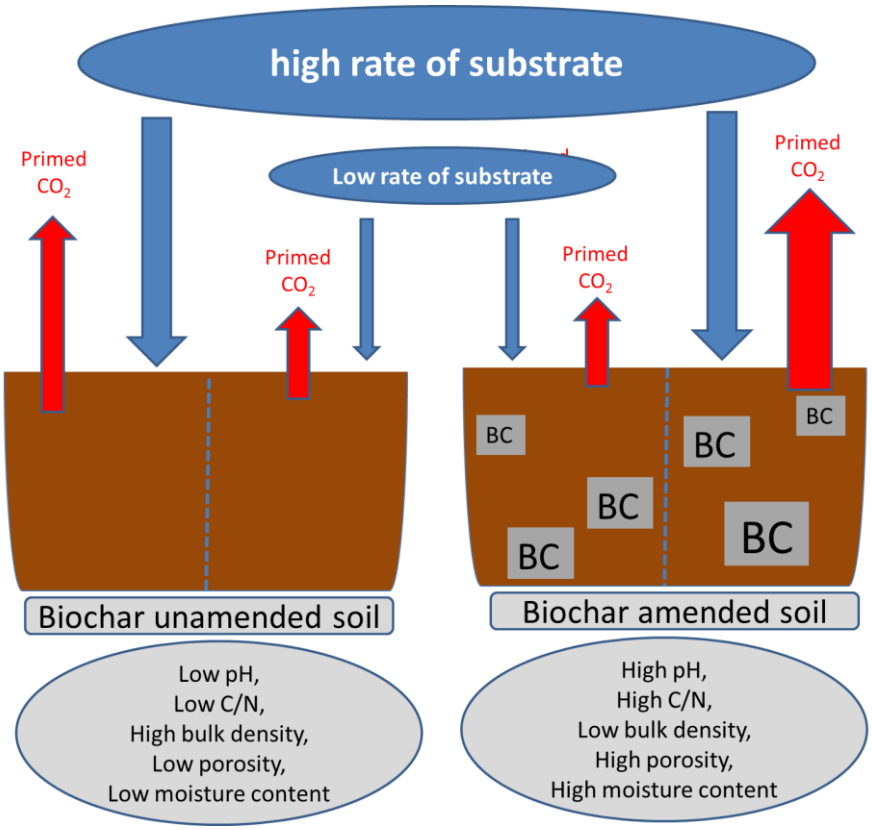
Graphical Abstract



Priming effects in biochar enriched soils using a three-source-partitioning approach: ^{14}C labelling and ^{13}C natural abundance

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Abstract

The changes to soil properties due to biochar addition may affect both the direction and magnitude of priming effects. However, the mechanisms involved in biochar induced priming effects still remain largely unknown due to the limitation of methods to separate more than two carbon (C) sources (e.g. soil, biochar, substrate). We combined ^{14}C labeling with ^{13}C natural abundance to separate the total CO_2 from i) native soil organic C (SOC, C_3 signature), ii) added glucose (^{14}C labeled) and iii) biochar (C_4 signature).The primed soil CO_2 emissions following a large addition of glucose (1000 mg glucose kg^{-1} soil) to one Chinese and one German Luvisol soil were much larger (140% and 53% respectively) in a soil recently amended with maize derived biochar (pyrolyzed at 400°C), compared to non amended soil. Glucose addition at a lower rate (100 mg C kg^{-1} soil) produced no significant differences in priming effects of native soil organic matter between the biochar amended and non-amended soils. Glucose also caused priming of biochar decomposition, with an additional C_4 biochar loss of between 270 µg CO_2-C g^{-1} and 540 µg CO_2-C g^{-1} depending on soils and glucose concentrations. Approaches using two stable isotopes (^{13}C and^{12}C) have previously been limited to partitioning two sources (biochar C and soil organic C). Here, for the first time, ^{14}C labeling was combined with ^{13}C natural abundance to partition three C sources in a biochar amended soil. By partitioning soil CO_2 emissions derived from SOC, from added biochar and glucose decompositions, this study provides a better understanding of the priming effects following addition of substrates to biochar amended soil, to approximate to the true complexity of biochar enriched soils.

Key words: *biochar, priming effect, three C sources, ^{14}C labelling, ^{13}C natural abundance*

1. Introduction

The short term increases or decreases in the mineralization of soil organic C caused by the addition of organic substrates are known as positive or negative priming effects (Kuzyakov et al., 2000). Previous studies showed that added biochars could induce either positive or negative priming effects (Luo et al., 2011; Zimmerman et al., 2011). Negative priming effects were attributed to the physical protection of soil organic matter (Maestrini et al., 2014b). Singh et al. (2014) showed that, at 10 months following biochar addition to soil, about 70% was recovered in the free light fraction, while 20% was in the aggregate-occluded and 6% in the mineral associated fraction. The protection of soil organic C from mineralization might be due to direct adsorption on biochar surfaces or organo-mineral associations promoted by biochar (Singh and Cowie, 2014). However, some studies indicated that physical protection through soil aggregation might be weakly linked to biochar induced priming effects (Kerre et al., 2016). Positive priming effects occur in the early stages following biochar incorporation, and are reported to be over quite rapidly in many studies (Jones et al., 2011a; Maestrini et al., 2014a; Whitman et al., 2014; Zhao et al., 2013; Zimmerman et al., 2011). These short term positive priming effects, due to increased mineralization of soil organic C, are largely attributed to stimulation of microbial activity by the labile C contained within the biochar, or abiotic release of CO_2 from carbonates in the ash (Maestrini et al., 2014b; Smith et al., 2010; Zimmerman et al., 2011). The positive priming effects observed following the addition of biochar are of similar magnitude (between 50 - 500 µg C g^{-1} soil) to other priming effects following the addition of labile organic C, which stimulates microbial activity in the short term (Jones et al., 2011a; Zhao et al., 2013). Thus, biochar induced priming effects are generally believed to be either positive or negative in the short term while mainly negative after labile C has been utilized in the long term.

After the short term mineralization of bio-available C from the biochar, the effects of biochar on soil properties, e.g. water-filled pore spaces, habitat, soil aeration, moisture, pH and nutrient availability may persist during the medium (1 year) to long term (above 3 years) period). In addition to environmental factors, e.g. temperature

77 (Benbi et al., 2014; Fang et al., 2014), soil priming effects can also be strongly
78 affected by soil properties, e.g. soil pH, aggregate stability, organic matter and
79 nutrient availability (Blagodatskaya and Kuzyakov, 2008). For example, a highly
80 significant positive correlation between soil primed CO_2 (expressed as a percent of
81 added C) and soil pH (range 3 to 8) was found by Blagodatskaya and Kuzyakov
82 (2008). As soil properties might be changed due to biochar addition, the soil organic C
83 mineralization in biochar free soil and biochar amended soil may respond differently
84 to added substrates. Thus, in addition to the short term effects of biochar on soil
85 organic C mineralization, following addition of substrate to the newly established
86 biochar soil system, the direction or magnitude of the soil priming effect might differ
87 due to the changes in soil properties caused by the biochar.
88 The first report on biochar causing soil priming effects was criticized because
89 isotopic labelling was not used. It was therefore not possible to separate the different
90 SOM pools involved (Lehmann and Sohi, 2008; Wardle et al., 2008). Since then,
91 stable isotope techniques have been used to distinguish between the mineralization of
92 biochar and soil organic C mineralization in many studies (Jones et al., 2011a;
93 Maestrini et al., 2014a; Maestrini et al., 2014b; Zimmerman et al., 2011). Until now,
94 most studies designed to investigate biochar induced soil priming effects have used
95 two approaches: 1) addition of unlabeled biochar to unlabeled soil (Wardle et al.,
96 2008), and 2) addition of ^{13}C (Jones et al., 2011b; Zimmerman et al., 2011) or ^{14}C
97 labeled biochar to unlabeled soil (Kuzyakov et al., 2009). However, quantitatively
98 partitioning systems using two stable isotopes (^{13}C and^{12}C) have been limited to only
99 two sources (biochar C and soil organic C). Recently there has been increasing
100 interest in the effects of biochar on priming effects involving three C sources e.g. soil,
101 biochar and plants(Weng et al., 2015; Whitman et al., 2014). Whitman et al. (2014)
102 partitioned the CO_2-C evolved from three sources of biochar, plant root exudates and
103 soil organic C using only two isotopes, by assuming an extreme scenario whereby
104 only one C source was mineralized from the combined C sources of biochar plus
105 root exudates, which gave the upper (root exudates) and lower (biochar) values of the
106 mineralization of C4 materials and soil organic C mineralization. Whitman and

