, 14 and 49 (36 incubation jars per sampling date) to analyze the microbial biomass C and dissolved organic C.

2.3. Chemical analyses

The amount of trapped CO_2 was determined by the titration of 1 ml of the NaOH solution with 0.1 M HCl against phenolphthalein after adding 1 ml of 0.5 M BaCl_2 solutions. Another 1 ml of the NaOH solution was added to 6 ml Rothscint scintillation cocktail (Roth Company, Germany), and well mixed by vortex. After decay of chemiluminescence (after 24 h), the ^{14}C activity was determined by a liquid scintillation counter (LS6500 Multi-Purpose Scintillation Counter, 217 Beckman, USA). The ^{14}C counting efficiency was approximately 92% and the ^{14}C activity measurement error did not exceed 2%.

The soil microbial biomass C was determined by chloroform fumigation extraction. The procedure (Wu et al., 1990; Vance et al., 1987) was adapted according to Malik et al. (2013). Briefly, a non-fumigated control consisting of 10 g of moist soil was shaken with 40 ml of 0.05 M K_2SO_4 for 1 h at 200 rev min^{-1} , centrifuged at $3000 \text{ rev min}^{-1}$ for 10 min, and filtrated. An additional 10 g of moist soil was fumigated with chloroform for 24 h, then the chloroform was removed and the soil was extracted as described for the control. The soil extracts were passed through a $0.45 \mu\text{m}$ membrane filter and the dissolved organic C concentration in the extracts was measured with a TOC/TIC analyzer (Dimatec, Essen, Germany). The soil water content was determined in another 10 g soil, which was dried at 105 °C. Microbial biomass C was calculated by taking the difference between the C content of the fumigated and the non-fumigated soil and dividing by a K_{EC} factor of 0.45 and is presented as percent of dry soil. The extract from the non-fumigated soil was used as a measure of the dissolved organic C. After pre-incubation, three replicates of each treatment were destructively sampled to analyze microbial biomass C and dissolved organic C. The ^{14}C activities of microbial biomass C and dissolved organic C were measured in 3 ml aliquots added to 6 ml Rothscint scintillation cocktail (Roth Company, Germany) after the decay of chemiluminescence using the liquid scintillation counter (see above).

2.4. Calculations and statistics

The SOM-derived CO_2 was calculated as follows:

$$\text{SOM-derived CO}_2 = \text{total CO}_2 - \text{Glucose-derived CO}_2$$

The glucose-derived CO_2 ($\text{CO}_{2\text{glucose}}$) was calculated according to the specific ^{14}C activity of the added glucose ($^{14}\text{C}_{\text{glucose}}$, DPM), where $^{14}\text{CO}_{2\text{glucose}}$ is the activity measured in the CO_2 efflux (DPM):

$$\text{Glucose-derived CO}_2 = \text{C}_{\text{glucose}} \times ^{14}\text{CO}_{2\text{glucose}} / ^{14}\text{C}_{\text{glucose}}$$

Glucose mineralization was calculated as a percentage of the initial input ^{14}C activity. The

glucose-C incorporated into microbial biomass C and dissolved organic C was also expressed as a percentage of the initial input ^{14}C activity.

We use $\sum \text{total CO}_2$, $\sum \text{glucose-derived CO}_2$ and $\sum \text{SOM-derived CO}_2$ to represent the cumulative CO_2 from three sources after several days' incubation in this study.

The priming effects are expressed as the difference of the SOM-derived CO_2 from soil with glucose addition and CO_2 from soil without glucose addition (Blagodatskaya et al., 2007).

$$\text{PE } (\mu\text{g C g}^{-1} \text{ soil}) = \text{SOM-derived CO}_{2\text{amended}} - \text{SOM-derived CO}_{2\text{unamended}}$$

Table 3

Production of $\sum \text{total CO}_2$ and $\sum \text{SOM-derived CO}_2$ after 49 days across aggregate size classes, crushing and glucose amendment treatments.

