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Title: Enhanced priming of old, not new soil carbon at elevated atmospheric CO_2

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Abstract

Rising atmospheric CO_2 concentrations accompanied by global warming and altered precipitation patterns calls for assessment of long-term effects of these global changes on carbon (C) dynamics in terrestrial ecosystems, as changes in net C exchange between soil and atmosphere will impact the atmospheric CO_2 concentration profoundly. In many ecosystems, including the heath/grassland system studied here, increased plant production at elevated CO_2 increase fresh C input from litter and root exudates to the soil and concurrently decrease soil N availability. Supply of labile C to the soil may accelerate the decomposition of soil organic C (SOC), a phenomenon termed 'the priming effect', and the priming effect is most pronounced at low soil N availability. Hence, we hypothesized that priming of SOC decomposition in response to labile C addition would increase in soil exposed to long-term elevated CO_2 exposure. Further, we hypothesized that long-term warming would enhance SOC priming rates, whereas drought would decrease the priming response.

We incubated soil from a long-term, full-factorial climate change field experiment, with the factors elevated atmospheric CO_2 concentration, warming and prolonged summer drought with either labile C (sucrose) or water to assess the impact of labile C on SOC dynamics. We used sucrose with a $^{13}C/^{12}C$ signature that is distinct from that of the native SOC, which allowed us to assess the contribution of these two C sources to the CO_2 evolved. Sucrose induced priming of SOC, and the priming response was higher in soil exposed to long-term elevated CO_2 treatment. Drought tended to decrease the priming response, whereas long-term warming did not affect the level of priming significantly.

We were also able to assess whether SOC-derived primed C in elevated CO_2 soil was assimilated before or after the initiation of the CO_2 treatment 8 years prior to sampling, because CO_2 concentrations were raised by fumigating the experimental plots with pure CO_2 that was ^{13}C-depleted compared to ambient CO_2. Surprisingly, we conclude that sucrose addition primed

decomposition of relatively old SOC fractions, i.e. SOC assimilated more than 8 years before sampling.

1. Introduction

The global terrestrial soil organic carbon (SOC) pool is the largest terrestrial carbon (C) pool and constitutes a C stock that is more than twice the size of the atmospheric CO_2-C pool (IPCC, 2013). Therefore, even relatively moderate fluctuations in net C exchange between soil and atmosphere will impact the CO_2 concentration in the atmosphere profoundly. Faced by rising atmospheric CO_2 levels and the anticipated climatic changes that will result from this rise, we need to better understand how such changes will influence SOC decomposition and CO_2-release from terrestrial organic C pools.

At least two factors that can potentially alter SOC decomposition, namely nitrogen (N) availability and input of fresh plant C, are expected to change with rising CO_2 levels. Supply of fresh plant derived C into the soil matrix may accelerate the decomposition of SOC and decrease soil C stocks (Fontaine et al., 2004); a phenomenon termed 'the priming effect'. Priming effects induced by root exudation and rhizodeposition can cause an up to 350 % increase in SOC decomposition compared to the root free soil (Cheng et al., 2014). Even so, most C-cycling models do not consider the influence of priming and living roots on SOC decomposition rates (Cheng et al., 2014), perhaps as a result of limited knowledge about underlying mechanisms and factors influencing the magnitude of priming. However, the few attempts that have been made to date to represent plant-induced priming of SOC decomposition have resulted in improved model performance, also regarding global change effects (Cheng et al., 2014, Perveen et al., 2014). It is therefore evident that models predicting future climatic conditions and atmospheric CO_2 levels, as well as our means of mitigating the effects of rising CO_2 levels, depend on more in-depth understanding of feedbacks between climate, elevated atmospheric CO_2 levels, and SOC decomposition caused by priming.

Common plant physiological responses to elevated CO_2 comprise enhanced photoassimilation of C, increased root volume, and increased input of plant C to the soil in the form of root exudates and rhizodeposits (Hungate et al., 1997; Adair et al., 2009; Albert et al., 2011; Arndal et al., 2013, 2014). Since the extent of priming seems to depend on the concentration of labile C inputs, with no or low priming at low concentrations (Blagodatskaya & Kuzyakov, 2008; Gude et al., 2012) and gradually increasing priming with increasing concentrations (Blagodatskaya & Kuzyakov, 2008; Paterson & Sim, 2013) up to a point of saturation (Guenet et al., 2010; Xiao et al., 2015), it can be expected that priming will increase at elevated atmospheric CO_2 concentration due to higher inputs of labile plant C to soils (Paterson et al., 2008). Accordingly, elevated atmospheric CO_2 has been shown to increase decomposition of SOC in grasslands (Xie et al., 2005; Niklaus & Falloon, 2006) as well as forests (Phillips et al., 2012). However, there are also examples where input of labile plant C resulted in negative priming (Sullivan & Hart, 2013; Cheng et al., 2014), i.e. inhibited the decomposition of SOC. A possible reason to the contradictory findings is that the magnitude and direction of priming is dependent on the nutrient status of the soil. In fact, it has been suggested that the decomposition of SOC in response to input of labile C is driven by enhanced microbial demand for nutrients retained in soil organic matter (SOM) (Paterson, 2009; Philips et al., 2012). Accordingly, Bengtson et al. (2012) demonstrated a strong link between rhizodeposition, SOM decomposition and gross N mineralization in a coniferous forest soil, while Fontaine et al. (2004, 2011) found that soil C losses caused by priming increased when soil microbes are nutrient limited. In line with these findings, a recent meta-analysis concluded that input of labile C enhanced decomposition of native SOM, but only in soils with low nitrogen (N) content (Zhang et al., 2013). Since it has been observed that increased plant N demand at elevated atmospheric CO_2 commonly leads to decreased soil N availability (Luo et al., 2004; Larsen et al., 2011), this can also explain why priming can be expected to increase under elevated atmospheric CO_2 conditions.

However, in order to fully appreciate how priming will influence the net ecosystem exchange of C in a high CO_2 world we also need to consider climatic parameters, such as temperature and precipitation patterns that are also undergoing changes that are expected to continue for the decades to come (IPCC, 2013). In Northern Europe we expect an annual mean temperature increase between 0.75 and 0.1 °C over the coming 20 years and more extreme precipitation patterns, for instance prolonged summer droughts according to the IPCC RCP4.5 scenario (IPCC, 2013). Hence, evaluation of elevated CO_2 impacts on priming of SOC decomposition must consider projected temperature and precipitation scenarios.