Lehmann (2015) introduced a dual-isotope approach to partition soil CO_2 emissions derived from soil organic C, added biochar and root respiration. However, it still remains challenging for three C sources to be separated by traditional approaches using only two stable isotopes (^{13}C and ^{12}C). More complex methodological approaches which are able to discern three C sources in biochar enriched system would provide a much needed and valuable research tool in future research (Weng et al., 2015; Whitman et al., 2014).

Based on the biochar induced changes in soil properties, e.g. pH increase, aeration, moisture dynamics, C availability, nutrients status, our hypothesis is that soil organic C mineralization may respond differently to the addition of fresh organic C in unamended and biochar-amended soils. Our main aim was to test the possibility of adopting the three-source-partitioning approach by combining ^{14}C labelling with ^{13}C natural abundance in soil with three C sources (native soil organic C, glucose-C and biochar-C). The second aim was to investigate the differences in amounts of primed CO_2 following the addition of ^{14}C glucose to an unamended (soil and glucose) and biochar amended (soil, glucose and biochar) soil.

2. Materials and methods

2.1 Soils, biochar and glucose addition

Soils were sampled (0-23 cm) with a Dutch auger, from Zhejiang province, China (Soil A) and north-west of Gottingen, Germany (Soil B). Both soils were classified as Luvisols according to World Reference Base (WRB) system with soil classification scheme. The two soils were analyzed in different labs but with identical procedures. The soil samples were hand-picked to remove obvious plant debris and roots, sieved at field moisture (<2 mm) and subsequently adjusted to 40% of water holding capacity (WHC). Soil pH was measured at a soil:$CaCl_2$ ratio of 1:2.5 (weight/weight). Air-dry soil (10 g, <2 mm) and 25 ml of $CaCl_2$ (0.01 M) were shaken together for 1 min and left to settle for 30 min, which was repeated once more before pH was determined with a pH electrode. Total C and N of the soils (air-dried, milled <200 μm)

were determined by dry combustion (LECO CNS 2000, LECO Corporation, Michigan, USA). The natural $\delta^{13}C$ (‰) abundance of the soils (air-dried, milled <200 μm) was determined on an elemental analyser-isotope ratio mass spectrometer (Sercon Ltd, Crewe, UK). All measurements are given on an oven-dry weight basis (o.d., 105 °C, 24 h). Soil A had a pH ($CaCl_2$) of 7.0, a bulk density of 1.3 g cm^{-3}, an organic C content of 60.5 g kg^{-1}, a total N content of 3.8 g kg^{-1} and a $\delta^{13}C$ value of -26.7 ‰ (C3-source). Soil B had a pH ($CaCl_2$) of 6.0, a bulk density of 1.4 g cm^{-3}, an organic C content of 12.4 g kg^{-1},,a total N content of 1.3 g kg^{-1} and a $\delta^{13}C$ value of -27.4 ‰ (C3- source). More information about soil B including its properties and management were reported by Kramer et al. (2012).

The biochar was made from maize straw (C4-source), previously dried at 105 °C for 24 h, milled < 1 mm, contained within a sealable retort with N_2 flowing to limit O_2, and pyrolysed in a Carbolite CWF 1200 furnace at 400°C for 30 minutes. Biochar was analysed in the same way as the soils, except that the pH was measured at a soil:$CaCl_2$ ratio of 1:10 (weight/weight). The biochar analytical values are given in Table 1.

Uniformly labelled ^{14}C glucose as substrate (corresponding to 50 KBq g^{-1} soil) was prepared 12 hours before the incubation study started. The $\delta^{13}C$ of the glucose was -9.905‰.

2.2 Experimental design and layout

The experimental design comprised 15 treatments: 2 unamended soils (A and B), 2 biochar amended soils (soils A and B amended with biochar), plus biochar alone supplied with dissolved soil organic C (DOC) solution (1:20 soil: water ratio). All of these 5 treatments were supplied with 3 additions (water and 2 glucose levels). Soils from both treatments (C3 soil plus C4 biochar, and C3 soil) were amended with distilled water as a control to permit the calculation of glucose-induced priming effects. Also, the soil (C3) alone incubated with distilled water or glucose was used as a reference to estimate the $\delta^{13}C$ shifts between the pools due to ^{13}C isotopic fractionation. The biochar enriched soil (C3+C4) treated solely with water was used as a control to estimate the changes in $\delta^{13}C$ due to preferential utilization of

^{13}C-enriched biochar C. Each treatment was replicated 4 times for setup and incubation, and 3 out of 4 replicates were randomly chosen for ^{14}C and ^{13}C analysis.

To determine the effect of added glucose on the mineralization of soil organic C in biochar amended soils (C3+C4) and biochar free soils (C3), biochar (50 mg C g^{-1} soil) and ^{14}C labelled glucose were added to the two C3 soils (Soil A and B). The biochar were added to soils at the rate of 50 mg C g^{-1} soil, as in our previous study (Luo et al., 2011). The biochar derived from C4 maize straw (milled <1 mm) was incorporated into the two soils. Both soils (A and B) were separately amended with dried C4 biochar and carefully mixed in plastic bags to homogenize them. Uniformly labelled ^{14}Cglucose was added to a solution of unlabeled glucose to reach the final concentration of 5.7 KBq g^{-1} soil. ^{14}C-labelled glucose was applied to soil at 100 mg C kg^{-1} (glucose low, GL) or 1000 mg C kg^{-1} soil (glucose high, GH) in water to provide final soil moisture contents of 50% WHC. NH_4NO_3 was added, in solution, at 170 mg N kg^{-1} soil to avoid any N limitation. In order to estimate the δ^{13}C value of biochar, it was supplied with dissolved soil organic C (DOC) (soil free), the measured δ^{13}C value of released CO_2 from biochar plus DOC treatment is the biochar δ^{13}C value. The δ^{13}C of the biochar can be found in Table 1.

Each moist soil sample (20 g, o.d. basis) was incubated in a 100 ml glass jar. The soils were adjusted to 40% WHC and pre-incubated for one week at 20 ^{o}C. After pre-incubation, uniformly labelled ^{14}C glucose and distilled water were added to reach a final soil moisture content of 50% of WHC. Four empty jars served as blanks. Then the jars were incubated in the dark at 20 ^{o}C for 28 days. During the incubation, the CO_2 evolved from the soils was trapped by 3 ml of 1.0 M NaOH solution in small placed on the soil surface, and exchanged at 1, 3, 5, 7, 14, and 28 days. Aliquots of NaOH from the three randomly chosen replicate vessels from each treatment were used to measure the ^{14}C activity, ^{13}C-CO_2 (‰) and total amount of trapped CO_2.