Treatments	$\sum \text{total CO}_2$ (mg C g^{-1} aggregate)	$\sum \text{total SOM-derived CO}_2$ (mg C g^{-1} aggregate)	$\sum \text{total SOM-derived CO}_2$ (% of SOC in aggregate)
>2 mm	0.72(0.02)a	0.68(0.02)a	5.11(0.22)a
2–0.25 mm	0.79(0.03)a	0.74(0.04)a	4.90(0.30)a
< 0.25 mm	0.59(0.02)b	0.54(0.02)b	4.22(0.11)b
G0	0.63(0.01)b	0.63(0.02)b	4.47(0.10)b
GL	0.65(0.02)b	0.64(0.02)b	4.54(0.23)b
GH	0.85(0.03)a	0.74(0.05)a	5.22(0.17)a
Intact	0.72(0.02)a	0.68(0.03)a	4.82(0.31)a
Crush	0.75(0.03)a	0.70(0.02)a	4.66(0.31)a

Numbers in bracket represent standard error of the means ($n=3$); G0: without glucose addition, GL: low glucose addition, GH: high glucose addition; different low-case letters indicate significant differences among across aggregate size classes, crushing or glucose amendment treatments.

where SOM-derived $\text{CO}_{2\text{amended}}$ was SOM-derived CO_2 efflux from the jar with glucose addition, SOM-derived $\text{CO}_{2\text{unamended}}$ production from the jar without glucose addition.

All measurements were performed in triplicate. Significant differences ($p < 0.05$) among treatments were determined using analysis of variance (ANOVA) with SAS.

3. Results

3.1. Total and SOM-derived CO_2 efflux

The $\sum$ total CO_2 efflux during the first 3 days was mainly affected by level of glucose addition. This effect declined with the time. By contrast, the effect of aggregate sizes on the $\sum$ total CO_2 emission increased by a factor of 10 after 49 days compared with 3 days (Table 1). The $\sum$ total CO_2 production after 49 days was 21–23% higher in the macroaggregates (>0.25 mm) than in the microaggregates (<0.25 mm) ($p < 0.05$; Table 3). Aggregate crushing did not affect the $\sum$ total CO_2 efflux for any of the aggregate size classes and also across aggregates sizes and glucose amendment treatments during the experiment ($p > 0.05$; Table 3).

The effects of aggregate size and glucose level accounted for 15% and 52% variations in the $\sum$ SOM-derived CO_2 production after 3 days, respectively, while for 24% and 12% after 49 days, respectively (Table 2). The $\sum$ SOM-derived CO_2 production followed a similar trend as the $\sum$ total CO_2 efflux (Table 3).

3.2. Glucose-derived CO_2 efflux

The level of glucose addition dominated the differences in $\sum$ glucose-derived CO_2 production during the first 3 days, accounting for 71% variation (Table 1). The effects of aggregate size and glucose level, accounted for 15% and 62% variations in the $\sum$ glucose-derived CO_2 production after 49 days, respectively (Table 1). The $\sum$ glucose-derived CO_2 production increased with glucose addition ($p < 0.05$; Fig. 1). When considering the average of intact and crushed aggregates, the $\sum$ glucose-derived CO_2 production was 26% higher in the GH treatment than in the GL treatment after day 3, while 14% higher after day 49 ($p < 0.05$; Fig. 1). The maximal mineralization rate occurred before the first 6 h for the GL, and it was between 6 h and 24 h at the GH level (Fig. 1).

Aggregate crushing caused significantly more $\sum$ glucose-derived CO_2 over 49 days in macroaggregates (>2 mm and 0.25 – 2 mm) at both levels of glucose addition ($p < 0.05$; Fig. 1). By contrast, aggregate crushing had no effect on $\sum$ glucose-derived CO_2 production over 49 days in microaggregates at either level of glucose addition ($p > 0.05$; Fig. 1).

3.3. Microbial biomass C and dissolved organic C

The initial microbial biomass C content was 38% higher in the macro- than in the micro-aggregates at day 0 (before glucose addition, Fig. 2). A rapid decline of microbial biomass C without

Fig. 1. Cumulative ($\sum$ glucose-derived CO_2) and rate of glucose CO_2 -C production over the incubation in individual aggregate sizes (>2 mm, 2 – 0.25 mm, <0.25 mm), crushing and glucose amendment (GL, GH) treatments. Error bars represent standard error of the means ($n = 3$); GL: low glucose addition, GH: high glucose addition. Different low-case letters indicate significant differences among treatments within an aggregate size on each sampling day.

