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Soil organic carbon status after eight years of CO_2 enrichment and its vulnerability to substrate-induced priming of three cropland soils

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Highlights

- Elevated CO_2 does not increase soil organic C after 8 years
- Long-term eCO_2 increases C distribution to the >53 μm fraction
- Elevated CO_2 increases the priming effect in the Calcarosol
- SOC stability depends on soil C, N availability and texture
- Croplands are more likely to be CO_2 sources than sinks in future climate change

Abstract

Elevated CO_2 (eCO_2) may enhance soil organic carbon (SOC) sequestration via greater input of photosynthetic carbon (C). However, greater rhizodeposits under eCO_2 would also stimulate microbial decomposition of native SOC. This study aimed to examine the status and stability of SOC in three Australian cropland soils after long-term CO_2 enrichment. Samples (0-5 cm) of cropping Chromosol, Vertosol and Calcarosol soils were collected from an 8-year Free-Air CO_2 Enrichment (SoilFACE) experiment and were used to examine SOC dynamics by physical fractionation and ^{13}C isotopic tracing. Compared to the ambient CO_2 (aCO_2) (390-400 μmol mol^{-1}), 8 years of elevated CO_2 (eCO_2) (550 μmol mol^{-1}) did not increase SOC stock of all soils, but accelerated SOC turnover with 12% more C in the coarse soil fraction and 5% less C in fine

fraction. Elevated CO_2 also enhanced the vulnerability of SOC to ^{13}C-glucose-induced priming but this effect was only significant in the coarse textured Calcarosol topsoil. Elevated CO_2 increased labile C (coarse C fraction, +13%), soil pH (+0.25 units) and decreased available N (-30%) in the Calcarosol, which stimulated the microbial growth (+28%), leading to an enhanced priming effect. Despite with greater priming, the Chromosol and Vertosol lost proportionally less SOC when compared to the Calcarosol probably due to more chemical and physical protection of C in the Chromosol and Vertosol soils but not pH influence. In conclusion, the effect of long-term eCO_2 enrichment on soil C and N availability in cropping soils was soil type dependent with the coarse textured Calcarosol being more vulnerable to substrate-induced decomposition of its SOC.

Keywords: Free-air CO_2 enrichment (FACE), ^{13}C-glucose, Physical fractionation, Priming effect, SOC stability

1. Introduction

Anthropogenic activities have resulted in continuous increases in atmospheric CO_2 concentration since the Industrial Revolution and the concentration will continue to increase in a long time (Chesney et al., 2013). Higher atmospheric CO_2 generally stimulates plant photosynthesis and enhances plant production (Ainsworth and Long, 2004; Ellsworth et al., 2004; Kimball, 2016). Elevated CO_2 (eCO_2) also enhances the input of photosynthetic carbon (C) to belowground compartments in the form of root exudates (Phillips et al., 2006), root biomass and other rhizodeposits (Pendall et al., 2004). This process provides a potential strategy to slow down the on-going increment of atmospheric CO_2 by sequestrating more atmospheric C belowground. Alternatively increased input of recently fixed C belowground (e.g. root exudates) is important to a range of soil microbial processes, leading to potential stimulation of microbial growth and/or activity and shifts in microbial community structure (Cheng et al., 2012; Langley et al., 2009; Vestergård et al., 2016). These changes may either increase or decrease decomposition of original soil C (Xu et al., 2017, 2018). Soil C sequestration and decomposition together determine whether eCO_2 would affect soil C stocks and the capacity of soils to mitigate climate change (de Graaff et al., 2009, 2006a; Lal, 2008).

The net effect of CO_2 enrichment on SOC stock has been examined in several long-term Free-air CO_2 Enrichment (FACE) experiments. Soil organic C content have been found to be either unaffected (Keiluweit et al., 2015; van Groenigen et al., 2006), increased (Luo et al., 2006) or decreased (Carney et al., 2007; Hoosbeek et al., 2004; Langley et al., 2009) by CO_2 enrichment. A recent meta-analysis suggested that eCO_2 only increases the accumulation of new soil C in the short

term (< 1 year) (van Groenigen et al., 2017), indicating that RPE may not be just a short-term microbial response and could be more important in the long run. The relative contribution of soil C sequestration and decomposition under eCO_2 may also affect SOC turnover in the long run (Loiseau and Soussana, 1999; Sulman et al., 2014). For example, Xie et al. (2005) found eCO_2 caused more C stored in less protected soil physical fractions (> 53 μm) after 9 years of CO_2 enrichment in a grassland system. The ^{13}C isotopic tracing technique further revealed that new C replaced 26.5% of soil C pool after 10 years of CO_2 enrichment at the same site (van Kessel et al., 2006). This finding suggests that, in the long run, eCO_2 renews SOC pools with more newly-added photosynthetic C in less-protected soil fractions while the native C (old C) is progressively decomposed until an equilibrium is achieved (Keiluweit et al., 2015; van Kessel et al., 2006). The newly-added C is readily utilised by microbes due to low chemical complexity (Ruiz-Dueñas and Martínez, 2009) and less physiochemical protection (Rasmussen et al., 2006; Six et al., 2002). The renewed SOC pools resulting from long-term eCO_2 would thus be more vulnerable to microbial decomposition (referred to low stability thereafter) when compared to aCO_2. It remains unclear however whether changes in SOC content and turnover resulting from long-term CO_2 enrichment would affect the vulnerability of SOC to microbial decomposition.

Soil type and properties such as C and N status, pH and texture can directly affect microbial growth, activity and/or community structure and indirectly via influences on substrate and nutrient availability to soil microorganisms (Hamer and Marschner, 2005a, 2005b; Kuzyakov et al., 2007). For example, soil C:N ratio and N content could affect the direction and magnitude of priming effect (PE) – the difference in microbial decomposition of SOC with or without external substrate addition (Fontaine et al., 2003; Kuzyakov et al., 2000) and higher PEs are normally found in higher C and N soils (Kuzyakov et al., 2000; Liu et al., 2017; Qiao et al., 2015). Low soil pH normally affects microbial composition with less bacterial community and/or decreases enzymatic activity, thus limiting microbial decomposition (Aye et al., 2016; Rousk et al., 2010; Wang et al., 2016). Soils with fine textures often exhibit high C retention capacity and stability because small pores harbour less O_2 for anaerobic decomposers and SOC is largely bounded with clays and/or protected within aggregates (Procter et al., 2015; Reis et al., 2014; Six et al., 2002). Most CO_2 experiments however, especially FACE experiments, examine only one soil type, so it is unclear how soil type and CO_2 interactively influence SOC dynamics.