2.3 Chemical analysis

2.3.1 Soil CO_2 emission and δ^{13}C (‰)

The concentrations of CO_2 trapped in the NaOH solutions were measured by

titrating 0.5 mL NaOH with 0.1 M HCl with phenolphthalein as an indicator after addition of 0.5 M $BaCl_2$ (Zibilske, 1994). To determine the $\delta^{13}C$ (‰) of the trapped CO_2-C, 2 ml aliquots of the NaOH were added to 3 ml 1.5 M $BaCl_2$ in vials (Aoyama et al., 2000). The resulting $BaCO_3$ precipitates were then filtered and trapped on glass fibre filters (90mm, Whatman GF/A, UK), carefully rinsed with water and dried overnight (80 °C). The precipitates were scraped off the filters, weighed (5 mg) into tin capsules and analysed for $\delta^{13}C$ on an elemental analyser-isotope ratio mass spectrometer (DELTA V plus IRMS, Thermo Fisher Scientific, Bremen, Germany).

2.3.2 Glucose derived-^{14}C in CO_2 pool

The ^{14}C activity of CO_2 trapped in NaOH was measured in a scintillation cocktail (Rotiszint Eco PlusCarl Roth, Germany) after decay of the chemiluminescence using a 1450 LSC & Luminescence Counter MicroBetaTriLux (Perkin Elmer Inc., USA). The ^{14}C counting efficiency was 87% and the ^{14}C activity measurement error did not exceed 2%.

2.4 Calculation

To partition three sources of CO_2-C, an approach combining ^{14}C labelling with ^{13}C natural abundance, the calculation of Blagodatskaya et al. (2011) and Tian et al. (2016) was used. Initially, the amount of glucose-derived C (C_G-derived) was calculated based on the radioactivity of the evolved $^{14}CO_2$ ($^{14}C_{curr}$, DPM), the amount of added glucose (C_G), and the radioactivity of the applied glucose ($^{14}C_G$, DPM):

$$C_G\text{-}derived = {}^{14}C_{curr} * C_G / {}^{14}C_G \quad (1)$$

Then, the amount of SOM-derived C was calculated as:

$$C_{SOM\text{-}derived} = C_{total} - C_{G\text{-}derived} \quad (2)$$

Where C_{total} is the total amount of C in the evolvedCO_2.

Secondly, the $\delta^{13}C$ (‰) values of SOM-originated C in each pool ($\delta^{13}C_{SOM\text{-}derived}$) were calculated based on a mass balance equation according to Balesdent and Mariotti (1996).

The $\delta^{13}C$ signature of glucose-derived C (see below) was subtracted from the total

lδ^{13}C signature, considering the contribution of the amount of glucose-originated C estimated in the first step based on ^{14}C:

$$\delta^{13}C_{SOM-derived} = \frac{(\delta^{13}C_{total} \cdot C_{total} - \delta^{13}C_{G-derived} \cdot C_{G-derived})}{C_{total} - C_{G-derived}} \quad (3)$$

where $\delta^{13}C_{total}$ and $\delta^{13}C_{G\text{-}derived}$ are the δ^{13}C-values of the total and glucose-derived C. The former value was measured experimentally as described in section 2.3.1. The δ^{13}C of $G_{\text{-derived}}$ was assumed to be equal to the δ^{13}C of glucose (-9.905‰).

The contributions of soil organic C (C3) and biochar C (C4) were then calculated based on the glucose-corrected δ^{13}C signature ($\delta^{13}C_{SOM\text{-}derived}$) at each corresponding date. The amount of C4- derived CO_2-Cwas calculated from:

$$C_{C4-derived} = \frac{C_{SOM-derived}(\delta^{13}C_{SOM-derived} - \delta^{13}C_{C3-ref})}{\delta^{13}C_{C4-material} - \delta^{13}C_{c3-materia}} \quad (5)$$

Where $\delta^{13}C_{C3\text{-}ref}$ is the δ^{13}C value in the reference C3 soil at the corresponding sampling data.

The $C_{C3\text{-}derived}$ was then calculated by subtracting the $C_{C4\text{-}derived}$ from the total amount of C.

The glucose induced priming effects in the biochar amended soils, with and without glucose addition (C3+C4) were calculated based on the changes in the δ^{13}C signature and the amount of extra CO_2 evolved after ^{14}C-glucose addition compared with the treatment without glucose.

$$PE = Biochar\ soil - C_{glucose\ amended} - Biochar\ soil - C_{without\ glucose} \quad (6)$$

Then the glucose induced priming effects in unamended soils (C3) were calculated from

$$PE = soil\ derived\ C_{glucose\ amended} - soil\ derived\ C_{without\ glucose} \quad (7)$$

The changes in the δ^{13}C signature due to preferential substrate utilization of labile and ^{13}C-enriched biochar C (compared with ^{13}C depleted soil C), were considered to provide the correct assessment of the priming effect (Blagodatskaya et al., 2011). The dynamic changes in δ^{13}C due to preferential utilization were estimated in biochar enriched soil (C3+C4) treated solely with H_2O. Therefore, the priming effects were calculated separately for C3 and C4 carbon sources (C3-PE and C4-PE, respectively) considering the changes in the contribution of soil C (C3) and biochar C (C4) in the

controls:

$$C3 - PE = C_{C3}^{amended} - C_{C3}^{unamended}$$

$$\boldsymbol{C4 - PE = C_{C4}^{amended} - C_{C4}^{unamended}}$$

2.5 Statistics

Data meeting assumptions of normality and equality of variances were analyzed by ANOVA. Data not meeting the assumptions were logarithmically transformed and analyzed by ANOVA. A one-way analysis of variance was undertaken to determine the significance ($p<0.05$) of differences between the treatments of glucose induced priming effects with and without biochar (SPSS 19.0). LSD was chosen for the multiple comparisons between different treatments.

3 Results

3.1 CO_2 release from soil

Soils which received glucose gave much higher CO_2 fluxes than soils not receiving glucose, during the 28 days of incubation (Fig. 1, $P < 0.001$), with significant differences between the two glucose concentrations. The soils with addition of 100 µg C glucose g^{-1} soil evolved lower concentrations of CO_2, (less than 1000 µg C g^{-1} soil) while the soil receiving glucose 1000 µg C g^{-1} soil at evolved much more CO_2 (more than 1500 µg C g^{-1} soil) over 28 days of incubation (Fig. 1).

At 100 µg glucose C g^{-1} soil, rapid C mineralization occurred in the first few days, then glucose mineralization ceased (Fig. 1a and c), while glucose-derived CO_2 continued to be evolved after 28 days of incubation at 1000 µg C g^{-1} soil. In addition, glucose at the low (GL) and high (GH) concentrations evolved different amounts of CO_2, at 29.9 and 285.0 µg C g^{-1} soil, respectively, over 28 days of incubation. Percentage mineralization of added glucose in GL and GH treatments were similar (29.4 % and 30.2 % of added glucose respectively). Compared to biochar free soil, biochar amended soil gave higher CO_2 emissions ($P < 0.05$), with 300.1 and 298.5 µg C g^{-1} evolved from soil A and B, respectively (Fig. 1b). The addition of GH resulted

