Do rhizosphere priming effects enhance plant nitrogen uptake under elevated CO₂?

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13 **Abstract**

14 Numerous studies suggest that rhizosphere priming can mediate decomposition of soil organic
15 matter (SOM), but direct evidence of priming-induced soil SOM decomposition on plant N uptake
16 under elevated CO₂ (eCO₂) is very rare. By using a continuous dual labelling technique with
17 ¹³C-depleted CO₂ and ¹⁵N-enriched soil, we investigated priming of SOM decomposition and its
18 relationship with plant N uptake of C4 and C3 grasses from a grassland ecosystem under eCO₂. We
19 observed that eCO₂ induced increases in plant biomass, plant N uptake, rhizosphere priming, and
20 total SOM decomposition in both grasses at an early plant life stage. Increased total SOM
21 decomposition was positively related with plant N uptake by both C4 and C3 grasses under eCO₂.
22 However, the C3 grass was more dependent on N acquired from rhizosphere priming of SOM than
23 the C4 grass. Our findings highlight that plant N uptake could be enhanced under eCO₂ via
24 accelerated SOM decomposition, and rhizosphere priming effects on SOM decomposition could
25 play a more important role in N availability of the C3 grass in comparison with the C4 grass.

26

27 **Keywords**

28 Decomposition; elevated CO₂; plant N uptake; rhizosphere priming; soil organic matter; stable
29 isotopes

30 **1. Introduction**

31 Plants play important roles in exchanging ecosystem-atmosphere C and regulating climate (Le
32 Quéré et al., 2015). Elevated CO₂ (eCO₂) has been found to increase plant production and promote
33 C allocation to plant rhizospheres, which could help to mitigate rising atmospheric CO₂
34 concentration (Luo et al., 2006; Nie et al., 2013a). As suggested by the progressive nitrogen
35 limitation hypothesis, however, the positive response of plants to eCO₂ may decline over time as
36 soil N becomes increasingly limiting under eCO₂ (Luo et al., 2004). As a result, sustained growth of
37 plants under eCO₂ might require supply of exogenous N to meet the high N requirement of plants.

38 Recent studies suggest that interactions between plant roots and soil can increase SOM
39 decomposition, thereby enhancing N mineralization from SOM under eCO₂ (Dijkstra et al., 2008;
40 Phillips et al., 2011). This positive feedback of soil N cycling to eCO₂ is mainly explained by
41 eCO₂-induced increase in plant rhizodeposition coupled with an increase in belowground
42 decomposition activity (Kuzyakov 2002; Cheng et al., 2014). Therefore, priming of SOM
43 decomposition may considerably affect soil N supply and feedback to plant N availability (Hu et al.,
44 2001). However, most experiments testing rhizosphere priming effects have focused primarily on
45 soil respiration and the ecosystem C budget (Kuzyakov 2002; Cheng et al., 2014). Our
46 understanding of the effect of priming-induced change in SOM decomposition on plant N
47 availability is limited, despite its importance for long-term ecosystem sustainability under climate
48 change (Gardenas et al., 2011; Dijkstra et al., 2013; Cheng et al., 2014).

49 A growing body of evidence suggests that plant priming effects have positive effects on plant N
50 availability in various ecosystems under eCO₂. For example, Phillips et al. (2011) found that an

51 eCO₂-induced increase in root-derived C was positively correlated with microbial release of
52 extracellular enzymes involved in N mineralization in a pine forest. Zak et al. (2011) applied an
53 inorganic ¹⁵N dilution method to show a positive feedback of soil N cycling to increased plant
54 productivity due to eCO₂ in northern forests. In addition, Carrillo et al. (2014) found that
55 eCO₂-induced change in SOM decomposition can alter plant tissue N concentration and its
56 stoichiometry in a grassland. However, direct evidence of effects of priming-mediated soil SOM
57 decomposition on plant N uptake which largely determines N availability is very rare (Gardenas et
58 al. 2011, Cheng et al. 2014). Efforts to disentangle the feedbacks of plant N uptake to eCO₂ would
59 greatly improve our understanding of the close linkage between rhizosphere priming effects and
60 plant N availability under climate change.