The objective of this study was to investigate the effect of long-term CO_2 enrichment on SOC stability in three cropping soils with contrasting physicochemical properties. Soil samples (0-5 cm) were collected from an 8-year FACE experiment (SoilFACE) established in a semiarid temperate

environment. Soil C decomposition dynamics were examined by incubation with ^{13}C-labelled glucose. We hypothesised that (1) long-term eCO_2 would shift SOC pools with increases in newly-input C and decreases in protected C; (2) as a result, the SOC under long-term eCO_2 would be more vulnerable to substrate-induced microbial decomposition and (3) this effect would be more pronounced in soils with low SOC and coarse texture.

2. Materials and methods

2.1 Study site – the field experiment

The SoilFACE experiment is located in Horsham, Victoria, Australia (36º44′57″S, 142º06′50″E). The SoilFACE has been performed for continuous 8 years since 2009. The experiment comprised two CO_2 concentrations, three soil types and four replicates. Pure CO_2 was injected daily on the upwind side of the bunker from sun rise to sunset during the growing seasons to achieve an elevated CO_2 concentration (eCO_2, 550 ± 30 μmol mol^{-1}) than the ambient CO_2 concentration (aCO_2, 390 ± 10 μmol mol^{-1}). More details of the FACE facility can be found in Mollah et al. (2009).

Intact soils of a Chromosol, Vertosol, and Calcarosol (Isbell and NCST 2016) were collected from paddocks, packed into soil columns (diameter 30 cm, length 100 cm) and then sank into bunkers with the surface of soils columns being kept at the ground level. These soils represented three major soil types in dryland cropping systems in South-Eastern Australia. The starting soil pH, total C and N were measured in Jin et al. (2017). The Chromosol had pH 4.5, total C 48.9 mg g^{-1} and total N 4.0 mg g^{-1}, the Vertosol pH 7.3, total C 9.4 mg g^{-1} and total N 0.8 mg g^{-1} and the Calcarosol pH 5.7, total C 4.4 mg g^{-1} and total N 0.4 mg g^{-1}. Soil texture was reported by Butterly et al. (2016). The Chromosol had 15.9% sand, 65.8% silt and 18.3% clay, the Vertosol 12% sand, 36.9% silt and 51.1% clay and the Calcarosol 84.5% sand, 10% silt and 5.5% clay. Field capacities of the Chromosol, Vertosol and Calcarosol were 0.46, 0.44 and 0.12, respectively. The top of the soil columns was kept at the ground level of the paddock. The research site has an annual rainfall of 462 mm, minimum mean temperature of 7.6 ºC and maximum mean temperature of 22.3 ºC (Jin et al., 2017), which is characterised as a Mediterranean type climate (Mollah et al., 2009).

A pulse-wheat rotation was adopted in the SoilFACE. Briefly, field pea (*Pisum sativum* L. cv. PBA Twilight) was grown in the year of 2009, 2011, canola (*Brassica napus* L. cv. Hyola 50) in 2013 and 2015 and wheat (*Triticum aestivum* L. cv. Yitpi) in 2010, 2012, 2014 and 2016. Urea-N was supplied at the annual rate of 50 kg N ha^{-1} for wheat and canola and P fertiliser was applied as triple superphosphate at 15 kg P ha^{-1} a^{-1} for all crops. Crop residues were chopped to < 2 mm and returned

to their respective soil columns after removing grain at maturity. Possible residue loss during summer time was avoided by mesh-netting.

2.2 Soil sampling

Soil sampling was conducted in June 2017. Surface soils (0–5 cm) were taken from the 4 soil columns of each soil type within each bunker and composited. Soil samples were kept cool, sieved through a 2-mm sieve and stored field-moist at 4 ºC after transporting back to laboratory. Subsamples were taken for measurements of basic properties, i.e. pH, available N, total C and N, and microbial biomass C and N.

2.3 Physical fractionation

Soil organic C was partitioned into coarse and fine fractions according to Baldock et al. (2013). Briefly, 5 g of each soil sample was sieved to ≤ 2 mm and then mixed with 20 ml of sodium hexametaphosphate solution (5 g L^{-1}). The soil and solution mixture was shaken for 16 h on a reciprocating shaker (OM6, Ratek Instruments, Boronia, Australia) at 180 rpm with an amplitude of 2.5 cm to disperse soil aggregates before sieving with a 53 μm-size sieve. Soil particles on the surface of the sieve (coarse fraction > 53 μm) was back-washed into pre-weighed container and samples that passed through the sieve (fine fraction < 53 μm) was also recovered and transported into pre-weighed containers. Both fractions were dried in an oven at 50 °C to get the dry weights. The coarse fraction was homogenised and ball-milled and the fine fraction was ground by hand using a mortar and pestle. Both fractions were analysed for organic C concentration (mg C g^{-1} particles) using an Elemental Analyser (PerkinElmer EA2400, Branford, Connecticut, USA). The proportion (%) of soil organic C allocated to coarse or fine fraction was calculated as coarse organic C or fine organic C (mg C g^{-1} soil) divided by the sum of coarse organic C and fine organic C.

2.4 Laboratory incubation

Soil samples were pre-incubated in plastic bags at 25 ºC for 16 days prior to application of treatments to acclimate microorganisms. During the pre-incubation, fresh air was flushed daily to maintain the aerobic condition and the soil moisture was kept at 50% field capacity by adding milli-Q water.

After the pre-incubation, ^{13}C enriched glucose (99 atom%, Sigma-Aldrich, USA) was mixed with equivalent ^{12}C glucose to produce a 5 atom% ^{13}C-glucose solution. Two sets of 30 g (fresh mass equivalent) pre-incubated soils were weighed into mesh-ended soil cores. One set of the soils was mixed with the glucose solution at the rate of 500 μg C g^{-1} soil weekly over six consecutive weeks.

The incubation system was kept unconfined and dried overnight at 25 ºC when needed to enable the addition of glucose solution without saturating the soil (Morrissey et al., 2017). The overnight water loss was determined in a preliminary experiment to calculate the volume of solution needed to bring the soil moisture to 80% of field capacity. Another set of the pre-incubated soils, without the glucose addition, was included as controls. During the first addition, the substrate solution was applied evenly onto the surface of the soil, allowed to settle for 15 min and then mixed with a spatula. The same procedure was conducted for the control samples.