281 in the largest soil CO_2 evolution in the biochar enriched soil B, at 1534.2 µg C g^{-1} soil
282 (Fig. 1d), which was not significantly different from the CO_2 evolved from the
283 corresponding treatment in Soil A (1468.3 µg C g^{-1} soil) (Fig. 1b).
284
285 **3.2 Partitioning the total soil CO_2-C evolved.**
286 By combining ^{14}C labelling with ^{13}C natural abundance measurements
287 (Blagodatskaya et al., 2011), it is possible to partition the CO_2-C from ^{14}C glucose,
288 native soil organic C and biochar (Fig. 2). In both soils with glucose addition, the
289 cumulative C4-derived CO_2 was greater than the CO_2 efflux derived from C3-soil
290 organic C at the beginning of the incubation +8 (day 3) but there was no significant
291 difference ($P > 0.05$) by the end of the incubation. The mineralization of C4-biochar *C*
292 mainly occurred during the first 7 days of incubation. For example, in Soil B,
293 significantly more biochar C ($P < 0.001$) was mineralized at day 7 (368.8 µg C g^{-1})
294 compared to day 3 (272.7 µg C g^{-1}) with GL addition and from 413.9µg C g^{-1} to 550.8
295 µg C g^{-1} with GH addition over the same period, but only another 10 % (GL) and 4 %
296 (GH) of biochar C were mineralized from day 7 to day 28 (Fig. 2). In both soils,
297 biochar mineralized very slowly and no biochar mineralization was detected after day
298 7. Soil organic C (C3) mineralization depended largely on the glucose addition rate,
299 irrespective of soil type. The GL rate mainly stimulated C3 soil mineralization during
300 the first 14 days (314 µg C g^{-1} in soil A and 332 µg C g^{-1} in Soil B, respectively), and
301 produced much lower mineralization rates (49.2 µg C g^{-1} in Soil A and 27.3 µg C g^{-1}
302 respectively in Soil B from day 14 to 28, while the high concentration of glucose (GH)
303 caused continuous C3 soil organic C mineralization with no decrease in rate, with the
304 CO_2 evolved increasing from 346.2 µg C g^{-1} to 486.9 µg C g^{-1} (Soil A) and 435.8 µg C
305 g^{-1} to 574.2 µg C g^{-1} (Soil B) during the corresponding period from day 14 to 28 (Fig.
306 2).
307
308 **3.3 Glucose induced priming effect**
309 Following the addition of glucose at the high rate (GH), different trends of soil
310 organic C mineralization occurred in unamended and biochar amended soil. The soil

311 organic C loss was hardly detectable in any treatments apart from the GH plus biochar
312 enriched soil, where the continuous mineralization of soil organic C extended after the
313 28 day incubation period (Fig. 3). The GH induced soil primed C losses in the biochar
314 enriched soil during 28 days of incubation were 457.0 and 423.5 µg CO_2-C g^{-1},
315 respectively, in soil A and B (Fig. 3 b, d and Fig. 4), which were 140% and 53%
316 higher respectively than the primed soil CO_2 in biochar free soil (Fig. 3b,d).
317 Following GL addition, no significant differences occurred between biochar free and
318 biochar enriched Soil A (Fig. 3a) during the 28 day incubation period. The combined
319 addition of biochar and GL caused 169.0 µg CO_2-C g^{-1} soil organic C - mineralization
320 while GL alone produced 160.9 µg CO_2-C g^{-1} of soil primed CO_2, during the 28 days
321 of incubation (Fig. 3a).

322 Glucose alone also caused biochar priming effects. GL gave an additional C4
323 biochar loss of 270.5 µg CO_2-C g^{-1}soil (Soil A) and 300.9 µg CO_2-C g^{-1} soil (Soil B),
324 while GH caused C4 biochar losses of 539.8 µg CO_2-C g^{-1} soil (Soil A) and 482.6 µg
325 CO_2-C g^{-1}soil (Soil B), respectively (Fig. 4).

326

327 **4 Discussion**

328 **4.1 Approaches to partition three C sources in a biochar rich soil system**

329 A three-source-partitioning approach of combining ^{14}C labelling with ^{13}C natural
330 abundance has been adopted in some studies to partition three C sources in soil
331 systems (Blagodatskaya et al., 2011). This yielded advantages such as 1) Compared
332 with the natural abundance of ^{13}C, making the analyses difficult, the use of highly
333 labeled ^{14}C substrates e.g. glucose-C permits the precise and accurate estimation of
334 the different soil pools which contribute to soil CO_2 fluxes (Blagodatskaya et al., 2011;
335 Whitman and Lehmann, 2015), 2) One fewer treatment is necessary to partition
336 sources, compare to the usage of dual treatments with different isotope ratios for the
337 same component (Whitman and Lehmann, 2015; Kuzyakov and Bol, 2004), 3)
338 Estimation of interactions between the soil C pools is direct, without complicated
339 3-way subtraction (Blagodatskaya et al., 2011; Kuzyakov and Bol, 2004). Our results
340 demonstrated this approach by partitioning soil CO_2 emissions derived from soil

organic C, added biochar and substrate (glucose in this study). This helps us to better understand the soil priming effects following substrate addition to biochar enriched soils.

4.2 Priming effects in biochar free and enriched soil

A 'real' priming effect reflects an increase in mineralization of soil organic C whereas an 'apparent' priming effect is due to the increased turnover of microbial C (Blagodatskaya and Kuzyakov, 2008). In this study, the 'apparent' priming effect caused by C pool substitution could be largely excluded as the microbial biomass C contents (data not published) were less than the primed CO_2 in all of the treatments (Fig 3).

Previous studies focused on short term (two weeks) (Jones et al., 2011a; Luo et al., 2011), medium (half a year) (Zimmerman et al., 2011) and long term (8 years) (Kuzyakov et al., 2014) biochar induced priming effects in unperturbed soils. Based on a review study of the 650 data points from 18 studies of biochar induced priming effects, the most positive priming effect occurred in the first 20 days while negative priming appeared in a later stage (Maestrini et al. (2014b). This might indicate that the initial loss of primed C following biochar incorporation would be compensated by greater C stabilization in the long term. This, however, does not reflect the situation in natural soil ecosystems, as substrates are ubiquitous in all soils under agricultural, grassland and forest managements. Our present work on biochar-induced priming effects considers this process following the incorporation of substrates in biochar enriched soil. We used glucose in this study. However, future study should focus more on naturally occurring substrates e.g. plant residues or root exudates. We found that the added glucose gave higher priming effects (53% and 140% more unlabeled C evolved) from two soils which had previously received biochar, compared to biochar free soil (Fig. 3). In another recent study, the combined addition of maize derived biochar and maize feedstock caused 430 µg CO_2-C g^{-1} while maize alone gave 350 µg CO_2-C g^{-1} soil primed CO_2, during 180 days of incubation (Kerre et al., 2016). Whitman and Lehmann (2015) also found 23% higher soil organic C mineralization

induced by roots when biochar was present. However, Whitman et al. (2014) found that biochar additions counteracted positive priming effect induced by maize residues, eliminating net C losses by decreasing soil organic C decomposition (48% lower). This might be due to the limited amount of C released from roots into the rhizosphere (Wichern et al., 2007). Similarly, in our study, a low concentration of glucose (GL) caused slightly higher (4% in Soil A and 34% in Soil B) primed soil organic C in a biochar amended soil compared to an unamended soil (Fig 3, 4 and 5). If we assume a continuous input of easily available substrates (e.g. rhizo-deposits) to the soil organic matter pool over a large time scale, the loss due to priming following substrate additions to biochar amended soils cannot be ignored. Clearly, extrapolation of the effects of glucose in our experiment to field conditions must be done with caution. However, further research will compare different substrates of differences in chemical composition, e.g. root exudates and litter, on soil priming effects in biochar enriched soils. In addition, studies of the effect of continuous input of small amounts of substrates into biochar enriched soils are required.