61 Grassland ecosystems, covering 40% of the Earth's land surface, support many productive
62 agricultural and natural areas, and provide habitat and food for livestock and humans (Morgan et al.,
63 2008; Gibson 2009). In particular, the continued ability of grasslands to provide sufficient nutrients
64 to livestock as climate changes is uncertain (Morgan et al., 2008). For example, in a North
65 American grassland, eCO₂ altered soil N dynamics and above- and below-ground stoichiometry,
66 leading to changes in grass performance (Dijkstra et al., 2010; Carrillo et al., 2014). However, C3
67 and C4 grasses have contrasting responses to eCO₂ in terms of their productivity, nutrient use
68 efficiency, and digestibility of herbage available to grazing livestock (Morgan et al., 2004; Soussana
69 and Luescher 2007; Lattanzi et al., 2010). Therefore, understanding of eCO₂-induced shifts in N
70 uptake of C3 and C4 grasses should throw a new light on rangeland use and management under
71 climate change.

72 By using a novel dual-isotope continuous labelling technique with ^{13}C -depleted CO_2 and
73 ^{15}N -enriched soil, we conducted a laboratory microcosm experiment with two common North
74 American grasses (C4 *Bouteloua gracilis* and C3 *Hesperostipa comata*), to test whether
75 priming-mediated SOM decomposition under eCO_2 can affect plant N uptake and whether C3 and
76 C4 grasses have different responses of rhizosphere priming to eCO_2 .

77

78 2. Materials and methods

79 2.1. Experimental setup

80 The C4 *B. gracilis* and C3 *H. comata* we selected in this experiment are widespread grasses of the
81 central and southern Great Plains, USA, and are utilized by wildlife as well as domestic cattle and
82 sheep (Lecain et al., 2000; Derner et al., 2006). The soil we used was enriched in ^{15}N ($0.5 \text{ g m}^{-2} \text{ }^{15}\text{N}$
83 applied as 99.9 atom% ^{15}N ammonium nitrate in April, 1997) from a prior experiment in a
84 shortgrass prairie at the USDA-ARS Central Plains Experimental Range, Colorado, USA (Dijkstra
85 et al., 2008). About 15 years after ^{15}N was added (and 9 years after the completion of the original
86 experiment), soil was collected from the top 15-cm and sieved (mesh size 2 mm) to remove roots
87 and homogenize the soil. The soil is a Remmit fine sandy loam (Ustollic Camborthid) with 0.8%
88 organic C in the top 15 cm. We leached the soil with DI water in large buckets with small
89 waterspouts to decrease nutrient availability. After that, the soil was air-dried and passed through a
90 2-mm sieve to further remove plant residues, soil fauna and other coarse materials, and then
91 homogenized to attain a composite sample. Before our experiment, the initial soil inorganic N
92 ($\text{NH}_4^+ + \text{NO}_3^-$) content was determined on 2-M KCl extracts measured on an AlpKem Flow Solution

IV Automated wet chemistry system. Soil inorganic $\delta^{15}\text{N}$ was determined using the diffusion method with (precombusted) GF/D glass fiber filter papers (Brooks et al., 1989). All samples were analysed for C, N, ^{13}C and ^{15}N on a Thermo Finnigan Delta Plus XP mass spectrometer with a Costech 4010 elemental analyser inlet (or gas bench for respiration samples) (Thermo Finnigan, Bremen, Germany). Soil organic N was estimated as the difference between total and inorganic N, and soil organic $\delta^{15}\text{N}$ was determined by mass balance. The initial soil inorganic N ($\text{NH}_4^+ + \text{NO}_3^-$) and organic N concentrations were 0.14 ± 0.002 and $0.41 \pm 0.03 \text{ mg g}^{-1}$, respectively ($n = 8$; t -statistic < 0.0001). The proportion of inorganic N is somewhat higher than in a similar undisturbed soil (Carrillo et al., 2012), possibly owing to mineralization during long-term storage. The $\delta^{15}\text{N}$ values of inorganic and organic N of initial soil were 445.7 ± 12.5 and $587.5 \pm 10.8\text{‰}$, respectively ($n = 8$; t -statistic < 0.0001). Therefore, we took advantage of the distinct $\delta^{15}\text{N}$ values to estimate the relative sources of plant N (N from the original inorganic pool vs. N mineralized from SOM).