Soil cores were then transferred into 1-L air-tight Mason jars, each containing a vial of 8 ml NaOH (1 *M*) that acted as a CO_2 trap and another vial of 8 ml milli-Q water to maintain humidity during the incubation. In the following weeks, the same amount of glucose solution or water was added evenly on the surface of soils using syringes but without thorough mixing to avoid the effect of perturbations. Four jars containing only the NaOH traps and water vials were also incubated as blanks. The jars were sealed and incubated at 25 ºC for 6 weeks. The NaOH traps were renewed weekly, and NaOH solutions in the old traps were transferred into 10 ml air-tight vials and kept at 4 ºC to avoid CO_2 absorbance from the atmosphere. The jars were flushed with fresh air before replacing new traps and re-sealed. The incubation jars were kept open to aid water evaporation overnight so that the addition of glucose and water did not over-saturate the soil cores. Soil samples were destructively harvested and then stored at 4 ºC at the end of the incubation for subsequent chemical analysis.

2.5 Soil respiration and its ^{13}C abundance

Two millilitres of NaOH solution was back-titrated with 0.25 *M* HCl to determine the CO_2 evolved from each soil core using the phenolphthalein indicator after precipitation of the carbonate with 8 ml of 0.25 *M* $BaCl_2$. Another 2 ml of NaOH was precipitated with 2 ml $SrCl_2$ (1 *M*) solution to form $SrCO_3$ precipitates (Cheng, 1998). The suspension was then mixed with 15 ml milli-Q water and adjusted to a neutral pH using 0.25 *M* HCl to prevent the absorbance of CO_2 and the formation of $Sr(OH)_2$. The precipitates were centrifuged and rinsed three times with milli-Q water and oven-dried at 60 °C for 72 h, and analysed for $\delta^{13}C$ value using an Isotope Mass Spectrometer (Sercon 20-22, Gateway, Crewe, UK) at one, two, three, four and six weeks after incubation.

2.6 Determination of priming effect (PE)

A two-pool mixture model was used to separate the total soil respiration (C_{total}) into glucose-derived CO_2-C (C_{glu}) and soil-derived CO_2-C (C_{soil}) based on the following equations:

$$f = (\delta^{13}C_{glu} - \delta^{13}C_{total}) / (\delta^{13}C_{glu} - \delta^{13}C_{soil})$$

$$C_{soil} = C_{total} \times f$$

$$PE = C_{soil} - C_{control}$$

where $\delta^{13}C_{glu}$ is the $\delta^{13}C$ value of the glucose solution which is 5 atom% throughout the incubation; $\delta^{13}C_{total}$ is the $\delta^{13}C$ value of the total soil respiration measured from the $SrCO_3$ precipitates; $\delta^{13}C_{soil}$ is the ^{13}C natural abundance of the soils (Table 1); $C_{control}$ is the basal soil respiration. The priming effect caused by glucose addition was calculated as the difference between soil-derived CO_2-C (C_{soil}) in the glucose-amended soil and soil basal respiration ($C_{control}$).

2.7 Soil analysis

Soil samples before and after the incubation were analysed for chemical and biological properties. Briefly, soil pH was measured after shaking the soil in 0.01 *M* $CaCl_2$ (1: 5 w/v) for 1 h. Concentrations of C and N in ball-milled samples were analysed using an elemental analyser (PerkinElmer EA2400, Branford, Connecticut, USA), and $\delta^{13}C$ value by an Isotope Mass Spectrometer (Sercon 20-22, Gateway, Crewe, UK). Fresh soils after field collection were extracted with 2 *M* KCl at a 1:5 (w/v) soil to solution ratio to determine mineral N (nitrate + ammonium). Fresh soils were also analysed for water content, K_2SO_4-extractable organic C (EOC) and inorganic N (EIN) and microbial biomass C (MBC) and N (MBN). Soil microbial biomass C (MBC) was determined by the chloroform-fumigation and extraction method (modified after Vance et al., 1987). Briefly, fumigated and non-fumigated soil samples were extracted with 0.5 *M* K_2SO_4 (1: 5, w/v) and the extracts were filtered through Whatman no. 41 filter paper and measured for EOC using a TOC Analyser (GE Sievers InnovOx, Boulder, Colorado, USA). Microbial biomass C was calculated as the difference of EOCs between the fumigated and non-fumigated extracts using a conversion factor of 0.45 (Brookes et al., 1985). The fumigated and non-fumigated soil extracts were also autoclaved at 120 ºC for 30 min and analysed for total inorganic N (NO_x^--N + NH_4^+-N) using a Flow-inject Analysis System (Lachat Instruments, Loveland, Colorado, USA). The MBN was calculated as the difference in NO_x^--N + NH_4^+-N between the fumigated and non-fumigated soil extracts by a conversion coefficient of 0.54 (Vance et al., 1987).

2.8 Statistical analysis

Data were presented as means of four replicates except the results of $\delta^{13}C$ which were means of three replicates. Tests of normal distribution and homogeneous variances were conducted with skewed data being either log_{10}- or square-root-transformed. Two-way ANOVA was adopted to test the effects of CO_2 history, soil type and their interactions on soil properties before incubation and soil respiration-related measurements. Three-way ANOVA was adopted to test the effects of CO_2 history, soil type, substrate and their interactions on soil properties after incubation. Significant

differences of means were detected at $P < 0.05$ using the least significant difference test (LSD) and signified using low-case letters. All statistical analyses were conducted in Genstat (v17, VSN International, Hemel Hempstead, UK).

3. Results

3.1 Soil properties after long-term field CO_2 enrichment

The total soil C and N concentrations differed between the soils. The Chromosol had 3.6 and 9.5 folds more C and N than the Vertosol and the Calcarosol, respectively (Table 1). The effect of 8 years of eCO_2 treatment on soil C and N concentration varied with soil type. Soil C was not uniformly increased by eCO_2 despite greater biomass production in this treatment (data not shown). Soil total N concentration was reduced by eCO_2 in the Chromosol by 9.5% but not in other soils. Elevated CO_2 had no effect on the ^{13}C abundance of the three soils, meaning that the CO_2 used in the SoilFACE experiment had a similar ^{13}C isotopic composition to the atmospheric CO_2. The Vertosol had the highest $\delta^{13}C$ value (-24.7‰), followed by the Calcarosol (-25.6‰) and the Chromosol lowest (-26.7‰) (Table 1).

The Chromosol showed the highest soil available N (27.8 μg g^{-1}) which was 8.1 and 6.2 fold greater than that of the Vertosol and Calcarosol, respectively (Table 1). On average, soils under long-term eCO_2 had lower available N when compared to aCO_2, in particular for the Chromosol (Table 1). The EOC of the Chromosol was 3.4 and 4.6 times higher than that of the Vertosol (46 μg g^{-1}) and Calcarosol (34 μg g^{-1}), respectively (Table 1). Elevated CO_2 decreased the EOC in the Chromosol by 20% (Table 1).