Fig. 2. Changes in microbial biomass C and dissolved organic C over the incubation in individual aggregate size (>2 mm, 2–0.25 mm, <0.25 mm), crushing and glucose amendment (GL, GH) treatments

Error bars represent standard error of the means ($n = 3$); GL: low glucose addition, GH: high glucose addition. Different low-case letters indicate significant differences among treatments within an aggregate size on each sampling day.

glucose addition was observed during the first 3 days for all three aggregates size classes ($p < 0.05$) and thereafter it leveled off. By contrast, the microbial biomass C generally increased in the treatments with glucose addition (GL and GH) from day 0 to day 3, and increased further by day 14 in the high glucose addition. Thereafter, the microbial biomass C gradually declined (Fig. 2). The microbial biomass C content in all aggregate size classes was always lower under G0 treatments as compared with treatments with glucose addition (GL and GH) starting from day 3 to day 49 ($p < 0.05$, Fig. 2).

When compared with the G0 control, glucose addition (GL and GH) decreased the dissolved organic C content at day 3 as much as 56% ($p < 0.05$; Fig. 2). The dissolved organic C showed a slight decrease to 49 days in the G0 control, while it slightly increased from 14 to 49 days in the GH treatment.

3.4. Glucose incorporation into microbial biomass and dissolved organic matter

The percentage of glucose-C incorporated into microbial biomass decreased from day 3 to 14, except in intact >2 mm and <0.25 mm under GL level ($p < 0.05$; Fig. 3). The percentage of glucose-C incorporated into microbial biomass decreased with an increase in the glucose level for all the intact and crushed aggregate sizes during the experiment ($p < 0.05$; Fig. 3). Aggregate crushing decreased glucose-C incorporation into microbial biomass in macroaggregates (Fig. 3). More glucose-C (27%) was incorporated into microbial biomass in intact >2 mm aggregates

than in crushed aggregates at day 14 ($p < 0.05$). ^{14}C incorporation into microbial biomass was always higher for the intact medium aggregates (2–0.25 mm) than for the crushed aggregates ($p < 0.05$), except at day 49 for the GH treatment. There was no effect of crushing on glucose-C incorporation into microbial biomass in microaggregates.

The glucose-derived ^{14}C in dissolved organic matter varied between 0.23% and 0.9% of the ^{14}C input, and it decreased with time (Fig. 4). In contrast to ^{14}C in the microbial biomass, there was no significant difference of ^{14}C in the dissolved organic C between the different glucose levels and intact and crushed aggregates except for day 14, where the crushed aggregates with high glucose addition showed higher ^{14}C values among all size classes.

3.5. Priming effect after 49 days of incubation

The PE was mainly positive after 49 days for all treatments, except for intact 2–0.25 mm and <0.25 mm aggregate size classes with low glucose addition where a negative PE was measured (Fig. 5). The PE increased with an increased glucose level for the intact aggregates ($p < 0.05$; Fig. 5). For the intact samples, the PE was higher in the size >2 mm than in the 2–0.25 mm and <0.25 mm under GL level. The PE was lowest in microaggregates (<0.25 mm) under GH level. The PE was independent on aggregates sizes after aggregate crushing.

Aggregate crushing increased the PE in all aggregate sizes under GL level ($p < 0.05$; Fig. 5). By contrast, the PE decreased in macroaggregates (>0.25 mm) under GH level after crushing.

Fig. 3. Changes in the percentage of glucose-derived C in microbial biomass C over the incubation in individual aggregate size (>2 mm, 2–0.25 mm, <0.25 mm), crushing and glucose amendment (GL, GH) treatments. Error bars represent standard error of the means ($n = 3$); GL: low glucose addition, GH: high glucose addition. Different low-case letters indicate significant differences among treatments within an aggregate size on each sampling day.

4. Discussion

4.1. Total and SOM-derived CO_2 efflux

The level of glucose had the greatest effect on the $\sum$ total CO_2 production during the experiment, independent of the aggregate sizes (Tables 1 and 2). The production of $\sum$ SOM-derived CO_2 was higher under high versus low glucose level (Table 3). This increase of $\sum$ SOM-derived CO_2 production was ascribed to a higher microbial biomass (Fig. 2) and possibly to more extracellular enzymes produced after the glucose addition, which resulted in more rapid mineralization of the substrate and breakdown of the native soil organic C (Degens and Sparling, 1996).