Low soil moisture generally reduces soil microbial activity (Moyano et al., 2013) and may also reduce rhizosphere priming of SOC decomposition (Dijkstra & Cheng, 2007). It can, therefore, be expected that prolonged summer droughts will decrease priming. However, as plants have better water use efficiency at elevated CO_2 (Field et al., 1995; Ainsworth & Rogers, 2007), drought impacts on microbial activity are in some cases less severe when combined with elevated CO_2 (Kassem et al., 2008). The combined effect of elevated CO_2 and drought on the magnitude of priming is to our knowledge not known.

Likewise, little is known about temperature effects on priming. The only study to date that has systematically addressed the temperature dependency of priming found the process to be non-responsive to temperature variations (Ghee et al., 2013). In general, even moderate warming enhances the activity of heterotrophic microbial SOM decomposers (Wang et al., 2014). Therefore, in systems with low N availability temperature dependent stimulation of microbial activity could enhance the need for microbial N acquisition through SOM decomposition and increase priming. However, the priming response to warming may very well depend on soil moisture conditions, since warming enhances evaporation. This could potentially exacerbate negative effects of drought on soil microbial activity.

Previous studies demonstrated that elevated CO_2 changed C turnover dynamics of different fractions of SOM. Elevated CO_2 increased the content of recently assimilated C in both coarse and fine particulate fractions of SOM, but decreased the content of older C in more physically protected, fine particulate organic matter and mineral-associated organic matter (Hofmockel et al., 2011). This suggests that elevated CO_2 elicits priming of older relatively stable rather than recent SOC pools. In the current experiment we are able to test this hypothesis, as we raised the atmospheric CO_2 concentration by fumigating with CO_2 that was ^{13}C-depleted compared to the naturally occurring atmospheric CO_2. Therefore, C fixed in elevated CO_2 treatments was ^{13}C-depleted compared to the C assimilated before CO_2 treatment started and compared to the C pools of ambient CO_2 treatments (Reinsch & Ambus, 2013). A comparison of the isotopic composition of primed SOC-derived CO_2-C from elevated CO_2 and ambient CO_2 treatments can therefore reveal if primed C derives from C fixed before or after the initiation of CO_2 fumigation.

The aim of this study was to test the effects of long-term elevated CO_2 exposure, warming and annual extended drought events on potential priming in a nutrient-poor temperate heath/grassland, where long-term elevated CO_2 exposure has reduced the relative N content of organic inputs (Larsen et al., 2011; Arndal et al., 2013, 2014; Vestergård et al., 2015). Moderate warming has also prolonged the plant growth season with two weeks in the spring at the site (Kongstad et al., 2012). If an extended growth period also enhances plant N uptake over the season, this could potentially intensify microbial N demand. We hypothesize, in accordance with other reports (van Groenigen et al., 2005; Xie et al., 2005; Niklaus & Falloon, 2006), that potential priming of soil C is enhanced in soil exposed to elevated CO_2, where plant production and hence C input to the soil is enhanced and the relative N availability has declined. We further hypothesize that warming enhances priming in soil exposed to elevated CO_2, because we expect that an earlier onset of spring growth and

147 enhanced microbial activity under warming further reduced N availability. We hypothesize that
148 summer drought, which is expected to reduce microbial activity, will reduce priming in soil exposed
149 to elevated CO_2. Finally, we expect that warming augments this effect of drought. Further, we will
150 clarify if primed soil C is recently fixed or of older origin.
151
152 Addition of labile carbohydrates is a common method to assess and compare potential priming
153 activity between different soils and treatments (Wu et al., 1993; Zyakun & Dilly, 2005; Garcia-
154 Pausas & Paterson, 2011; Paterson & Sim, 2013; Reinsch et al., 2013). If the $^{13}C/^{12}C$ signature of
155 the added carbohydrate is distinct from that of native SOC it is possible to assess the contribution of
156 these two C sources to the respiratory CO_2 evolved. In the present study we estimated potential
157 priming by incubating soils with labile sucrose, a common constituent of root exudates (Grayston et
158 al., 1998), with a $^{13}C/^{12}C$ ratio that is distinct from the isotopic ratio of the native SOC.
159
160 **2. Materials and Methods**
161
162 *2.1 Field site*
163 Our field experiment was carried out in an unmanaged temperate heath/grassland in North Zealand
164 c. 50 km northwest of Copenhagen, Denmark (55°53'N, 11°58' E). The soil is a Cambic Arenosol
165 (FAO classification) developed on a nutrient-poor sandy deposit. The organic layer is 5-10 cm thick
166 and the pH is around 5. From 1975-2005 the average annual precipitation was 610 mm and the
167 mean annual temperature was 8 °C (Danish Meteorological Institute). From 2005 to 2013 the mean
168 annual precipitation was 742 mm with a range between 648 and 894 mm and the mean annual
169 temperature was 9.7 °C with a range between 7 and 10 °C. The prevailing species are the grass
170 *Deschampsia flexuosa* (c. 70 % coverage) and the dwarf shrub *Calluna vulgaris* (c. 30 % coverage)
171 intermixed with other grasses, herbs, mosses and lichens (Kongstad et al., 2012).

172

173 *2.2 Climate manipulation treatments*

174 Climate and CO_2 manipulations, aimed to simulate climatic conditions and atmospheric CO_2 levels
175 that are predicted for Denmark in 2075, were initiated in October 2005. The global change factors
176 drought (D), warming (T) and CO_2 concentration (CO_2) were manipulated individually and in all
177 possible combinations. The CO_2 concentration was increased with 120 ppm based on the average of
178 predicted concentrations in 2075 in five atmospheric CO_2 stabilization scenarios (SP450, SP550,
179 SP650, SP750 and SP1000) (IPCC 2007). The temperature increase chosen was the average of
180 predicted temperature responses for Northern Europe (IPCC 2007), and drought manipulations were
181 also based on the predictions in the IPCC 2007 report. The CO_2 concentration was increased *in-situ*
182 via the free-air carbon dioxide enrichment (FACE) technique in octagon shaped plots (octagons)
183 during daytime hours. Extended spring/summer droughts were imposed using moveable curtains to
184 exclude precipitation for a period of ~1 month during spring/early summer each year. Drought
185 curtains reduced precipitation by 7.6 ± 2.1 % (mean ± SD) annually. In 2013, the drought period
186 was conducted between 29th of April and 27th of May. From 27th of May to sampling, June 5th-6th,
187 the site only received a few mm precipitation, effectively extending the drought period until
188 sampling. Passive night time warming was achieved via moveable curtains that covered the
189 experimental plots during night time hours and prevented heat loss to the atmosphere. The warming
190 effect at 20 cm above ground surface ranged between 0.5 ^{o}C and 1.5 ^{o}C over the year (Scherber et
191 al., 2013). Warming curtains were withdrawn during rainfall.
192 The experiment is a full-factorial split plot design organized in 6 blocks. One block contains two
193 octagons each of 6.8 m diameter, one exposed to ambient CO_2 concentration and one exposed to
194 elevated CO_2 concentration, respectively. Each octagon is divided into four plots, which amounts to
195 a total of 48 plots. Within each octagon, one plot is subjected to the drought treatment, one is
196 subjected to warming, and a third plot is subjected to the combined drought and warming treatment.