Elevated CO_2 increased the MBC in the Calcarosol by 28% but did not affect either MBN or the C:N ratio (Table 1). The highest MBC (400 μg g^{-1}) was found in the Vertosol, followed by the Chromosol (250 μg g^{-1}) and the Calcarosol (54 μg g^{-1}) (Table 1). However, the Chromosol and the Vertosol had similar MBN (37.1 and 34.8 μg g^{-1}, respectively), leading to a higher MBC:N ratio in the Vertosol than the Chromosol. The MBN of the Calcarosol was 11.8 μg g^{-1}, resulting in a MBC:N of 4.5.

The three soils differed greatly in their soil pH. The Vertosol had a neutral pH of 7.2 compared to pH 5.0 in the Calcarosol and 4.4 in the Chromosol (Table 1). Long-term CO_2 enrichment increased the soil pH in the Calcarosol by 0.3 units (Table 1).

3.2 SOC fractionation

The Calcarosol had the highest proportion of SOC in the coarse fraction and lowest in the fine fraction among all the soils with the Chromosol and Vertosol showing a similar C distribution between the two fractions (Table 2). When averaged across soil types, eight years of CO_2 enrichment increased the proportion of SOC in the coarse fractions by 12% whereas it decreased in the fine fraction by 5% (Table 2), indicating a faster turnover of SOC under eCO_2. Long term eCO_2 treatment decreased the N concentration in both soil fractions of the Calcarosol and the C concentration in the fine fraction. It also decreased the N concentration in fine fraction of the Chromosol.

3.3 Soil respiration, its $\delta^{13}C$ value, priming effect and SOC stability

Total CO_2 efflux from all controls gradually decreased with incubation time. The soil respiration rate was in the order of Chromosol > Vertosol > Calcarosol during the whole incubation (Fig. 1a, Table S1). Total soil C respired at the end of the incubation totalled 706, 447 and 224 μg CO_2-C [g soil]$^{-1}$ for the Chromosol, Vertosol and Calcarosol, respectively, with CO_2 history having no effect (Fig. 1b, Table S1). Glucose addition increased total CO_2 efflux for all treatments when compared with the controls (Fig. 1). Moreover, weekly addition of glucose raised the CO_2 efflux rate after three weeks of incubation, with increases greatest in the Vertosol and Calcarosol (Fig. 1a). The total CO_2 efflux in the glucose treatments was largest for the Chromosol and the lowest for the Calcarosol; the exception occurred during the last two weeks of the incubation when the Vertosol had similar CO_2 efflux rates to the Chromosol (Fig. 1a, Table S1). The CO_2 history had no effect on the total CO_2 efflux in the glucose amended treatment (Fig. 1, Table S1). The cumulative CO_2 efflux was 2.6, 2.2 and 1.6 mg CO_2-C [g soil]$^{-1}$ for the Chromosol, Vertosol and Calcarosol, respectively, at the end of 6-week incubation (Fig. 1b).

The $\delta^{13}C$ value of the total CO_2 efflux increased gradually over time for the Calcarosol and Vertosol, indicating an increase of contribution of glucose-derived CO_2 to total CO_2 efflux with time. However, the $\delta^{13}C$ value of total CO_2 efflux from the Chromosol showed a slight decrease after the third week (Fig. 2). The Calcarosol had the highest $\delta^{13}C$ value of total CO_2 efflux throughout the incubation, followed by the Vertosol and then the Chromosol; the exception was during the first week when the Vertosol and the Chromosol had similar ^{13}C abundances of total CO_2 (Fig. 2, Table S2). The effect of CO_2 history on the $\delta^{13}C$ value of total CO_2 depended on both soil type and sampling time. Specifically, the long-term CO_2 enrichment decreased the $\delta^{13}C$ value of total CO_2 from all soils at week two, and that of the Chromosol at week three and four (Fig. 2, Table S2).

A positive priming effect (PE) was found for all treatments and the PEs increased with incubation time after the fourth week (Fig. 3a). The effect of soil type was significant across time with the Chromosol having the highest PE, followed by the Vertosol and then the Calcarosol (Figs. 3a, 4, Table S2). Although the Calcarosol tended to have higher PE under eCO_2, the difference was only statistically significant at week four (Figs. 3a, 4, Table S2). By the end of the incubation, weekly addition of glucose yielded more soil-derived CO_2-C compared to its corresponding control, with an increase of 827, 739 and 554 μg C $[g\ soil]^{-1}$ for the Chromosol, Vertosol and Calcarosol, respectively (Fig. 4).

Soil priming per gram of indigenous SOC (Fig. 3b) reflects the vulnerability of native SOC to microbial decomposition upon external substrate addition. The Calcarosol exhibited the highest primed C per SOC, followed by the Vertosol and the Chromosol lowest (Fig. 3b, Table S2). The CO_2 history had no effect on the primed C per SOC in both Chromosol and Vertosol, whereas in the in the Calcarosol it increased, especially at week two, three and four (Fig. 3b, Table S2).

3.4 Soil properties after incubation

After 6-weeks of incubation, the EOC was decreased by 11%, 25% and 29% for the Chromosol, Vertosol and Calcarosol soils, respectively (Tables 1 and 3). Elevated CO_2 had no significant effect on EOC. The addition of glucose substantially increased the EOC in the Calcarosol (Table 3), indicating that microorganisms cannot deplete this large pool of substrate C and/or this C was not bounded to soil particles.

For the controls, the Chromosol had a K_2SO_4-extractable inorganic N (EIN) of 86 μg g^{-1} which was 3.2 and 1.3 fold higher than that of the Vertosol and the Calcarosol, respectively (Table 3). The addition of glucose depleted the EIN (Table 3) when compared with controls.

After the 6-week incubation with glucose, the MBC was 2.0, 1.6 and 3.7 times greater than that measured in the no-glucose added treatments in the Chromosol, Vertosol and Calcarosol soils, respectively (Table 3), suggesting microbes in the Calcarosol were more limited by substrate before the incubation. The MBN in glucose-added samples was 2.0, 1.2 and 1.2 times higher than the MBN measured when no-glucose had been added to the Chromosol, Vertosol and Calcarosol soils, respectively (Table 3). As a result, glucose addition increased the MBC:N of the Calcarosol by 95% (Table 3), which means the microorganisms were more N-limited in the glucose-added Calcarosol. Moreover, by comparison to the other two soils, the Chromosol had the lowest MBC:N (6.9, Table 3) probably due to the highest available N and total N.