When the easily available glucose was consumed, the starving microorganisms switched to utilize SOM according to SOM accessibility in various aggregates fractions (Dungait et al., 2012). Thus, the contribution of aggregate sizes to the $\sum$ total

CO_2 emission increased by a factor of 10 after 49 days as compared with 3 days (Table 1). The observed increase of $\sum$ SOM-derived CO_2 in the macro- versus in the micro-aggregates after 49 days across both intact and crushed aggregate sizes (Table 3), is in agreement with previous studies, which reported that micro-aggregates provided the most protection to the associated SOM (Elliott, 1986; Six et al., 1999, 2002; Denef et al., 2007; Kimura et al., 2012). By contrast, several studies showed that CO_2 production was greatest from the smallest aggregates (Seech and Beauchamp, 1988; Drury et al., 2004; Sey et al., 2008) or that there was no difference between macro- and micro-aggregates (Rabbi et al., 2014). The observed inconsistency among previous studies on the effects of aggregate sizes on mineralization may be due to differences in the organic C content or the microbial community composition in various soils (Kimura et al., 2012). In addition, macro- and micro-aggregates in the same soil could have similar chemically stable SOC (Rabbi et al., 2014).

Fig. 4. Changes in the percentage of glucose-derived C in dissolved organic C over the incubation in individual aggregate size (>2 mm, 2–0.25 mm, <0.25 mm), crushing and glucose amendment (GL, GH) treatments. Error bars represent standard error of the means ($n = 3$); GL: low glucose addition, GH: high glucose addition. Different low-case letters indicate significant differences among treatments within an aggregate size on each sampling day.

Contrary to our hypothesis, aggregate crushing did not increase the $\sum$ total CO_2 and $\sum$ SOM-derived CO_2 production (Tables 2 and 3). Mineralization studies of crushed versus intact aggregates indicated that aggregate-protected pools are more labile than unprotected pools because protected pools are less exposed to microbial decay (Cambardella and Elliott, 1994). Our observation contradicts previous studies that reported an increase in readily decomposable C after aggregate disruption (Gregorich et al., 1989; Hassink, 1992). Similar to our study, Elliott (1986) did not find evident effects of crushing on CO_2 production from various aggregates under long-term cultivated soils, but they found significant effects in native soils. Drury et al. (2004) reported that aggregate crushing did not result in increased CO_2 production from soil under continuous corn cropping. These differences may be ascribed to the different characteristics of SOM and aggregates

depending on ecosystems and management practices. Hence, to better understand processes and mechanisms of how aggregate disruption affect SOM cycling and stability, it is necessary to conduct more specific climate/soil/crop systems studies in order to draw field site-specific conclusions in future.

4.2. Glucose mineralization and incorporation into labile organic C fractions

Within 3 days, 27–42% of the added glucose was mineralized, and up to 60% of the glucose was mineralized within 49 days. These results are comparable with previous studies (Saggar et al., 1999; Ohm et al., 2007; Hill et al., 2008). High glucose addition increased the percentage of glucose-C mineralized to CO_2 (Fig. 1), but it decreased the proportion of the

Fig. 5. Priming effect in individual aggregate size (>2 mm, 2–0.25 mm, <0.25 mm), crushing and glucose amendment (low GL, high GH) treatments after 49 days. Error bars represent standard error of the means ($n=3$). Different low-case letters indicate significant differences among all treatments.

added glucose incorporated into microbial biomass, compared to the low glucose level (Fig. 3). Such a short-time effect can be explained by the switch from substrate limitation of CO_2 production in GL treatment (glucose-C corresponded to 10% of microbial C) to a limitation by the amount of microbial biomass in GH (glucose-C corresponded to 100% of microbial C) (Schneckenberger et al., 2008). Over a long-term, however, the increased proportion of mineralized ^{14}C -glucose along with the low proportion of added glucose incorporated into microbial biomass in high versus low glucose treatments may indicate an increase in microbial turnover (Blagodatskaya et al., 2011) and decreased C use efficiency (CUE) (Creamer et al., 2014). The CUE was defined as the ratio of the glucose-derived biomass C to the sum of the glucose-derived biomass C and the Σ glucose-derived CO_2 (Herron et al., 2009). Previous studies have reported that the resource use efficiency increases with rising C limitations (Nguyen and Guckert, 2001; Bremer and Kuikman, 1994). In this study, we found that the CUE was 1.4–2.0 times greater in the lower versus higher glucose treatments in individual aggregate sizes and across all aggregates size classes (Supplementary Table 1).