197 The treatment, which is not subjected to any of the global change treatments, represents ambient
198 conditions (A). For further details regarding online measurements, treatments and experimental
199 setup, see Mikkelsen et al. (2008).
200 Characteristics of the soil in the treatments at sampling in June 2013 are shown in Table 1. SOC
201 content in 0-10 cm depth was higher at elevated CO_2 than at ambient CO_2, and at elevated CO_2
202 drought further increased the SOC content.
203
204 *2.3 Soil sampling and incubation*
205 Within each of the 48 plots, an undisturbed area of 0.5 m × 0.5 m was selected for this experiment.
206 Areas were chosen to contain an approximately equal amount of *C. vulgaris* and grasses (mainly
207 *D. flexuosa*) at initiation of the experimental setup in 2003. Unfortunately, an error in the
208 experimental procedures forced us to discard samples from one of the blocks, and we therefore
209 present data from five blocks (n = 5).
210 Soil cores were taken on the 5th-6th of June 2013 with an 8.7 cm diameter cylinder auger to 10 cm
211 depth. The soil was sieved (2 mm) in the field to separate roots from the soil and kept under cool
212 conditions (5 °C) until use. Two weeks after sampling, two 117 mL serum flasks per soil sample
213 were each added 4 g (fw) soil. We added 10 mL water or 10 mL of sucrose solution (4 g L^{-1}) to
214 each of the paired flasks, respectively. Assuming an average bulk density of 1.24 g cm^{-3} in the 10
215 top cm of the soil the sucrose added corresponds to an input of 572 g C m^{-2}. This is well above the
216 total annual C input to the soil, given that the annual net primary production roughly corresponds to
217 350 g C m^{-2} (Chapin III et al., 2002). Hence, during the incubation, the soil was fully water
218 saturated, and microorganisms were at no risk of experiencing C limitation. We used sugar cane-
219 derived sucrose with a $^{13}C/^{12}C$ ratio of $\delta^{13}C$ = -12 ‰, as it is distinct from the $\delta^{13}C$ value (-28.5 ‰)
220 of the C3 plants in the area (Reinsch & Ambus, 2013). This enables us to distinguish between CO_2
221 derived from the added sucrose and from SOC. To eliminate the initial CO_2-content of the flasks,

the flasks were sealed, evacuated (< 10% air remaining) and refilled to atmospheric pressure with CO_2-free atmospheric air (Alphagaz Luft 1, Air Liquide, Denmark). Evacuation and refilling was repeated twice and finally 20 mL extra CO_2-free air was added. We incubated the flasks on a shaker (4 h, 20 °C). Following incubation, we sampled 19 mL headspace gas with a syringe and flushed an evacuated 5.9 ml Exetainer vial (Labco Scientific, High Wycombe, UK) with the gas sample leaving the vial at atmospheric pressure for subsequent analyses of CO_2 concentration and isotopic $^{13}C/^{12}C$ ratio. Likewise, we sampled gas from four background control flasks, which only contained 10 mL water or 10 mL sucrose solution.

2.4 Soil and sucrose analysis

We dried 10 g soil at 103 °C to determine gravimetric soil water content. The total C content and $^{13}C/^{12}C$ isotopic ratio of soil and sucrose were measured in dried samples by Dumas combustion (1020 ºC) on an elemental analyser (CE 1110, Thermo Electron, Milan, Italy) coupled in continuous flow mode to a Finnigan MAT Delta PLUS isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany). Homogenized portions of 2 mg (sucrose) or 15 mg (soil) were weighed out into tin combustion cups for elemental analysis. Acetanilide (Merck, Darmstadt, Germany) and soil standards (Elemental Microanalysis, Okehampton, UK) were used for elemental analyser mass calibration. As working standard for isotope ratio analysis we used pure CO_2 gas calibrated against certified reference ^{13}C-sucrose (IAEA, Vienna, Austria). Performance of analysis (Qa/Qc) was assessed by the inclusion of reference samples of biological origin (Peach leaves (NIST 1547), National Institute of Standards and Technology, Gaithersburg, MD, USA).

2.5 CO_2 concentration and $^{13}C/^{12}C$ isotopic ratio

CO_2 concentrations and isotopic $^{13}C/^{12}C$ ratio were analysed on a DeltaV Advantage Isotope Ratio Mass Spectrometer (Thermo Scientific, Bremen, Germany) coupled in continuous flow mode to a

GasBench II. Two calibration gas mixtures of CO_2 in synthetic air were included in the analytical runs, *viz*. 362 ppm CO_2 at $\delta^{13}C$ = –2.7 ‰ vs. Vienna Pee Dee Belemnite (VPDB) and 356 ppm CO_2 at $\delta^{13}C$ = –29.3 ‰ (Messer Denmark, Padborg, Denmark). CO_2 concentrations and $\delta^{13}C$ were corrected according to the values measured in the background control treatments.

2.6 Data analysis

Because we eliminated the initial CO_2 content of the flasks before the soil incubation, we assessed respiration rates based on the CO_2 concentration measured after the incubation period. We calculated the sucrose-induced respiration as the difference between CO_2-C evolved in sucrose-amended and non-amended flasks.

We calculated the proportion of SOC-derived respiratory C (P_{SOC}) and sucrose-derived respiratory C (P_{suc}) in sucrose-amended flasks using a two end-member-mixing-model:

[Equation 1] $$P_{!"\#} = \frac{!^{!"}!_{!"\#\$}!!^{!"}!_{!"\#}}{!^{!"}!_{!"\#}!!^{!"}!_{!"\#}},$$

[Equation 2] $$P_{!"\#} = 1 - P_{!"\#} = 1 - \frac{!^{!"}!_{!"\#\$}!!^{!"}!_{!"\#}}{!^{!"}!_{!"\#}!!^{!"}!_{!"\#}},$$

where $\delta^{13}C_{samp}$ denotes the isotopic value of the CO_2 evolved in flasks amended with sucrose, $\delta^{13}C_{SOC}$ denotes the isotopic value of soil C, and $\delta^{13}C_{suc}$ is the isotopic value of the added sucrose (-12 ‰).