4. Discussion

4.1 The effect of CO_2 history on SOC

The effect of long term (8 year) treatment with eCO_2 on total SOC depended on soil type. Plants growing under eCO_2 condition generally exhibit increased rates of photosynthesis, and thus enhanced rates of belowground C input via altered root growth and rhizodeposition (Nie et al., 2013; Pendall et al., 2004; Phillips et al., 2011). It was assumed that the potentially greater input of C into belowground compartments under eCO_2 would result in a net overall increase in SOC stocks but we found that SOC remained unchanged or could decrease depending on the soil type. This net decrease appeared to result from eCO_2 induced changes in microbial decomposition. Priming effect has been assumed as a general process that could be stimulated by CO_2 enrichment due to a greater supply of easily-metabolised substrates, accounting for the increase in SOC decomposition under elevated CO_2 (Black et al., 2016; Langley et al., 2009; van Groenigen et al., 2014). Increased PE under elevated CO_2 is attributed to changes in quantity and quality of rhizodeposits (Phillips et al., 2011; Xu et al., 2017, 2018) and the subsequent effects on microbial growth, activity (Billings and Ziegler, 2005; Janus et al., 2005; Jin et al., 2014) and/or community composition (Carney et al., 2007). No increment in SOC under eCO_2 even after eight years of cultivation possibly reflect a counter-balancing effect of PE on SOC gains from enhanced productivity.

The enhanced input of C and increased decomposition under eCO_2 could potentially accelerate SOC turnover (van Groenigen et al., 2014, 2017). This is confirmed by the finding that eCO_2 generally increased C distributed in particulate soil organic matter rather than more-protected fine fractions after eight years of imposition. Similar results have been reported previously. For example, Hofmockel et al. (2011) reported that four years of CO_2 enrichment (200 μmol mol^{-1} above ambient concentration) speeded up SOC turnover in a forest soil with more C in the coarse particulate fraction when compared to aCO_2. Xie et al. (2005) also found more retention of newly-added C in particulate soil organic matter and enhanced decomposition of indigenous soil C in a clay-loamy Cambisol after 9 years of exposure to eCO_2 (600 relative to 350 μmol mol^{-1}) in a Swiss grassland. The results imply that the inevitable higher atmospheric CO_2 that will occur in future climates may actually accelerate soil C loss rather than sequestration from the atmosphere, resulting in a positive feedback effect. Whether the renewed SOC fractions by long-term CO_2 enrichment affects SOC stability to environmental changes in future is discussed below.

4.2 CO_2 history on SOC vulnerability to microbial decomposition

Glucose addition was expected to induce more C primed from cropping soils treated with long-term eCO_2, but the increase was only significant for the Calcarosol (Fig. 3a). Soil organic C of the Calcarosol was more sensitive to substrate-induced priming under eCO_2 than aCO_2 as shown by more primed C per unit mass of SOC (Fig. 3b). Elevated CO_2 increased the proportion of coarse organic C, a relatively labile C fraction primarily composed of plant residues, living organisms and debris of dead organisms. The increase might have contributed to the greater (28%) microbial biomass under eCO_2 when compared to aCO_2 in the Calcarosol (Table 1), thereby leading to the higher PE under eCO_2. In addition, the Calcarosol had 30% less available N under eCO_2 than under aCO_2. The relative N-limitation (higher C:N ratio of soil and microbes) under eCO_2 might have actually increased the decomposition of residue and soil organic matter for N requirement through biosynthesis of more extracellular enzymes (Craine et al., 2007; Chen et al., 2014). The higher microbial biomass N and lower microbial C-to-N ratio following the substrate incubation (Table 3) could result from the enhanced SOC decomposition and utilisation of soil-derived N. Lastly, long-term CO_2 enrichment increased soil pH by ~0.3 units in the Calcarosol, which might also contribute to the higher substrate-induced priming of SOC. In other studies, low pH has been found to affect microbial community composition and enzyme activity (Kemmitt et al., 2006; Rousk et al., 2010) and to decrease microbial degradation of soil organic matter (Aye et al., 2016; Wang et al., 2016). Long-term CO_2 enrichment could have also changed the SOC composition of the Calcarosol, as indicated by the higher C:N ratio in the coarse C fraction. Elevated CO_2 has been found to increase plant C:N (Nie et al., 2015) and perhaps the amounts of recalcitrant C compounds (Peñuelas et al., 1996, 1997; Poorter et al., 1997) of plant tissues. However, the changes in residue quality under eCO_2 is too small to induce any changes in microbial growth and/or their decomposing capacity (de Graaff et al., 2006b). The result corroborates with some previous findings that even with lower quality, residues from eCO_2 do not exhibit lower decomposition than those from aCO_2 (Hirschel et al., 1997; Knnops et al., 2007; Norby et al., 2001).

However, the PEs did not differ between aCO_2 and eCO_2 in the Chromosol and Vertosol with relatively high C, N and clay contents. Soil texture affects the stability and microbial accessibility of soil organic C. The Vertosol (and Chromosol), with a relatively high specific surface area and high amounts of reactive sites, could retain more organic materials (Parfitt et al., 2003) inside of soil aggregates to decrease microbial accessibility and enzymatic decomposition (Six et al., 2002). This might account for the lower primed C per unit mass of SOC in the Vertosol and Chromosol than in the Calcarosol. The result is consistent with the notion that PEs are normally greater in soils that have more C and N (Kuzyakov et al., 2000; Liu et al., 2017; Qiao et al., 2015). CO_2 history had no effect on PE of the Chromosol and Vertosol in our incubation experiment. Given that there was

no net SOC gain, albeit more C input under eCO_2, eCO_2 might have stimulated microbial decomposing capacities and the decomposition of crop residues in the field.

4.3. Temporal responses of priming effect

The weekly supply of glucose induced higher PE at a later stage of incubation when labile SOC became less available. Multiple glucose pulses might have changed the composition and/or C-use plasticity of microbial community with activation of previously inactive SOC-utilising decomposers which could secrete larger quantities of extracellular enzymes to decompose recalcitrant C (Kuzyakov, 2010; Mau et al., 2015; Morrissey et al., 2017). For example, using the quantitative stable isotope probing of DNA, Morrissey et al. (2017) discovered that the increase in PE by multiple glucose pulses was related to the shift of a wide phylogenetical group of taxa using SOC for growth. This could be attributed to microbial mining of soil organic matter for N (Chen et al., 2014; Fontaine and Barot, 2005) at the later stage of incubation as no synchronous N was supplied to the incubated soils in this and other studies (Mau et al., 2015; Morrissey et al., 2017). In contrast, small and even negative PEs have been reported in reponse to a single pulse addition, which has been related to preferential utilisation of glucose (Blagodatskaya et al., 2011; Mau et al., 2015; Morrissey et al., 2017) by opportunistic organisms and their nutritional competition with SOC-decomposers (Mau et al., 2015).