Aggregate disruption increased glucose mineralization in macroaggregates (Fig. 1), but decreased the percentage of added glucose incorporated into microbial biomass (Fig. 3). In theory, such results were not unexpected considering the fact that the smaller particles would result in a higher surface to volume ratio; therefore, there should be more glucose-derived CO_2 from microaggregates after crushing. However, we observed increased glucose mineralization in macroaggregates after crushing, but not in microaggregates. The higher percentage of glucose mineralization, but lower percentage of added glucose incorporated into microbial biomass, may also indicate the reduced CUE after aggregate disruption. The CUE was observed 12.6–25.1% significantly higher in intact than in crushed macroaggregates (across low and high glucose treatments) (Supplementary Table 1). Previous studies reported that the ratio of fungal:bacterial biomass has been shown to be particularly sensitive to soil disturbance, with lower ratios associated with increased intensity of cultivation (Bailey et al., 2002; Six et al., 2006). It is likely that fungal mycelia were more easily disturbed and that the bacteria were unaffected after aggregate disruption. Therefore, crushing may lead to a lower fungal:bacterial ratio with decreased fungal and increased bacterial abundance. Assuming that the fungal population metabolizes the substrate more efficiently than bacteria (Otten et al., 2001; Keiblinger et al., 2010), we suppose that crushing

decreased CUE by altering the soil microbial community structure. The decreased CUE after crushing was mainly pronounced in macroaggregates where the fungi presumably occur (Guggenberger et al., 1999; Zhang et al., 2014). Similarly, we also found that aggregate crushing did not affect the CUE (≈ 0.45) in microaggregates in this study (Supplementary Table 1).

4.3. Priming effect

The PE increased by approximately 50–210% at the high than at the low glucose level in all intact aggregates after 49 days incubation (Fig. 5), indicating a stronger stimulation of microbial biomass by higher primer amounts. This result is consistent with the view that when the added substrate C was lower than 15% of the microbial biomass C, the PE was lower than the PE induced when the substrate C accounted for 50–200% of the microbial biomass C (Blagodatskaya and Kuzyakov, 2008). The higher PE for intact larger macroaggregates (>2 mm) versus microaggregates (Fig. 5) indicated the macroaggregates may have more decomposable SOM as well as more microbial biomass (Fig. 2). Greater microbial biomass in macroaggregates versus microaggregates is consistent with results from previous studies (Gupta and Germida, 1988; Guggenberger et al., 1999).

The PE decreased in macroaggregates (>0.25 mm) after aggregate crushing under high glucose level (Fig. 5). This result may be partly explained by the decreased role of fungi due to disruption of fungal mycelia by crushing, assuming that fungi are important PE drivers (Fontaine et al., 2011) and that fungi occur mainly in macroaggregates (Guggenberger et al., 1999; Zhang et al., 2014). In addition, the bacteria may switch from decomposing hardly utilizable substrates such as SOM to metabolizing the added glucose (preferential substrate utilization) (Paterson et al., 2007; Kuzyakov, 2010). Altogether, we observed a decreased PE in macroaggregates under GH level. However, under GL level, the role of fungi for decomposing SOM was also decreased because fungal mycelia were more easily disturbed after aggregate crushing. However, the added glucose was not sufficient for sustaining bacteria growth (preferential substrate utilization was not possible), but it was enough to stimulate bacteria to decompose SOM, especially *r*-strategists (Blagodatskaya et al., 2007; Nottingham et al., 2009). Thus, we observed an increased PE in all aggregates sizes under GL level after aggregate crushing (Fig. 5). However, as reviewed by Kuzyakov (2010), the relationship between PE mechanisms and microbial community structure requires further studies.

5. Conclusions

The Σ total CO₂ production mainly depended on the amount of added glucose and aggregate size classes. High glucose addition increased Σ total CO₂, Σ glucose-derived CO₂ and Σ SOM-derived CO₂ production, but decreased the percentage of glucose-C incorporated into microbial biomass compared with the low glucose addition. The Σ SOM-derived CO₂ production was 16–21% greater from the macro- than from the micro-aggregates. The PE increased with an increase in glucose levels for all of the intact aggregates, showing stronger stimulation of microbial biomass by greater primer amounts. Aggregate crushing had no effect on total and Σ SOM-derived CO₂ production; however, it increased glucose mineralization and decreased the percentage of glucose incorporated into microbial biomass. Aggregate crushing increased the PE in all aggregates sizes under GL level. By contrast, there was a decreasing PE trend in macroaggregates (>0.25 mm) after aggregate crushing under GH level.

To sum up, our results indicate that soil aggregate size class has clear effects on C mineralization, whereas aggregate disruption affects the added labile C decomposition and priming effect. Future studies are required to integrate how the soil microbial community structure and PE changes after aggregate crushing.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.apsoil.2015.01.014>.

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