By calculating the total SOC-derived CO_2 evolved in sucrose-amended flasks and subtracting the basal respiratory CO_2 evolved, i.e. the CO_2 produced in non-amended flasks, we can calculate the amount of SOC primed in response to sucrose addition as follows:

[Equation 3] $\mathrm{C\ primed} = \mathrm{P_{!"\#}} \times [\mathrm{CO_{!"\#\$}}] - [\mathrm{CO_{!"!\#}}]$,

where $[CO_{2suc}]$ and $[CO_{2H2O}]$ denote the CO_2 evolved in flasks with and without sucrose, respectively. We present the priming effect as the increase in SOC-derived CO_2 with sucrose-amendment in relation to the SOC content of individual samples.

To assess whether primed C for elevated CO_2 octagons derived from C assimilated before or after the initiation of the FACE in 2003, we calculated the $\delta^{13}C$ of the primed SOC-derived CO_2, $\delta^{13}C_{primed}$. First, we calculated the $\delta^{13}C$ of the sucrose-induced respiratory CO_2, i.e. the additional CO_2 produced from sucrose and from SOC priming in response to sucrose addition, as the difference in ^{13}C respired with and without sucrose divided by the difference in CO_2 produced with and without sucrose:

[Equation 4] $\delta^{!"}\mathrm{C\ of\ sucrose\text{-}induced\ CO_!\text{-}production} = \frac{[!!_{!"\#\$}]\times!^{!"}!_{!"\#}-[!!_{!"!\#}]\times!^{!"}!_{!"\#}}{[!!_{!"\#\$}]-[!!_{!"!\#}]}$,

where subscripts “suc” and “H2O” denote CO_2 evolved in flasks with and without sucrose addition, respectively.

At the same time, $\delta^{13}C$ of sucrose-induced CO_2-production can also be expressed in relation to the proportional contribution of sucrose and SOC to the sucrose-induced CO_2-production:

293 [Equation 5] $\delta^{13}C$ of sucrose-induced CO_2-production $= P^*_{suc} \times \delta^{13}C_{suc} + P^*_{SOC} \times \delta^{13}C_{SOC\&}$,

294

295 where P^*_{suc} and P^*_{SOC} denote the proportions of sucrose-derived and SOC-derived CO_2 in the

296 sucrose-induced respiration, respectively. We know all the variables of equation 4, and by

297 combining equations 4 and 5, we can express $\delta^{13}C_{primed}$ as follows:

298

299 [Equation 6]

300 $$\delta^{13}C_{SOC\&} = \frac{\frac{[CO_2]\times\delta^{13}C - [CO_2]\times\delta^{13}C}{[CO_2]-[CO_2]} CO_2^* - \delta^{13}C \times CO_2}{CO_2^*} =$$

$$\frac{\frac{[CO_2]\times\delta^{13}C - [CO_2]\times\delta^{13}C}{[CO_2]-[CO_2]} CO_2^* - \delta^{13}C \times CO_2}{CO_2^*}.$$

301

302 We know that $\delta^{13}C_{suc}$ is -12 ‰, so we must calculate P^*_{suc}. We calculate P^*_{suc} based on the sucrose-

303

304 derived CO_2, $CO_{2sucrose\text{-}derived}$, and the total sucrose-induced respiration, i.e. the difference between

305 CO_2 evolved in sucrose-amended and non-amended flasks. This is possible, because we can

306 calculate $CO_{2sucrose\text{-}derived}$ as the product of the proportion of the sucrose-derived respiratory CO_2

307 (P_{suc}) in sucrose-amended flasks and the CO_2 evolved in sucrose-amended flasks:

308

309 [Equation 7] $P^*_{suc} = \frac{[CO_{2\,sucrose\text{-}derived}]}{[CO_2]\,[\delta^{13}C]} = P_{suc} \times \frac{[CO_2]}{[CO_2]\,[\delta^{13}C]}$.

310

311 We know P_{suc} from equation 1; hence:

312

313 [Equation 8] $P^*_{suc} = \frac{\delta^{13}C_{suc}\,CO_2 - \delta^{13}C_{SOC}\,CO_2}{\delta^{13}C_{suc}\,CO_2 - \delta^{13}C_{SOC}\,CO_2} \times \frac{[CO_2]}{[CO_2]\,[\delta^{13}C]}$,

314

and by inserting equation 8 in equation 6, we find that $\delta^{13}C$ of the primed SOC, $\delta^{13}C_{primed}$ can be calculated accordingly:

[Equation 9] $$\delta^{13}C_{primed} = \frac{\frac{[\delta^{13}C_{mix}\times(C_{mix}+C_{soil})]-[\delta^{13}C_{soil}\times C_{soil}]}{[(C_{mix}+C_{soil})-C_{soil}]}(C_{mix}+C_{soil})\times\frac{[(C_{mix}+C_{soil})-C_{soil}]}{[\delta^{13}C_{mix}\times(C_{mix}+C_{soil})]-[\delta^{13}C_{soil}\times C_{soil}]}\times\delta^{13}C_{soil}}{(C_{mix}+C_{soil})\frac{[\delta^{13}C_{mix}\times(C_{mix}+C_{soil})]-[\delta^{13}C_{soil}\times C_{soil}]}{[(C_{mix}+C_{soil})-C_{soil}]}\times\frac{[(C_{mix}+C_{soil})-C_{soil}]}{(C_{mix}+C_{soil})}}.$$

For one of the soil samples from the ambient treatment the $\delta^{13}C$ value of CO_2 evolved in the flasks was unexpectedly high, which suggests that the CO_2 partly originated from carbonate C. We therefore omitted this sample from data analyses. We tested the effects of elevated CO_2, drought and warming on all response variables with full factorial three-way ANOVAs. Homogeneity of variance was assessed with the Brown-Forsythe test. Data for basal respiration rate were log transformed prior to analysis to obtain homogeneity of variance. All statistical analyses were executed in Sigma Plot version 13.0.