4.4. Conclusions

Longer term (8 years) of exposure to eCO_2 did not increase SOC concentrations in the topsoil (0-5 cm) of three agriculturally important soil types in South-Eastern Australia, implying greater microbial decomposing capacities to mineralise plant residue and soil native C under eCO_2. Soil organic C would be more vulnerable to microbial decomposition in future higher CO_2 atmosphere, probably due to a higher proportion of the coarse SOC fraction, less available N and higher soil pH which stimulate microbial growth and depletion of N. This effect however was only observed in the Calcarosol which had lower clay and SOC concentrations and a higher proportion of unprotected C than the Chromosol and Vertosol. Our results imply that dryland soils in South-Eastern Australia are potentially likely to be net CO_2 sources than sinks in future higher CO_2 scenarios. At the same time, the priming effect appears to be a long-term soil process and should be considered in estimating the potential of terrestrial ecosystem C sequestration and global biochemical models to better understand global C cycling and its responses to climate change. This study focused on the effects of CO_2 history on microbial priming of native SOC and thus only surface soil (0-5 cm) with the highest microbial abundance and activity was studied. Future studies are needed to examine the effect of long-term eCO_2 on SOC dynamics at various depths of soil profiles.

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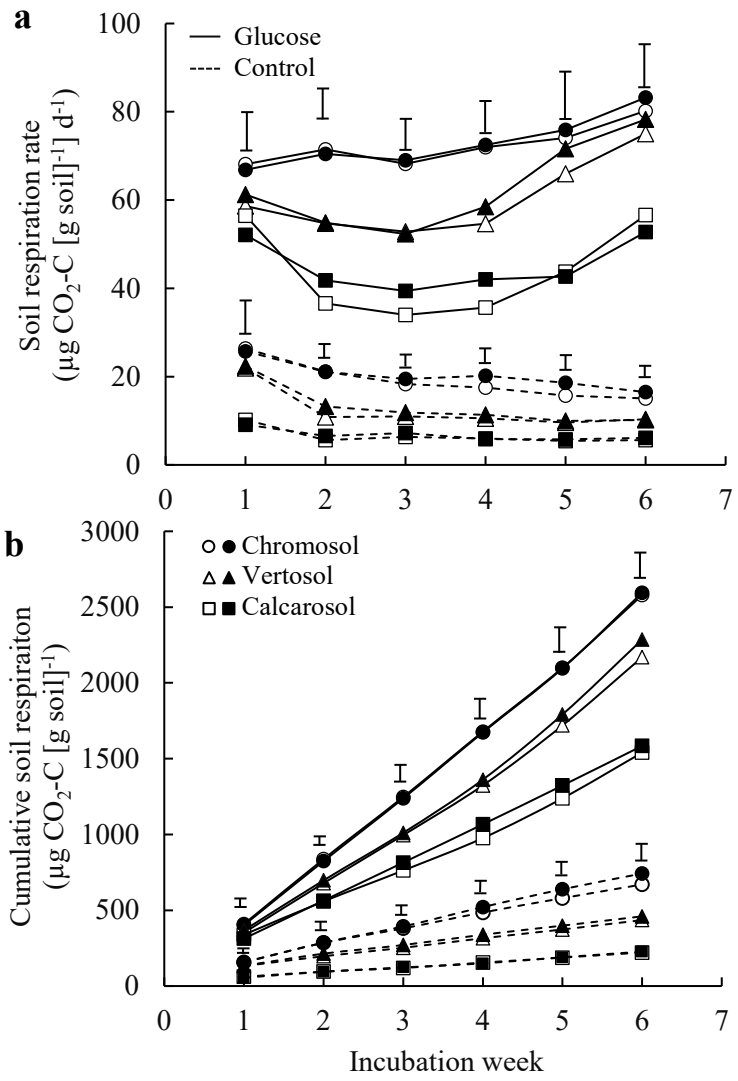

Fig. 1 Changes of soil respiration rates (µg CO_2-C [g soil]$^{-1}$ d^{-1}) (**a**) and cumulative soil respiration (µg CO_2-C [g soil]$^{-1}$) (**b**) over 6 weeks for samples that received weekly ^{13}C-glucose (solid lines) and samples that did not (dash lines). Open symbols represent aCO_2 (390 µmol mol^{-1}), solid symbols represent eCO_2 (550 µmol mol^{-1}), solid lines represent glucose-added soils and dash lines represent control soils. Vertical bars represent the least significant difference (P = 0.05) between means (n = 4) for each week, which were analysed separatedly for the glucose-added soils and contronls

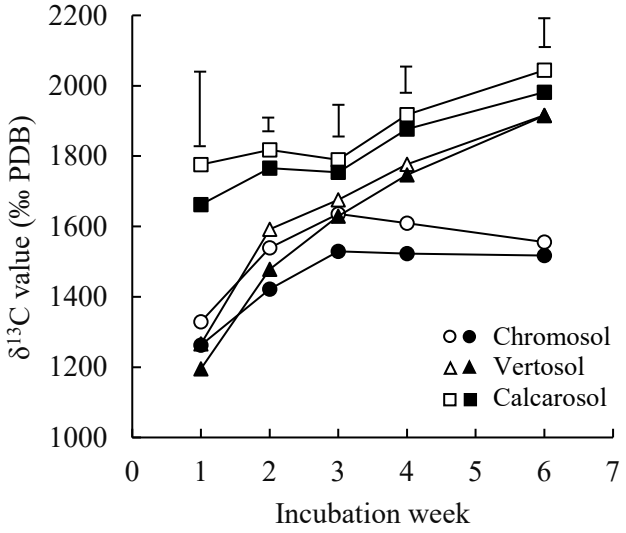

Fig. 2 Changes of $\delta^{13}C$ values (‰PDB) of total CO_2 efflux for samples that received weekly ^{13}C-glucose over 6 weeks. Open symbols represent aCO_2 (390 µmol mol^{-1}); solid symbols represent eCO_2 (550 µmol mol^{-1}). Vertical bars represent the least significant difference (P = 0.05) between means (n = 3) for each sampling time