4.3 Mechanisms involved in substrate induced priming effects in biochar enriched soil

The presence of living plants (Gregory, 2006) or addition of organic substances with various composition e.g. cellulose, (Fontaine et al., 2011) and sugar-cane sucrose (Nottingham et al., 2009) alter the soil physical, hydrological and chemical environment and associated biological processes thus altering soil organic C mineralization rates (Blagodatskaya and Kuzyakov, 2008). Compared with the substrates used in the above studies, biochar induces larger and longer significant changes in the physio-chemical characteristics of soil, (e.g. soil pH, bulk density, porosity and moisture content) due to its high stability (Chan et al., 2007; Sohi et al., 2010). Thus, in two C source systems, the short term priming effects following biochar addition are mainly due to labile C, while positive priming effects (of varying duration) in three C source systems can be explained by both increases in labile C and changes in soil physical and chemical properties, which might further activate certain

groups in the microbial biomass (Fig. 5).

A fresh substrate amendment can induce a shift of the microbial community towards the utilization of more easily decomposable C (Blagodatskaya and Kuzyakov, 2008). Microbial colonization within biochar was observed in several studies, and the most likely area for colonization is referred as the "charsphere" which is defined as the interface between biochar and soil, providing a unique and preferred soil micro-environment for microbial colonization (Luo et al., 2013). The substrate inputs in our study are likely to interact with microorganisms in the charsphere, resulting in greater amounts of biomass and higher activity which may possibly cause a faster turnover of soil organic C close to that specific area. We also found a faster response of bacteria following biochar application in the early stage and fungal dominance in the later stage (unpublished data). Fresh substrate alters the bacterial and fungal community structure during the incubation period in a "microbial succession", suggesting that the ecological functions of the bacterial and fungal community changes as soil conditions (mainly soil labile C and physical properties) change over time (Andrews and Harris, 1986; Blagodatskaya et al., 2007). We believe that succession from r-strategists to K-strategists occurred during the 28 days of incubation, as the growth of microorganisms becomes increasingly limited by resource deficiency (most glucose is decomposed in the first 1-3 days). K-strategists, instead of r-strategists, are better able to use other sources of C to produce biomass than r-strategists (Andrews and Harris, 1986; Blagodatskaya et al., 2007; Chen et al., 2016). The microbial succession can be more profound in biochar amended systems as the effects of biochar on soil properties are relatively long term. This can largely explain our results, showing that larger priming effects occurred in the later stages of incubation in biochar amended soil rather than in unamended soil (Fig. 3). However, this needs further research using modern approaches, e.g. Illumina MiSeq sequencing.

4.4 Environmental implications

Sufficient incorporation of biochar will increase total soil organic C and might help offset climate change (Sohi, 2012). Biochar, however, can cause priming effects,

which may partially offset its effects on C sequestration (Jones et al., 2011b). In many laboratory incubations, biochar induced priming effects ceased after a short period of incubation, and it is generally concluded that short term priming C losses due to biochar are negligible compared to the extra C incorporated into soil following biochar incorporation. However, this input of biochar into soil provides an additional surface area and, therefore, an important new microbiological niche (Noyce et al., 2016), for soil organic C to interact with other C sources, e.g. plant residues. Thus, with these fresh organic matter inputs and their repeated additions in a biochar-soil-plant system, we belive that the interactions between non-living and living organic C are more likely to occur and cause faster rates of soil organic C decomposition in the charsphere (Luo et al., 2013). If our results are validated in other systems with different soils, biochars and substrates, this C loss cannot be ignored and the C balance might need to be carefully recalculated in the long term by considering multiple labile organic C sources, *e.g.* root exudates, plant and animal residues etc., which commonly exist in nature (Zhu et al., 2014). Currently, we still do not know how substrates with different chemical compositions cause priming effects of different magnitudes. We believe this needs further systematic investigation. In addition, in nature, pulses of substrate are usual , e.g. inputs of litter and root exudates in most terrestrial ecosystems (Qiao et al., 2014) and are more likely to continuously prime soil organic C in biochar free systems than a single substrate input (Hamer and Marschner, 2005), we believe this might cause larger and continuous C losses.

5 Conclusions

We tested the possibility of adopting the three-source-partitioning approach by combining ^{14}C labelling with ^{13}C natural abundance in a biochar amended soil system. For the first time we were able to separate CO_2 emission from biochar, soil organic C and substrate (glucose) based on a combination of ^{14}C with ^{13}C addition to soil. We verified our hypothesis that soil organic C mineralization responds differently to the addition of fresh organic C in unamended and biochar amended soils. This study found that following ^{14}C labelled glucose addition, the accumulated primed soil C loss

during 28 days were 140% and 53% higher than the priming soil CO_2 in biochar free soils. In addition, glucose also caused priming of biochar decomposition, with an additional C_4 biochar loss of between 270 μg CO_2-C g^{-1} and 540 μg CO_2-C g^{-1} depending on soils and glucose concentrations. These results help us to better understand the soil priming effects following substrate addition in biochar amended soils. Long term field experiments are required with different soil and biochar types. However, some methodology problems remained to be overcome, for example, the use of ^{14}C under field conditions, and the high cost of large amounts of highly enriched ^{13}C labelled plants, etc.

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References

Andrews, J.H., Harris, R.F., 1986. R-Selection and K-Selection and Microbial Ecology. Advances in Microbial Ecology 9, 99-147.

Aoyama, M., Angers, D.A., N'Dayegamiye, A., Bissonnette, N., 2000. Metabolism of 13C-labeled glucose in aggregates from soils with manure application. Soil Biology & Biochemistry 32, 295-300.

Benbi, D.K., Boparai, A.K., Brar, K., 2014. Decomposition of particulate organic matter is more sensitive to temperature than the mineral asscsociated organic matter. Soil Biology & Biochemistry 70, 183-192.

Blagodatskaya, E., Yuyukina, T., Blagodatsky, S., Kuzyakov, Y., 2011. Three-source-partitioning of microbial biomass and of CO2 efflux from soil to evaluate mechanisms of priming effects. Soil Biology & Biochemistry 43, 778-786.

Blagodatskaya, E.V., Blagodatsky, S.A., Anderson, T.H., Kuzyakov, Y., 2007. Priming effects in Chernozem induced by glucose and N in relation to microbial growth strategies. Applied Soil Ecology 37, 95-105.

Blagodatskaya, E., Kuzyakov, Y., 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. Biology and Fertility of Soils 45, 115-131.

Chan, K.Y., Van Zwieten, L., Meszaros, I., Downie, A., Joseph, S., 2007. Agronomic values of greenwaste biochar as a soil amendment. Australian Journal of Soil Research 45, 629-634.

Chen, Y., Chen, G., Robinson, D., Yang, Z., Guo, J., Xie, J., Fu, S., Zhou, L., Yang, Y., 2016. Large amounts of easily decomposable carbon stored in subtropical forest subsoil are associated with r-strategy-dominated soil microbes. Soil Biology & Biochemistry 95, 233-242.

Fang, Y., Singh, B.P., Singh, B., 2014. Temperature sensitivity of biochar and native carbon mineralization in biochar-amended soils. Agriculture, Ecosystems & Environment 191, 158-167.

Fontaine, S., Henault, C., Aamor, A., Bdioui, N., Bloor, J.M.G., Maire, V., Mary, B., Revaillot, S., Maron, P.A., 2011. Fungi mediate long term sequestration of carbon and nitrogen in soil through their priming effect. Soil Biology & Biochemistry 43, 86-96.

Hamer, U., Marschner, B., 2005. Priming effects in different soil types induced by fructose, alanine, oxalic acid and catechol additions. Soil Biology & Biochemistry 37, 445-454.