3. Results

The soil-weight-specific basal respiration and sucrose-induced respiration, i.e. the extra CO_2 produced in sucrose-amended flasks compared to non-amended flasks, was on average c. 50 % higher in soil from elevated CO_2 plots, and this increase was more pronounced when drought and elevated CO_2 were combined (Table 2 and 3). This is in line with the higher SOC content at elevated CO_2, which was also highest when elevated CO_2 and drought were combined (Table 1). In contrast, warming did not affect basal or sucrose-induced respiration. The basal decomposition of SOC, expressed as the respiration activity per g SOC, was independent of treatments (Table 2 and 3). Likewise, the SOC-specific sucrose-induced respiration activity did not differ between treatments (Table 2 and 3).

339 Sucrose addition enhanced decomposition of native SOC (Fig. 1), hence priming occurred. At
340 ambient CO_2, sucrose enhanced the SOC decomposition rate with 35-49 μg C g SOC^{-1} h^{-1}. The
341 priming effect was significantly higher at elevated CO_2 (Table 3), where sucrose addition enhanced
342 SOC decomposition rate with 43-59 μg C g SOC^{-1} h^{-1}. There was a tendency towards reduced
343 priming in soils exposed to drought (P=0.11), but warming did not affect the level of priming (Fig.
344 1, Table 3).
345
346 As a consequence of the eight years of FACE with pure ^{13}C-depleted CO_2, the $\delta^{13}C$ of the total CO_2
347 efflux from elevated CO_2 soil (Fig. 2a) was significantly 3.0-5.4 ‰ lower than from ambient CO_2
348 soil in flasks without sucrose (Table 3). Addition of sucrose with the higher $\delta^{13}C$ of -12 ‰ raised
349 the $\delta^{13}C$ of the CO_2 evolved during the four-hour incubation. Nevertheless, CO_2 from elevated CO_2
350 octagons in the sucrose-amended flasks was still significantly 1.2-2.0 ‰ lower than CO_2 evolved
351 from ambient CO_2 soil (Fig. 2b, Table 3).
352 In contrast to the total CO_2 efflux (Fig. 2), the $\delta^{13}C$ of primed SOC-derived CO_2-C from elevated
353 CO_2 soil was not lower than the $\delta^{13}C$ of primed SOC-derived CO_2-C from ambient CO_2 soil
354 (Fig. 3). At ambient CO_2, the $\delta^{13}C$ of primed CO_2-C released from soils that were not exposed to
355 warming was significantly lower than the $\delta^{13}C$ of primed C in all other treatments (Fig. 3, Table 3).
356

357 **4. Discussion**

358 The soil C content in the upper 0-10 cm of the soil profile had increased with 12-22 % after eight
359 years of elevated CO_2 exposure in treatments without experimental drought exposure, and drought
360 further increased soil C content at elevated CO_2 (Table 1), which is consistent with the increased
361 root production at elevated CO_2 recorded in 2009-2010 at the same field site (Arndal et al., 2013).
362 This build-up of organic C resulted in larger basal and sucrose-induced respiration activities
363 expressed per soil weight, whereas the SOC-specific respiration did not respond to any of the

364 treatments (Table 2). Stimulating effects of elevated CO_2 on soil respiration rates (Fig. 1a) have
365 been reported at the current site of this investigation (Selsted et al., 2012), and are also well
366 described from other studies (Zak et al., 2000, van Groenigen et al., 2014).
367
368 As we hypothesized, the priming effect was more pronounced in soils exposed to elevated CO_2
369 (Fig. 1). With the increased soil C content and C:N ratios of aboveground (Vestergård et al., 2015)
370 and belowground (Arndal et al., 2013, 2014) organic inputs at elevated CO_2, it is likely that the
371 microbial N demand increased. Consequently, the enhanced priming and mineralization of SOC
372 may be a result of increased microbial N mining (Dijkstra et al. 2013; Chen et al. 2014). In line with
373 this, soils from elevated CO_2 plots at our field site exhibited higher activity of enzymes involved in
374 SOC degradation (Partavian et al., 2015). In a previous laboratory set-up with soil from the current
375 field site, Reinsch et al. (2013) assessed the temporal development of glucose-induced priming over
376 two weeks at 8°C and also found positive priming induced by labile C (glucose), with stronger
377 effects at elevated CO_2. The similar outcomes of the two studies demonstrate that the short-term (4
378 h) immediate priming response to labile C input, i.e. the priming capacity of the inherent microbial
379 community prior to microbial growth on the added labile substrate, is a relevant indicator also of
380 long-term priming effects. While Reinsch et al. (2013) only examined the occurrence of priming in
381 soils from a subset of the field treatments, i.e. the ambient (A), elevated CO_2 (CO_2) and the full
382 combination of all treatment factors ($TDCO_2$), we assessed the effects of all possible combinations
383 of the global change factors, i.e. elevated CO_2, warming and drought, on priming responses. In the
384 longer-term experiment, the priming effect diminished in soils exposed to elevated CO_2, drought
385 and warming in combination (Reinsch et al., 2013). In the present study we did not find a
386 comparable significant interaction between the three global change factors and potential priming of
387 SOC, although we note that the priming response in soils exposed to drought tended to be lower
388 than in soils that were not subjected to experimental drought.

389 In our investigation, we incubated soil samples from the different field treatments under
390 standardized conditions with respect to moisture, temperature and CO_2. Hence, we address whether
391 the long-term field manipulation of climate accommodated changes in the microbial decomposition
392 of SOC, which could be caused by altered availability and quality of SOC and N and/or altered
393 microbial community activity or composition. We sampled the soil immediately after the annual
394 drought treatment, where the water content in drought plots was still significantly reduced
395 (Table 1), and we hypothesized that reduced microbial activity after the drought would impair
396 priming. However, contrary to our expectation, both basal respiration and sucrose-induced
397 respiration per soil weight were enhanced by drought in combination with elevated CO_2, and
398 drought tended to increase the SOC-specific respiration (Table 2). This probably reflects a high
399 turnover of drought-decimated microorganisms upon re-wetting in the incubation experiment
400 (Groffman & Tiedje, 1988). On the other hand, drought tended to reduce the sucrose-induced
401 priming of SOC (Fig. 1). The stimulation of microbial activity upon re-wetting of soils after a
402 severe drought event thus appears uncoupled from the microbial priming of SOC in response to
403 labile C input.
404 Contrary to our hypothesis, long-term warming did not affect basal respiration, sucrose induced
405 respiration (Table 2), or potential priming (Fig. 1). In the field, warming enhanced microbial
406 abundance (Larsen et al., 2011, Haugwitz et al., 2014) and initiated earlier plant growth in the
407 spring (Kongstad et al., 2012). We expected this to result in decreased N availability, which would
408 be reflected in increased priming (Fontaine et al., 2004, 2011; Zhang et al., 2013), but we found no
409 evidence for this hypothesis. A possible reason is that eight years of warming and earlier onset of
410 spring growth of plants did not decrease soil N availability sufficiently to influence priming.
411 Further, at the field site the warming treatment only raised mean soil temperatures at 5 cm depth by
412 0.1-0.2 °C over the 3 months preceding the soil sampling (Vestergård et al., 2015). This is hardly a
413 temperature increase that would stimulate microbial activity considerably.