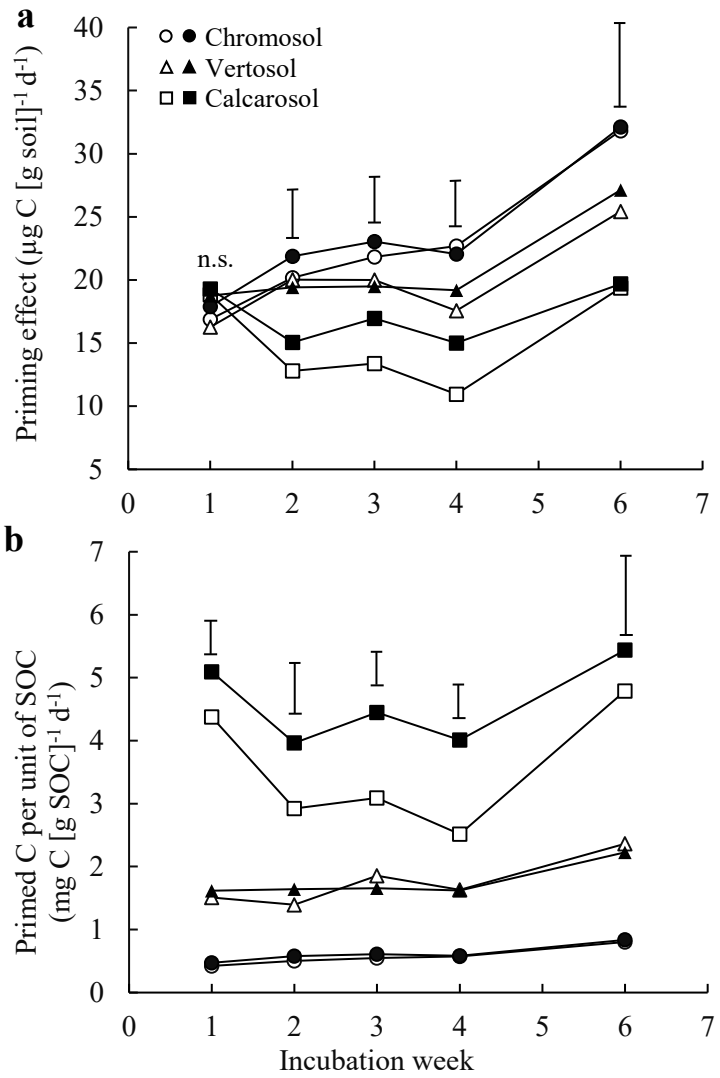

Fig. 3 Changes of priming effect (µg C [g soil]$^{-1}$ d^{-1}) (**a**) and primed C per SOC (mg C [g SOC]$^{-1}$) (**b**) caused by weekly glucose amendment over 6 weeks. Open symbols represent aCO_2 (390 µmol mol^{-1}); solid symbols represent eCO_2 (550 µmol mol^{-1}). Vertical bars represent the least significant difference (P = 0.05) between means (n = 4) for each sampling time

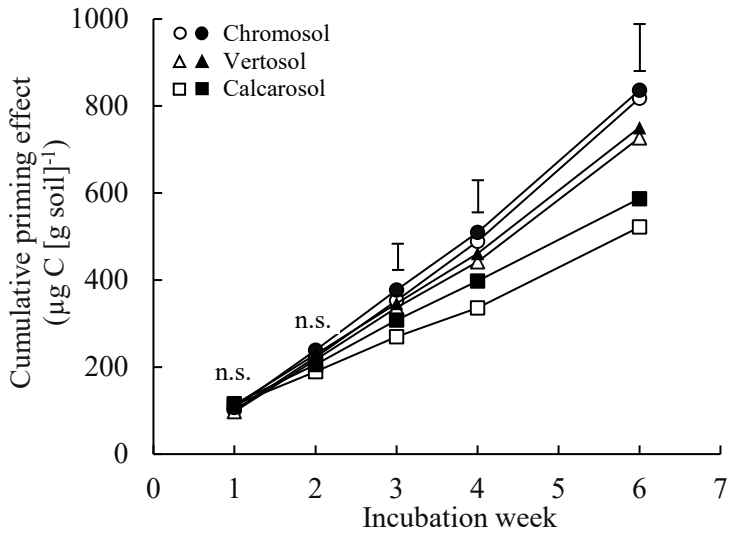

Fig. 4 Changes of cumulative priming effect (µg C [g soil]$^{-1}$) caused by weekly glucose amendment over 6 weeks. Open symbols represent aCO_2 (390 µmol mol^{-1}); solid symbols represent eCO_2 (550 µmol mol^{-1}). Vertical bars represent the least significant difference (P = 0.05) between treatment means (n = 4) for Weeks 3, 4 and 6. n.s. $P > 0.05$

Table 1 Physicochemical properties of the topsoil (0-5 cm) Chromosol, Vertosol and Calcarosol under either aCO_2 (390 µmol mol^{-1}) or eCO_2 (550 µmol mol^{-1}) for 8 consecutive years

Soil	CO_2	pH	Total C (mg g^{-1})	Total N (mg g^{-1})	$\delta^{13}C$ (‰PDB)	Available N (µg g^{-1})	EOC (µg g^{-1})	MBC (µg g^{-1})	MBN (µg g^{-1})	MBC:MBN
Chromosol	aCO_2	4.37d	40.8a	3.38a	-26.6b	30.7a	175a	258b	34.3a	7.6b
	eCO_2	4.52d	37.5a	3.06b	-26.8b	24.9b	140b	242bc	39.9a	6.4bc
Vertosol	aCO_2	7.16a	10.6b	0.86c	-24.8a	3.3d	47c	391a	33.6a	12.3a
	eCO_2	7.23a	11.2b	0.92c	-24.6a	3.6d	46c	409a	36.0a	12.1a
Calcarosol	aCO_2	4.95c	4.3c	0.36d	-25.4ab	5.3c	37d	47d	11.6b	4.1c
	eCO_2	5.20b	3.8c	0.33d	-25.9ab	3.7cd	32d	60c	12.1b	4.8bc
Significance level										
CO_2		**	n.s.	n.s.	n.s.	*	*	n.s.	n.s.	n.s.
Soil		***	***	***	**	***	***	***	***	***
CO_2×Soil		n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

EOC, K_2SO_4-extractable soil C; MBC and MBN, microbial biomass C and N.

Total C, total N, available N, EOC and MBC were log_{10}-transformated before statistical analysis.

Means (n = 4) with the same letters represent no significance at 0.05 level using the LSD test. n.s. $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 2 Concentrations of C and N (mg g^{-1} particle), C-to-N ratio (C:N) and the proportion (%) of soil organic C allocated to coarse (> 53 μm) and fine fraction (< 53 μm) separated from samples under either aCO_2 (390 μmol mol^{-1}) or eCO_2 (550 μmol mol^{-1}) for 8 consecutive years

Soil	CO_2	Coarse fraction (> 53 μm)				Fine fraction (< 53 μm)			
		C	N	C:N	%	C	N	C:N	%
Chromosol	aCO_2	50.4a	3.75a	13.5a	25.5c	36.5a	3.26a	11.2a	74.5a
	eCO_2	47.9a	3.49a	13.7a	28.9bc	32.9a	2.91b	11.3a	71.1ab
Vertosol	aCO_2	8.7b	0.70b	12.5ab	27.2c	9.5d	0.93e	10.3ab	72.8a
	eCO_2	8.5b	0.72b	11.6b	29.6bc	10.2d	1.00e	10.3ab	70.4ab
Calcarosol	aCO_2	1.4c	0.14c	9.6c	32.9ab	24.3b	2.46c	9.9b	67.1bc
	eCO_2	1.3c	0.11d	11.7b	37.3a	19.5c	2.05d	9.5b	62.7c
Significance level									
CO_2		n.s.	n.s.	n.s.	*	*	*	n.s.	*
Soil		***	***	***	***	***	***	**	***
CO_2×Soil		n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.