Jones, D.L., Murphy, D.V., Khalid, M., Ahmad, W., Edwards-Jones, G., DeLuca, T.H., 2011. Short-term biochar-induced increase in soil CO_2 release is both biotically and abiotically mediated. Soil Biology & Biochemistry 43, 1723-1731.

Kramer, S., Marhan, S., Ruess, L., Armbruster, W., Butenschoen, O., Haslwimmer, H., Kuzyakov, Y., Pausch, J., Scheunemann, N., Schoene, J., Schmalwasser, A., Totsche, K.U., Walker, F., Scheu, S., Kandeler, E., 2012. Carbon flow into microbial and fungal biomass as a basis for the belowground food web of agroecosystems. Pedobiologia 55, 111-119.

Kerre, B., Hernandez-Soriano, M.C., Smolders, E., 2016. Partitioning of carbon sources among functional pools to investigate short-term priming effects of biochar in soil: A ^{13}C study. Science of the Total Environment 547, 30-38.

Kuzyakov, Y., Bogomolova, I., Glaser, B., 2014. Biochar stability in soil: Decomposition during eight years and transformation as assessed by compound-specific 14C analysis. Soil Biology & Biochemistry 70, 229-236.

Kuzyakov, Y., Friedel, J.K., Stahr, K., 2000. Review of mechanisms and quantification of priming effects. Soil Biology & Biochemistry 32, 1485-1498.

Kuzyakov, Y., Subbotina, I., Chen, H., Bogomolova, I., Xu, X., 2009. Black carbon decomposition and incorporation into soil microbial biomass estimated by ^{14}C labeling. Soil Biology & Biochemistry 41, 210-219.

Lehmann, J., Sohi, S.P., 2008. Comment on "fire-derived charcoal causes loss of forest humus". Science 321, 1295.

Luo, Y., Durenkamp, M., De Nobili, M., Lin, Q., Brookes, P.C., 2011. Short term soil priming effects and the mineralisation of biochar following its incorporation to soils of different pH. Soil Biology & Biochemistry 43, 2304-2314.

Luo, Y., Durenkamp, M., De Nobili, M., Lin, Q., Devonshire, B.J., Brookes, P.C., 2013. Microbial biomass growth, following incorporation of biochars produced at 350 °C or 700 °C, in a silty-clay loam soil of high and low pH. Soil Biology & Biochemistry 57, 513-523.

Maestrini, B., Herrmann, A.M., Nannipieri, P., Schmidt, M.W.I., Abiven, S., 2014a. Ryegrass-derived pyrogenic organic matter changes organic carbon and nitrogen mineralization in a temperate forest soil. Soil Biology & Biochemistry 69, 291-301.

Maestrini, B., Nannipieri, P., Abiven, S., 2014b. A meta-analysis on pyrogenic organic matter induced priming effect. GCB Bioenergy 7, 577-590

Nottingham, A.T., Griffiths, H., Chamberlain, P.M., Stott, A.W., Tanner, E.V.J., 2009. Soil priming by sugar and leaf-litter substrates: A link to microbial groups. Applied Soil Ecology 42, 183-190.

Noyce, G.L., Winsborough, C., Fulthorpe, R., Basiliko, N., 2016. The microbiomes and metagenomes of forest biochars. Scientific Report 6, 26425.

Qiao, N., Schaefer, D., Blagodatskaya, E., Zou, X., Xu, X., Kuzyakov, Y., 2014. Labile carbon retention compensates for CO_2 released by priming in forest soils. Global Change Biology 20, 1943-1954.

Smith, J.L., Collins, H.P., Bailey, V.L., 2010. The effect of young biochar on soil respiration. Soil Biology & Biochemistry 42, 2345-2347.

Sohi, S.P., 2012. Agriculture. Carbon storage with benefits. Science 338, 1034-1035.

Sohi, S.P., Krull, E., Lopez-Capel, E., Bol, R., 2010. A review of biochar and its use and function in soil. Advances in Agronomy 105, 47-82.

Wardle, D.A., Nilsson, M.C., Zackrisson, O., 2008. Fire-derived charcoal causes loss of forest humus. Science 320, 629.

Weng, Z., Van Zwieten, L., Singh, B.P., Kimber, S., Morris, S., Cowie, A., Macdonald, L.M., 2015.

554 Plant-biochar interactions drive the negative priming of soil organic carbon in an annual ryegrass
555 field system. Soil Biology & Biochemistry 90, 111-121.
556 Whitman, T., Lehmann, J., 2015. A dual-isotope approach to allow conclusive partitioning
557 between three sources. Nature Communication 6, 8708.
558 Whitman, T., Zhu, Z., Lehmann, J., 2014. Carbon mineralizability determines interactive effects
559 on mineralization of pyrogenic organic matter and soil organic carbon. Environmental Science &
560 Technology 48, 13727-13734.
561 Wichern, F., Mayer, J., Joergensen, R.G., Müller, T., 2007. Release of C and N from roots of peas
562 and oats and their availability to soil microorganisms. Soil Biology & Biochemistry 39,
563 2829-2839.
564 Zhao, L., Cao, X., Masek, O., Zimmerman, A., 2013. Heterogeneity of biochar properties as a
565 function of feedstock sources and production temperatures. Journal of Hazardous Materials
566 256-257, 1-9.
567 Zhu, B., Gutknecht, J.L.M., Herman, D.J., Keck, D.C., Firestone, M.K., Cheng, W., 2014.
568 Rhizosphere priming effects on soil carbon and nitrogen mineralization. Soil Biology &
569 Biochemistry 76, 183-192.
570 Zimmerman, A.R., Gao, B., Ahn, M.-Y., 2011. Positive and negative carbon mineralization
571 priming effects among a variety of biochar-amended soils. Soil Biology & Biochemistry 43,
572 1169-1179.
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576 **Legends to Figures.**

577

578 Fig. 1. Cumulative CO_2 evolved from soil A with low concentration of at 100 mg C
579 kg^{-1} soil (graph 'a') and high concentration of additions at 1000 mg glucose-C kg^{-1} soil
580 (graph 'b'), and soil B with the same low (graph 'c') and high (graph 'd') concentration of
581 glucose addition. Bars indicate standard errors of the means (n=3).

582

583 Fig. 2. Contribution of three C sources to cumulative CO_2 evolved from soil A with
584 low at 100 mg C kg^{-1} soil (a) and high concentration of glucose addition at 1000 mg C
585 kg^{-1} soil (b), and soil B with the same low (c) and high (d) concentration of glucose
586 addition. The three sources of CO_2-C were: 1) Soil derived CO_2-C (C3-Soil), 2)
587 Biochar derived CO_2-C (C4-Biochar), and 3) Glucose derived CO_2-C (^{14}C). Error bars
588 indicate standard errors of the means (n=3).

589

590 Fig. 3 Glucose induced soil priming effects (PE) in unamended (grey bar) and biochar
591 amended soil (black) from soil A with low at 100 mg C kg^{-1} soil (a) and high
592 concentration of glucose addition at 1000 mg C kg^{-1} soil (b), and soil B with low (c)
593 and high (d) concentration of glucose addition.. Error bars indicate standard errors of
594 the means (n=3).

595

596 Fig. 4. Contribution of three C sources to cumulative CO_2 efflux from soil A with low
597 at 100 mg C kg^{-1} soil (a) and high concentration of glucose addition at 1000 mg C kg^{-1}
598 soil (b), and soil B with low (c) and high (d) concentration of glucose addition. The
599 three sources include: 1) C of added glucose (^{14}C-Glucose), 2) Soil-derived
600 $C_{glucoseunamended}$ and Biochar-derived $C_{glucoseunamended}$, and 3) the priming effect induced
601 by glucose addition (presented as the right segment of the pie-plot), and contribution
602 of the biochar-C (C4) and soil organic C (C3) to the primed C is shown as stacked
603 columns.