414

415 It is remarkable that the $\delta^{13}C$ of respiratory CO_2 derived from SOC priming in soils exposed to eight
416 years of elevated CO_2 with reduced ^{13}C was not lower than in ambient CO_2 soils (Fig. 3). In contrast,
417 the isotopic composition of total respired CO_2 from soils exposed to elevated CO_2 was ^{13}C-
418 depleted compared to CO_2 evolved from ambient CO_2 soils (Fig. 2). This shows that C assimilated
419 in the elevated CO_2 treatments was indeed decomposed in the soil basal respiration, whereas this
420 pool of newly assimilated C was not subject to primed decomposition; hence its decomposition was
421 apparently not energy limited. This implies that the primed C was assimilated more than eight years
422 before sampling. We can therefore add evidence to support previous statements that elevated CO_2
423 induces decomposition of older soil C (van Groenigen et al., 2005; Xie et al., 2005; Niklaus &
424 Falloon, 2006). Likewise, elevated CO_2 enhanced the formation of coarse particulate SOM (fresh
425 SOM) and decreased the fraction of physically protected SOM (old SOM) in forest soil (Hofmockel
426 et al., 2011) and in prairie soil (Procter et al., 2015). Given that old SOM pools contain significant,
427 yet (to a large extend) physically and chemically protected N stocks, this lends support to the
428 hypothesis that priming in response to labile C supply is a mechanism by which (some)
429 microorganisms gain access to a reservoir of N to meet their enhanced N demand under conditions
430 of ample C supply (Dijkstra et al., 2013; Chen et al., 2014). If enhanced priming at elevated CO_2 is
431 caused by increased microbial N demand, because more SOM with lower relative N content enters
432 the system at elevated CO_2, it is reasonable that priming should be directed towards SOM pools
433 with a higher N content, i.e. SOM pools incorporated into the system before the elevated CO_2
434 treatment was initiated.
435 Bulk SOC encompasses different pools of SOC of varying age and particle size, and the $\delta^{13}C$ of
436 these different pools vary considerably (Gerzabek et al., 2001). As expected, the SOC $\delta^{13}C$ was
437 decreased from -27.8 ‰ in ambient CO_2 soil to -29.3 ‰ in elevated CO_2 soil, whereas drought and
438 warming did not affect the isotopic composition of SOC. The lower $\delta^{13}C$ of the C primed in the

439 ambient plots and plots subjected to drought as a single factor compared to the other treatments
440 (Fig. 3), therefore suggests that sucrose-amendment primed the decomposition of different SOC
441 pools in the different treatments.
442 It has been argued that short-term incubations as employed in the current study reflects 'apparent'
443 rather than 'real' priming effects. Theoretically, apparent priming is a state, where the initial
444 enhanced respiratory pulse induced by labile C addition, derives from turn-over of microbial
445 biomass C rather than decomposition of SOC, i.e. part of the inherent microbial biomass C pool is
446 substituted by the added labile C. 'Real priming', on the other hand, describes the enhanced
447 decomposition of SOC after prolonged incubation with labile C (Blagodatskaya & Kuzyakov, 2008;
448 Blagodatsky et al., 2010). We argue, though, that the finding that the primed C was at least eight
449 years old is strong indication that even in our short-term incubation study, the addition of labile C
450 resulted in real priming; i.e. the enhanced decomposition of SOC. If the sucrose-induced priming
451 did indeed represent apparent priming, it would imply that the pool-substituted microbial biomass C
452 was more than eight years old. Microbial biomass turnover is on average much faster than eight
453 years, and it is quite unlikely that microorganisms grow preferentially on older C pools. Therefore,
454 we find it most plausible that the enhanced soil-derived CO_2-C flux represents real priming of SOC
455 rather than pool substitution of microbial biomass C.
456 It has been suggested that increased primary production at elevated CO_2 will enhance C
457 sequestration in terrestrial ecosystems and thus counteract the rise in atmospheric CO_2
458 concentration (Oren et al., 2001; Jastrow et al., 2005; Houghton, 2007). However, this and other
459 studies (Carney et al., 2007; van Groenigen et al., 2014) demonstrate that ecosystems exposed to
460 elevated CO_2 concentrations will be more prone to SOC decomposition triggered by labile C input.
461 This will thus reduce the anticipated increase in C sequestration. In our heath/grassland system
462 elevated CO_2 did enhance the C input to the system and hence the SOC pool (Table 1). However,
463 we demonstrate that labile C inputs accelerate the turnover of older SOC pools and alter C

dynamics of the system under elevated CO_2. Therefore, in the longer term, the net C balance of this and other systems in a high CO_2 world will depend on the extent to which the build up of new organic C will compensate for the increased loss of older organic C pools.

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731 **Figure legends**

732 Figure 1

733 Mean priming effect assessed as the increase in SOC decomposition rate in soils amended with
734 sucrose compared to soils incubated with water. Soils were collected from field plots in a Danish
735 grass/heathland exposed to 8 years of ambient conditions (A), annually repeated spring/early
736 summer drought (D), warming (T), elevated atmospheric CO_2 (CO2) and all possible combinations
737 of single factors. Error bars depict SE. *n*=4-5.

738

739 Figure 2

740 Mean $\delta^{13}C$ values of CO_2-C respired during incubation of soils collected from field plots in a
741 Danish grass/heathland exposed to 8 years of ambient conditions (A), annually repeated
742 spring/early summer drought (D), warming (T), elevated atmospheric CO_2 (CO2) and all possible
743 combinations of single factors. Soils were incubated with water (a) or with a sucrose solution (b).
744 Error bars depict SE. *n*=4-5.

745

746 Figure 3

747 Mean $\delta^{13}C$ values of SOC-derived CO_2-C primed by sucrose addition during incubation of soils
748 collected from field plots in a Danish grass/heathland exposed to 8 years of ambient conditions (A),
749 annually repeated spring/early summer drought (D), warming (T), elevated atmospheric CO_2 (CO2)
750 and all possible combinations of single factors. Treatments A and D, marked with asterisk, are
751 significantly different from the other treatments (Tukey $P<0.05$). Error bars depict SE. *n*=4-5.