Carbon and N in the coarse fraction were log_{10}-transformed and C in fine fraction was square-root-transformed before ANOVA.

Means (n = 4) with the same letters represent no significance at 0.05 level using the LSD test. n.s. $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 3 Concentrations of K_2SO_4-extractable C (EOC) and inorganic N (EIN), and microbial biomass C (MBC) and N (MBN), and MBC-to-MBN ratio (MBC:N) in soil after 6-week incubation with or without glucose amendment to cropping Chromosol, Vertosol and Calcarosol under ambient (aCO_2) (390 μmol mol^{-1}) or elevated CO_2 (eCO_2) (550 μmol mol^{-1}) for 8 years

Soil	CO_2	EOC (μg g^{-1})		EIN (μg g^{-1})		MBC (μg g^{-1})		MBN (μg g^{-1})		MBC:N	
		Control	Glucose	Control	Glucose	Control	Glucose	Control	Glucose	Control	Glucose
Chromosol	aCO_2	153b	172b	86.2a	8.8cd	221cd	505ab	30.8b	69.9a	7.7bc	6.8c
	eCO_2	128b	159b	85.6a	13.3c	278bc	501ab	41.6ab	72.7a	7.1c	6.9c
Vertosol	aCO_2	36d	53c	29.9b	7.3d	442ab	724a	49.9ab	61.2ab	10.0bc	11.8abc
	eCO_2	34d	57c	24.0b	7.8d	450ab	660a	48.7ab	59.6ab	9.8bc	11.0bc
Calcarosol	aCO_2	26de	602a	22.7b	4.8e	36e	154d	3.8d	5.4c	11.0bc	16.2a
	eCO_2	23e	472a	19.2b	4.4e	51e	168d	8.3c	8.7d	7.1c	12.2ab
Significance level											
CO_2		n.s.		n.s.		n.s.		*		n.s.	
Soil		***		***		***		***		***	
Substrate		***		***		***		*		*	
CO_2×Soil		n.s.		n.s.		n.s.		*		n.s	
Soil×Substrate		***		***		*		n.s.		*	

The CO_2× Substrate and CO_2×Soil×Substrate interactions are not significant at $P \leq 0.05$.

The data of EOC, EIN, MBC and MBN are log_{10}-transformed before ANOVA.

Means (n = 4) with the same letters represent no significance at 0.05 level using the LSD test. n.s. $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table S1 Main effects of soil type on soil respiration rate and cumulative soil respiration from the control and glucose-added soils at each week. The main effects of CO_2 history and the interactions between CO_2 history and soil type are not significant.

	Week 1		Week 2		Week 3		Week 4		Week 5		Week 6	
	Control	Glucose	Control	Glucose	Control	Glucose	Control	Glucose	Control	Glucose	Control	Glucose
	Soil respiration rate (μg CO_2-C [g soil]$^{-1}$ d^{-1})											
Chromosol	24.0	67.5	21.1	71.0	18.9	68.6	18.9	72.2	17.2	75.0	15.8	80.5
Vertosol	22.1	59.9	12.0	54.8	11.5	52.5	11.0	56.9	9.8	69.2	10.3	76.3
Calcarosol	9.7	54.3	6.1	39.2	6.8	37.9	5.9	38.8	5.6	43.2	5.9	54.4
Significance level												
CO_2	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Soil	***	***	***	***	***	***	***	***	***	***	***	***
CO_2×Soil	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Cumulative soil respiration (μg CO_2-C [g soil]$^{-1}$ [36 d]$^{-1}$)												
Chromosol	157	405	285	831	386	1242	501	1676	605	2098	701	2534
Vertosol	133	360	205	689	261	1004	327	1345	386	1761	447	2201
Calcarosol	58	326	95	561	121	788	152	1021	188	1281	223	1562
Significance level												
CO_2	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Soil	***	***	***	***	***	***	***	***	***	***	***	***
CO_2×Soil	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

n.s. $P > 0.05$; *** $P < 0.001$

Table S2 Main effects of CO_2 history, soil type and their interactions on $\delta^{13}C$ values of total CO_2 released, priming effect (PE), cumulative PE and PE per unit of soil organic C (SOC) at each week

Factors		Week 1	Week 2	Week 3	Week 4	Week 6
			$\delta^{13}C$ value			
aCO_2		1457	1649	1700	1768	1838
eCO_2		1373	1555	1637	1715	1804
Significance level						
CO_2		n.s.	***	*	*	n.s.
Soil		***	***	***	***	***
CO_2×Soil		n.s.	n.s.	n.s.	n.s.	n.s.
			PE (μg C [g soil]$^{-1}$ d^{-1})			
Chromosol		17.4	20.1	20.9	20.1	28.6
Vertosol		17.5	20.6	21.3	20.6	29.6
Calcarosol		19.1	13.9	15.2	13	19.5
Significance level						
CO_2		n.s.	n.s.	n.s.	*	n.s.
Soil		n.s.	***	***	***	***
CO_2×Soil		n.s.	n.s.	n.s.	n.s.	n.s.
			Cumulative PE (μg C [g soil]$^{-1}$ [36 d]$^{-1}$)			
Chromosol		104	220	345	466	772
Vertosol		105	234	361	485	793
Calcarosol		114	198	289	367	554
Significance level						
CO_2		n.s.	n.s.	n.s.	n.s.	n.s.
Soil		n.s.	n.s.	**	***	***
CO_2×Soil		n.s.	n.s.	n.s.	n.s.	n.s.
			PE per SOC (mg C [g SOC]$^{-1}$ d^{-1})			
Chromosol	aCO_2	0.42	0.50	0.55	0.57	0.90
	eCO_2	0.47	0.58	0.61	0.58	0.80
Vertosol	aCO_2	1.51	1.40	1.66	1.62	2.36
	eCO_2	1.62	1.64	1.86	1.63	2.04
Calcarosol	aCO_2	4.38	2.92	3.09	2.52	4.92
	eCO_2	5.09	3.96	4.45	4.01	4.99
Significance level						
CO_2		n.s.	n.s.	*	**	n.s.
Soil		***	***	***	***	***
CO_2×Soil		n.s.	*	**	***	n.s.

n.s. $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$