The experiment was carried out at the University of Wyoming, USA. After emergence of the second leaves on moist filter paper in glass Petri dishes, two seedlings of each grass were transferred to a pot filled with 600 g of soil (dry weight). We used a total of 15 pots (two grasses $\times$ five replicates + five unplanted pots) under each CO_2 treatment (ambient CO_2 and elevated CO_2). During the first week of the experiment, all pots (including unplanted plots) were rewetted to 25% gravimetric soil moisture content to enhance seedling growth. After that, the gravimetric water content in each pot was maintained at 15% (approximately 50% water holding capacity) using DI water, with no fertilizer additions. To simulate field conditions during the growing season, the climate-controlled chambers (Percival PGC-9/2, Percival Scientific, Perry, IN, USA) were set to a 14 h daytime period with light intensity of $700 \mu\text{mol m}^{-2} \text{ s}^{-1}$. The daytime and night-time

temperatures were 25°C and 18°C, respectively. We used a Li-250 light meter (LI-COR, Lincoln, NE, USA) and Telaire 7001 meters (Telaire, Goleta, CA, USA) to ascertain the reliability of light intensities and temperatures of the chambers every day. To achieve continuous ^{13}C -labelling of plant tissues, the chambers were modified to receive an influx of ^{13}C -depleted CO_2 ($\delta^{13}\text{C} = -33.1\text{‰}$) combined with an external air input which had been scrubbed by a soda lime column (Cheng and Dijkstra 2007). The CO_2 concentrations inside the chambers were calibrated by infrared CO_2 sensors (GMM220, Vaisala, Helsinki, Finland) and continuously monitored by Telaire 7001 meters (ambient CO_2 concentration: 371.9 ± 2.1 ppm (mean $\pm$ se)); elevated CO_2 concentration: 702.9 ± 8.7 ppm). The elevated CO_2 concentration was chosen because it is a scenario of climate change without emission control or reasonable management by the end of this century (“business as usual,” IPCC 2007). The $\delta^{13}\text{C}$ values of CO_2 inside chambers were continuously monitored by a Picarro G2101i $^{13}\text{CO}_2$ analyzer (Picarro, Sunnyvale, CA, USA). The chamber systems we used were shown to have high reliability and stability during comparative studies of plant genetics and eco-physiology (Haselhorst et al., 2011; Nie et al., 2015). Throughout the experiment, the $\delta^{13}\text{C}$ values of CO_2 were stable (ambient CO_2 : $-25.0 \pm 0.2 \text{‰}$; elevated CO_2 : $-24.9 \pm 0.2 \text{‰}$), and there was no significant daily difference in the $\delta^{13}\text{C}$ values of experimental chambers.

2.2. Harvest and measurements

We harvested during the rapid vegetative growth stage on 21 days after planting because of plant high nutrient requirement at this stage (Nie et al., 2011b; Carrillo et al., 2014). We collected plant and soil respiration by enclosing the pots in opaque, capped PVC chambers (45-cm height, 20-cm diameter) (Carrillo et al., 2014; Nie et al., 2015). Briefly, we sealed the bottom of chamber by placing it on a plastic dish containing water to impede gas loss, and scrubbed background CO_2 from

the air by circulating for 30 minutes through soda lime. Then we immediately collected an initial 30-ml air sample with a plastic syringe to ensure CO₂ scrubbing was complete. After 3 h we collected a final 30-ml air sample. Half of the final gas sample was injected into Exetainers and then analysed for $\delta^{13}\text{C}$ by a Thermo Finnigan Delta Plus XP isotope ratio mass spectrometer (Thermo Finnigan, Bremen, Germany) and the other half was analysed for CO₂ concentration by a Li-Cor 820 (LICOR Inc. Lincoln, NE). The continuous ¹³C-labeling of plant tissues allowed us to separate total soil respiration (C_{total}) into SOM-C (SOC) decomposition (C_{SOC}) and root respiration using the following model (Zhu and Cheng 2011):

$$C_{SOC} = C_{total} (\delta^{13}C_{root} - \delta^{13}C_{total}) / (\delta^{13}C_{root} - \delta^{13}C_{SOC}) \quad (1)$$

where $\delta^{13}C_{root}$, $\delta^{13}C_{total}$ and $\delta^{13}C_{SOC}$ are the $\delta^{13}C$ values of the root respiration, the total soil respiration in the planted treatments and the mean value of soil respiration in the corresponding unplanted treatments, respectively. For each CO₂ treatment, the $\delta^{13}C$ value of the root respiration was determined by growing plants in SOM-free sand. The $\delta^{13}C$ values used in this study are listed in Table 1. Priming of SOM decomposition was calculated as the difference in total SOM decomposition of each planted plot and mean SOM decomposition of unplanted pots under the same CO₂ level.