752

753

Table 1. Mean water content and C content in soils collected from field plots in a Danish grass/heathland exposed to 8 years of ambient conditions (A), annually repeated spring/early summer drought (D), warming (T), elevated CO_2 (CO_2) and all possible combinations of single factors. SE depicted in parentheses. *n*=4-5.

	Soil water content (%)		Soil C content (%)	
A	10.30	(0.42)	3.15	(0.29)
D	8.04	(1.32)	3.08	(0.32)
T	10.69	(1.00)	3.00	(0.23)
TD	6.71	(0.53)	2.89	(0.23)
CO_2	11.86	(0.50)	3.52	(0.33)
DCO_2	9.27	(0.69)	4.80	(0.41)
TCO_2	11.68	(1.16)	3.65	(0.60)
$TDCO_2$	11.24	(2.50)	5.75	(1.28)
Treatment effects	P_{CO2}=0.024 P_D=0.012		P_{CO2}=0.002 P_{CO2xD}=0.038	

Table 2. Mean basal and sucrose-induced respiration in relation to soil dry weight and soil organic C (SOC) content during incubation of soils collected from field plots in a Danish grass/heathland exposed to 8 years of ambient conditions (A), annually repeated spring/early summer drought (D), warming (T), elevated atmospheric CO_2 (CO_2) and all possible combinations of single factors. Sucrose-induced respiration is the difference between respiration activity in sucrose-amended and control samples. SE depicted in parentheses. *n*=4-5.

	Basal respiration ($\mu g\ CO_2$-C g $soil^{-1}\ h^{-1}$)		Sucrose-induced respiration ($\mu g\ CO_2$-C g $soil^{-1}\ h^{-1}$)		SOC-specific basal respiration ($\mu g\ CO_2$-C g $SOC^{-1}\ h^{-1}$)		SOC-specific sucrose-induced respiration ($\mu g\ CO_2$-C g $SOC^{-1}\ h^{-1}$)	
A	2.05	(0.38)	5.07	(0.51)	63.44	(6.45)	160.51	(3.57)
D	2.00	(0.31)	4.69	(0.78)	65.63	(10.02)	158.85	(30.94)
T	1.97	(0.24)	5.00	(0.53)	65.34	(4.99)	166.12	(9.85)
TD	1.84	(0.21)	3.98	(0.37)	64.05	(4.95)	138.67	(8.36)
CO_2	2.17	(0.16)	5.76	(0.73)	62.76	(4.45)	162.63	(9.50)
DCO_2	3.51	(0.47)	8.23	(1.04)	72.45	(5.20)	169.88	(11.04)
TCO_2	2.27	(0.49)	5.59	(1.03)	61.22	(5.32)	152.83	(13.68)
$TDCO_2$	4.39	(0.81)	8.49	(1.10)	80.18	(6.41)	162.39	(17.79)

Table 3. ANOVA table of effects of elevated CO_2 (CO_2), annually repeated spring/early summer drought (D), warming (T) and their interactions on respiration activity, sucrose-induced respiration activity, priming of soil organic C, isotopic composition of respiratory CO_2 evolved during 4 h incubation without or with sucrose and isotopic composition of SOC-derived primed C.

		Soil-weight specific basal respiration		Soil-weight specific sucrose-induced respiration		SOC-specific basal respiration		SOC-specific sucrose-induced respiration		Priming		δ^{13}C‰ of CO_2 without sucrose		δ^{13}C‰ of CO_2 with sucrose		δ^{13}C‰ of primed SOC-derived CO_2	
Source	*df*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
CO_2	1	13.70	**<0.001**	16.15	**<0.001**	1.23	0.275	0.42	0.520	8.61	**0.006**	65.22	**<0.001**	47.14	**<0.001**	5.97	**0.020**
D	1	5.88	**0.021**	2.93	0.097	2.61	0.116	0.369	0.548	2.65	0.114	4.13	0.051	0.02	0.888	3.58	0.068
T	1	0.04	0.835	0.09	0.771	0.16	0.691	0.46	0.504	0.77	0.386	1.02	0.320	0.07	0.793	1.27	0.269
D x CO_2	1	7.75	**0.009**	8.47	**0.007**	2.80	0.105	1.65	0.209	0.72	0.402	0.15	0.698	0.29	0.594	0.51	0.481
T x CO_2	1	0.29	0.592	0.14	0.709	0.02	0.900	0.10	0.758	2.32	0.138	3.03	0.091	1.44	0.239	4.40	**0.044**
T x D	1	0.13	0.718	0.01	0.926	0.14	0.711	0.13	0.721	1.71	0.200	0.25	0.621	1.02	0.320	0.12	0.730
T x D x CO_2	1	0.32	0.576	0.21	0.650	0.41	0.528	0.18	0.678	0.12	0.735	0.18	0.675	0.32	0.578	0.53	0.472
Error	31																