604

605 Fig. 5. Schematic overview of biochar induced soil priming effects in biochar
606 amended soils.

607

608

Table

Table 1: Biochar characterization

Total C (%)	C/N	C/H	C/O	Ash content (%)	DOC (mg g-1)	CEC (c mol/kg)	pH	Total K (mg/g)	$\delta^{13}C$
50.27 (3.99) [a]	30.31 (0.30)	14.70 (0.06)	2.57 (0.24)	20.2 (0.15)	0.600 (0.012)	116.20 (5.46)	10.06 (0.011)	8.60 (0.02)	-14.29 (0.02)

[a] Means ± standard error

Figure

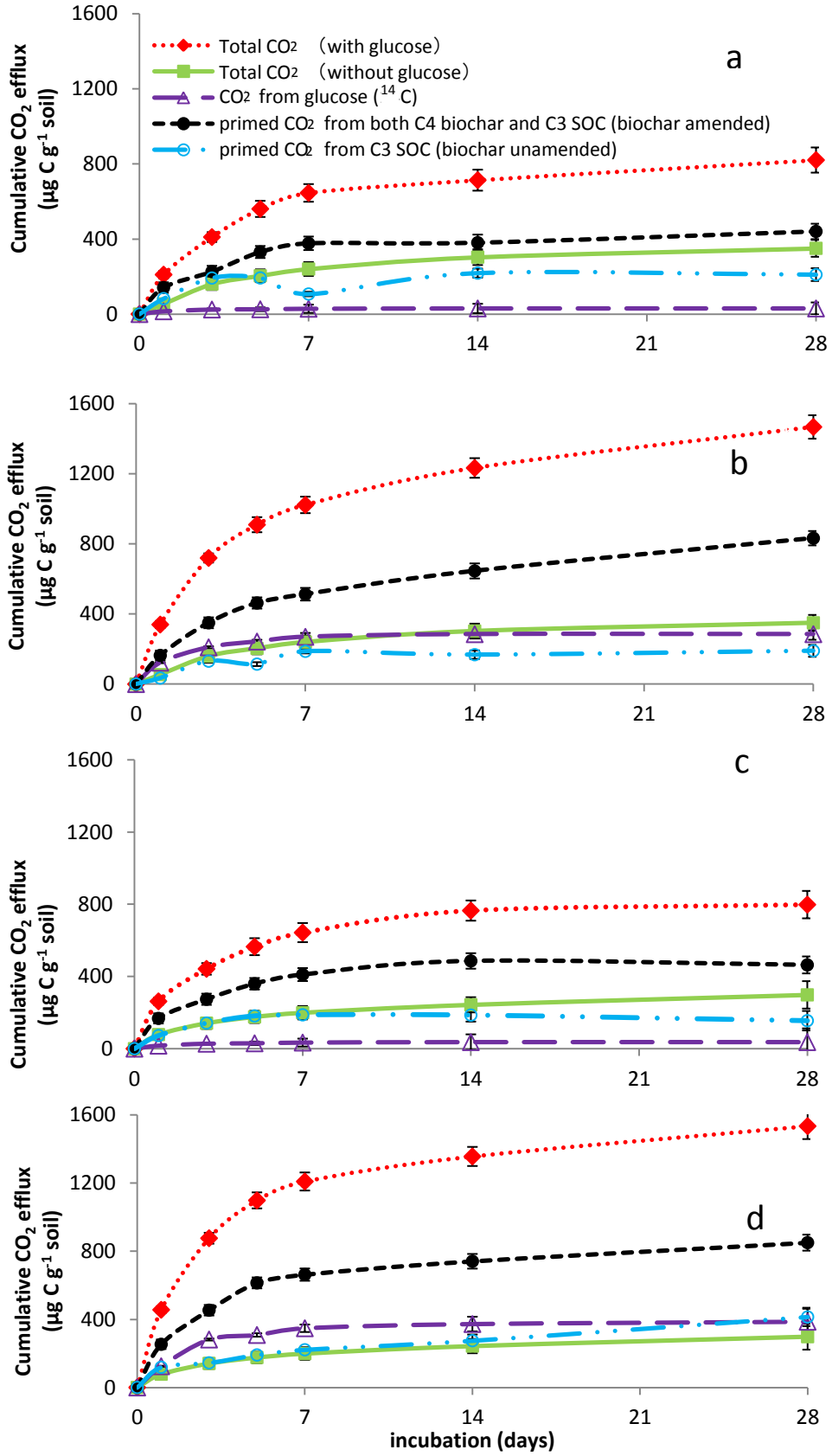

Fig. 1. Cumulative CO_2 evolved from soil A with low at 100 mg C kg^{-1} soil (a) and high concentration of glucose addition at 1000 mg C kg^{-1} soil (b), and soil B with low (c) and high (d) concentration of glucose addition. Bars indicate standard errors of the means (n=3).

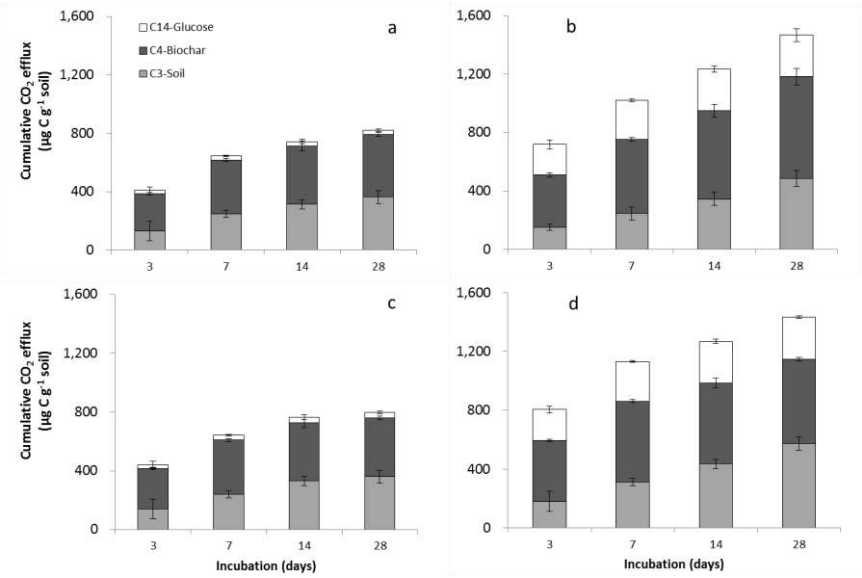

Fig. 2. Contribution of three C sources to cumulative CO_2 evolved from soil A with low at 100 mg C kg^{-1} soil (a) and high concentration of glucose addition at 1000 mg C kg^{-1} soil (b), and soil B with low (c) and high (d) concentration of glucose addition. The three sources of CO_2-C were: 1) Soil derived CO_2-C (C3-Soil), 2) Biochar derived CO_2-C (C4-Biochar), and 3) Glucose derived CO_2-C (^{14}C). Error bars indicate standard errors of the means (n=3).

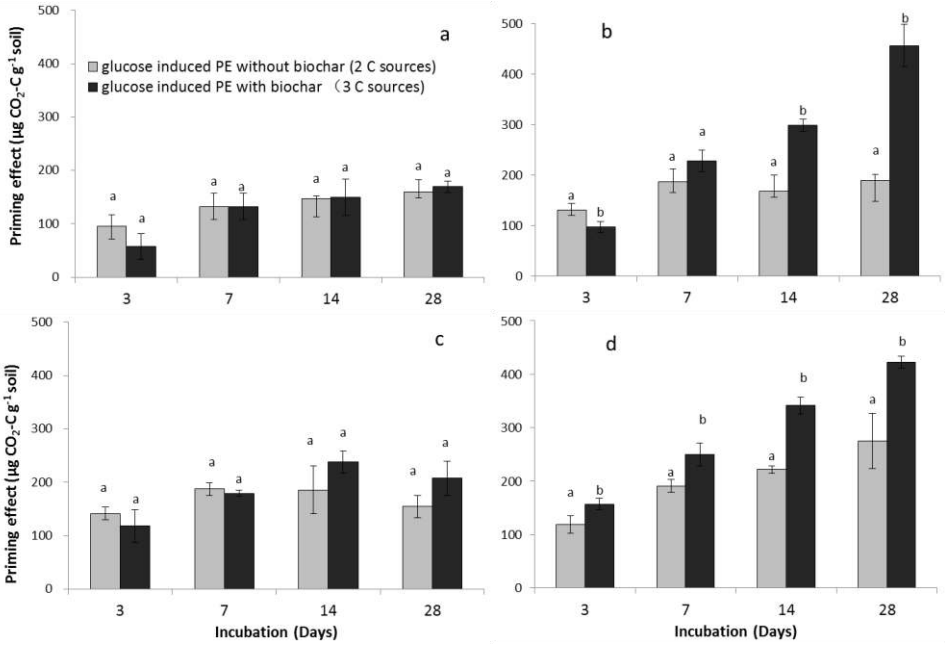

Fig. 3 Glucose induced soil priming effects (PE) in unamended (grey bar) and biochar amended soil (black) from soil A with low at 100 mg C kg^{-1} soil (a) and high concentration of glucose addition at 1000 mg C kg^{-1} soil (b), and soil B with low (c) and high (d) concentration of glucose addition.. Error bars indicate standard errors of the means (n=3).

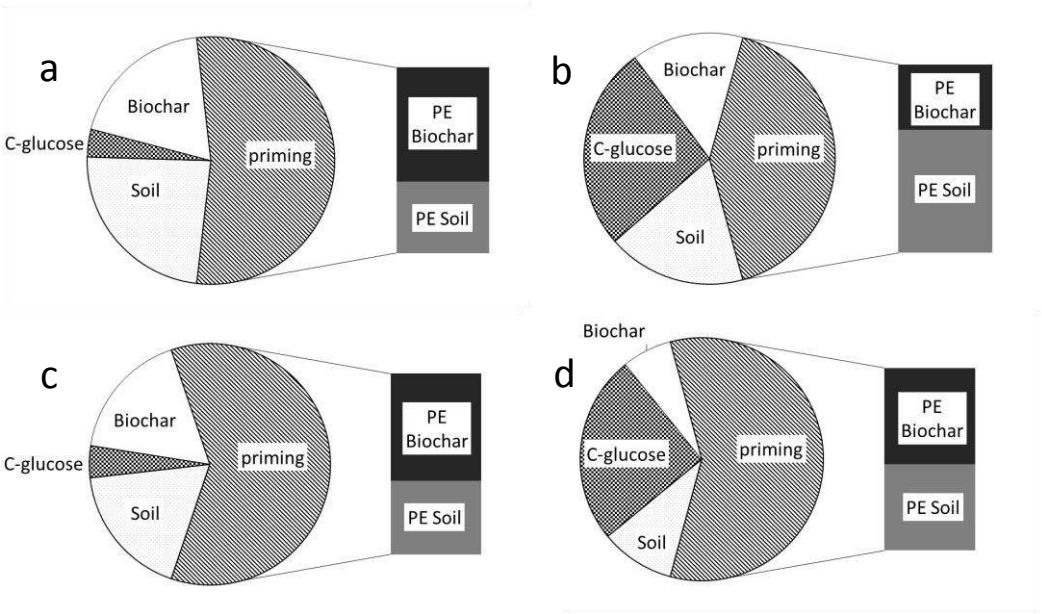

Fig. 4. Contribution of three C sources to cumulative CO_2 efflux from soil A with low at 100 mg C kg^{-1} soil (a) and high concentration of glucose addition at 1000 mg C kg^{-1} soil (b), and soil B with low (c) and high (d) concentration of glucose addition. The three sources include: 1) C of added glucose (^{14}C-Glucose), 2) Soil-derived $C_{glucose\ unamended}$ and Biochar-derived $C_{glucose\ unamended}$, and 3) the priming effect induced by glucose addition (presented as the right segment of the pie-plot), and contribution of the biochar-C (C4) and soil organic C (C3) to the primed C is shown as stacked columns.

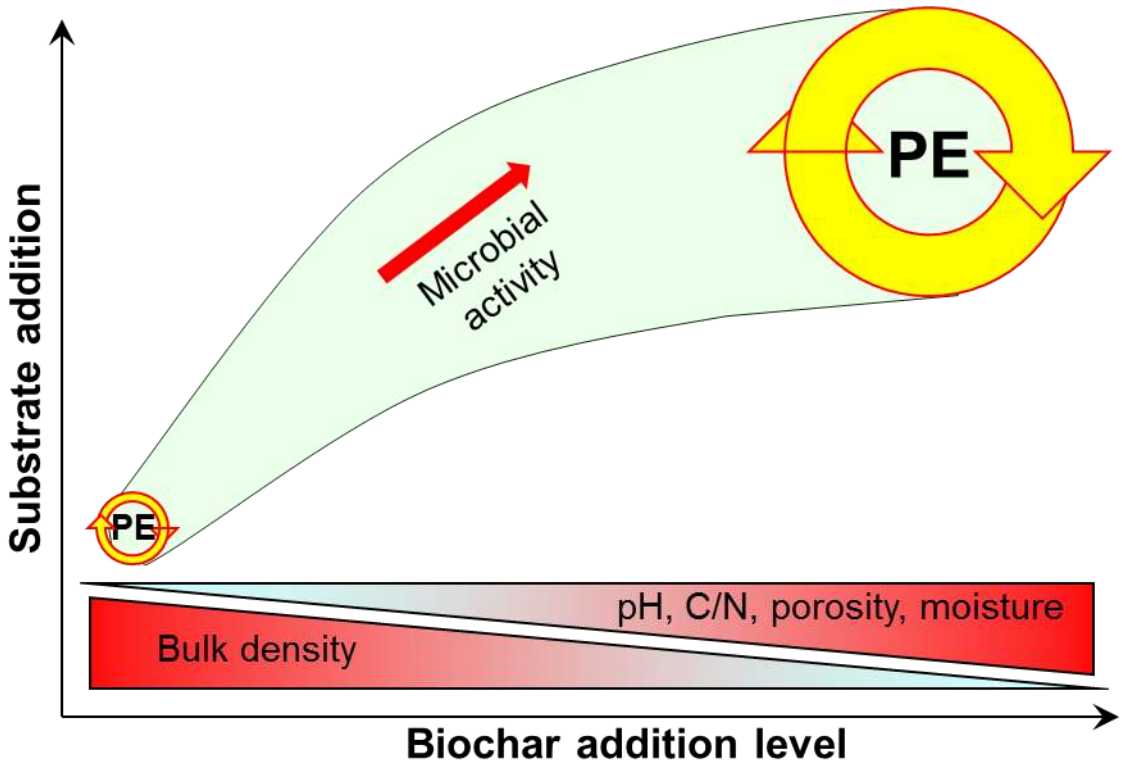

Fig. 5. Schematic overview of biochar induced soil priming effects in biochar amended soils.