Figure 1

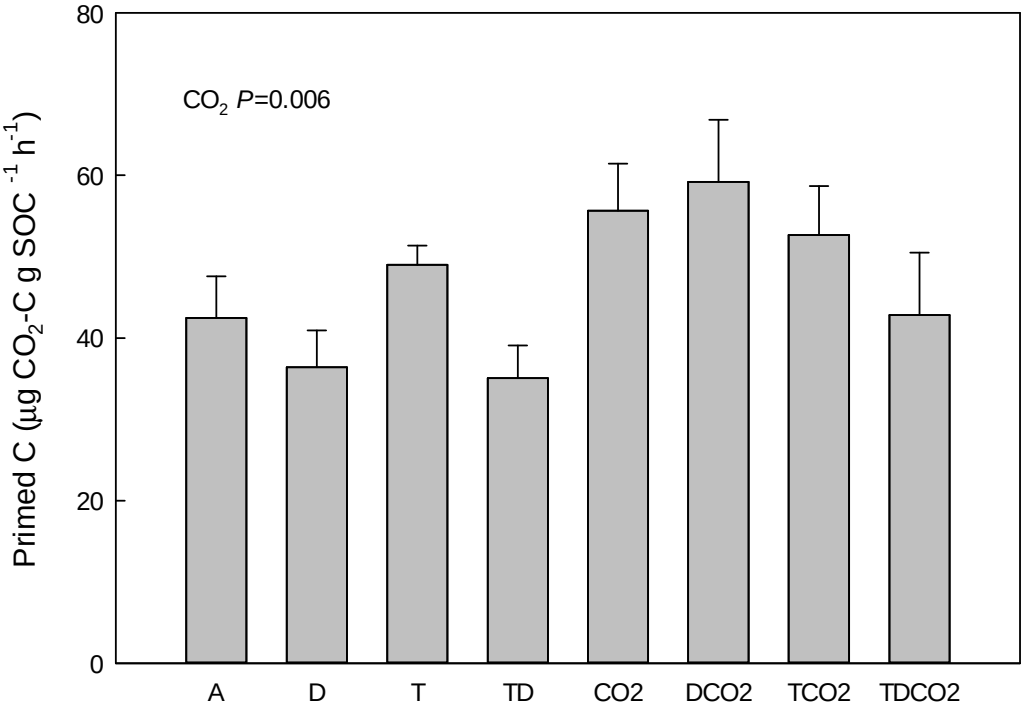

80
60
40
20
0
CO2 P=0.006
Primed C (µg CO2-C g SOC -1 h-1)
A
D
T
TD
CO2
DCO2
TCO2
TDCO2

Figure 2

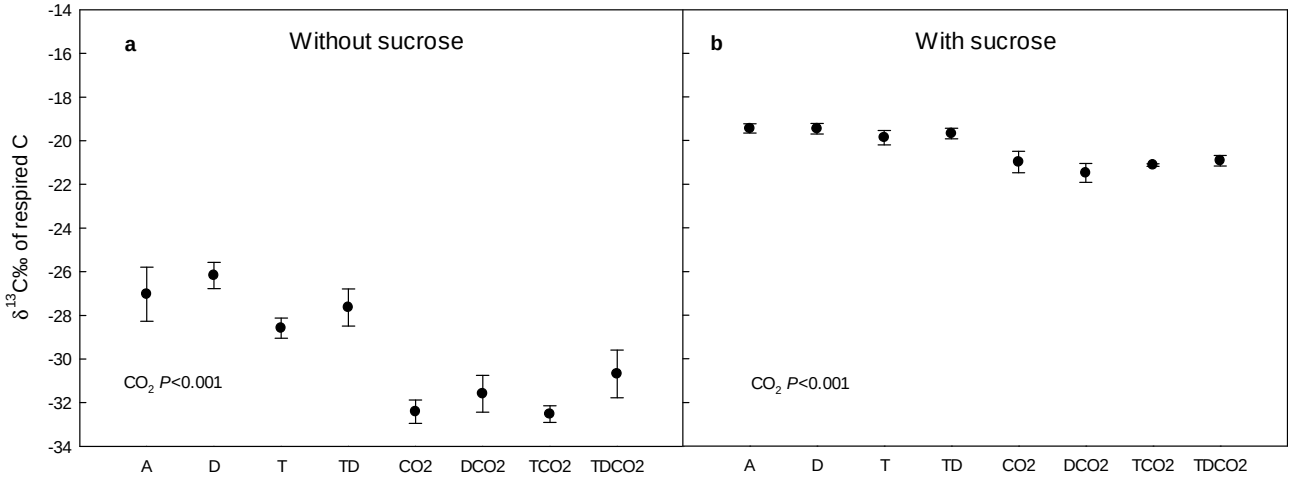
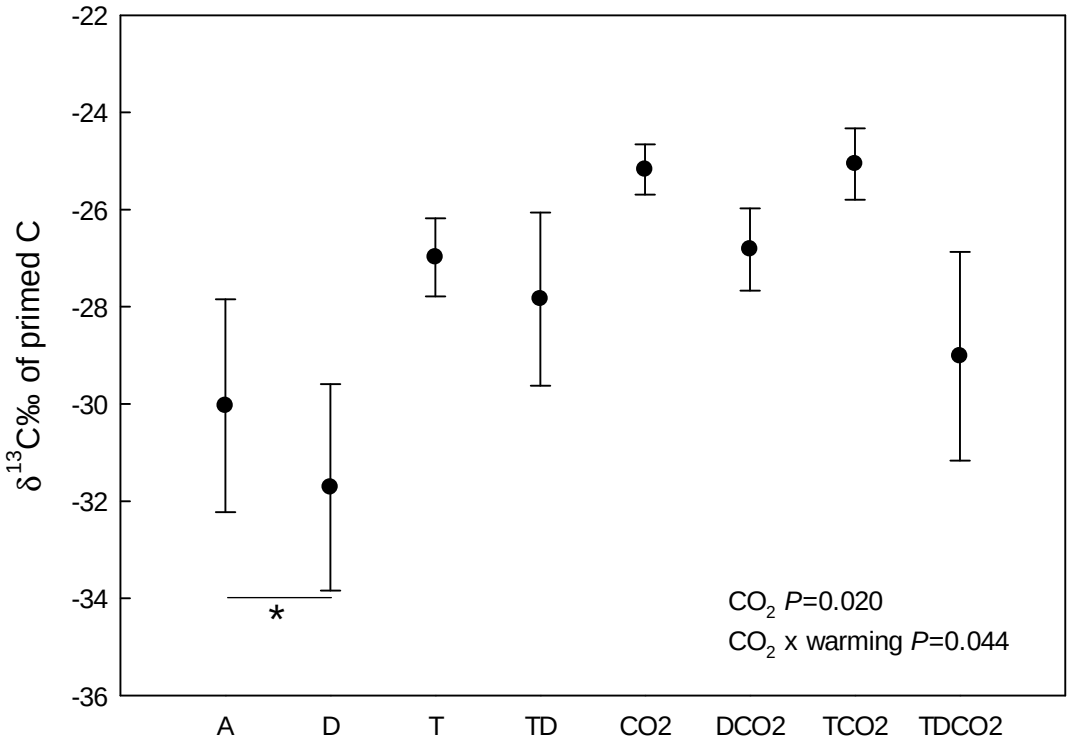

a
Without sucrose
b
With sucrose
δ13C‰ of respired C
-14
-16
-18
-20
-22
-24
-26
-28
-30
-32
-34
CO2 P<0.001
CO2 P<0.001
A
D
T
TD
CO2
DCO2
TCO2
TDCO2

Figure 3


-22
-24
-26
-28
-30
-32
-34
-36
δ13C‰ of primed C
*
CO2 P=0.020
CO2 x warming P=0.044
A
D
T
TD
CO2
DCO2
TCO2
TDCO2