After gas collection, the plants were harvested. Shoots and roots were dried at 60°C and weighed. The C/N concentrations, $\delta^{13}C$ and $\delta^{15}N$ values of both tissues were determined by a Thermo Finnigan Delta Plus XP isotope ratio mass spectrometer.

2.3. Statistical analyses

157 To determine the effects of eCO₂ and plant type on plant C/N pools, SOM decomposition, and
158 priming effects, we used a two-way ANOVA with eCO₂ and plant species as fixed effects by SPSS
159 22.0. *Post hoc* means were determined using Tukey HSD with the significance level (*P* value) set to
160 5%. Data not meeting assumptions of normality and homogeneity of variance were log-transformed
161 before statistical testing. Linear regression analyses were performed to evaluate relationships
162 between measurement variables by SPSS.

163

164 **3. Results**

165 eCO₂ was shown to significantly increase the growth of both C4 and C3 grasses ($P < 0.01$; Fig. 1a).
166 The mean positive biomass C response of C3 grass (+115%) to eCO₂ was stronger than that of C4
167 grass (+33%). Likewise, total plant N pools (shoots + roots) of both grasses were significantly
168 increased by eCO₂ ($P < 0.01$; Fig. 1b), suggesting eCO₂ increased plant N availability.

169 Because $\delta^{15}\text{N}$ value was significantly higher in soil organic N ($587.5 \pm 10.8\text{‰}$) compared to
170 inorganic N ($445.7 \pm 12.5\text{‰}$), we could determine relative contributions of soil inorganic N and
171 organic N released from SOM to plant N uptake. Our data showed that eCO₂ significantly increased
172 the $\delta^{15}\text{N}$ value in both grasses ($P < 0.01$; Fig. 1c), suggesting that eCO₂ might induce both grasses to
173 absorb a larger quantity of N from mineralized SOM than from inorganic N.

174 Moreover, the significant relationships between $\delta^{15}\text{N}$ value and N pool in both grasses (C4 grass: R^2
175 $= 0.55$, $P < 0.05$, Fig. 2a; C3 grass: $R^2 = 0.54$, $P < 0.05$, Fig. 2b) further suggest that the increase in
176 plant availability is accompanied by increased uptake from N released from SOM.

177 eCO₂ significantly increased total SOM decomposition for both grasses ($P < 0.001$; Fig. 3a). eCO₂
 178 also significantly affected priming of SOM decomposition (Fig. 3b). eCO₂ marginally and
 179 significantly increased priming of SOM decomposition for C4 ($P = 0.075$) and C3 ($P < 0.001$)
 180 grasses, respectively (Fig. 3b). However, eCO₂ significantly increased the percentage of priming of
 181 SOM decomposition of the total SOM decomposition only for the C3 grass (ambient CO₂: $21.1 \pm$
 182 3.7% ; elevated CO₂: $33.0 \pm 2.8\%$; $P < 0.05$). There was no difference in the percentages for the C4
 183 grass between the two CO₂ concentrations.

184 For the C4 grass, priming of SOM decomposition and $\delta^{15}\text{N}$ value did not show a statistically
 185 significant relationship across all C4 pots (Fig. 4a). For the C3 grass, priming of SOM
 186 decomposition was positively related with plant $\delta^{15}\text{N}$ value ($R^2 = 0.64$, $P < 0.01$, Fig. 4b),
 187 suggesting that rhizosphere priming induced by C3 grass could facilitate N mineralization from
 188 SOM. Total SOM decomposition was positively related with plant $\delta^{15}\text{N}$ for both C4 and C3 grasses
 189 (C4 grass: $R^2 = 0.40$, $P < 0.05$, Fig. 4c; C3 grass: $R^2 = 0.63$, $P < 0.01$, Fig. 4d).

190

191 **4. Discussion**

192 Coupled plant productivity and nutrient availability in the rhizosphere play a critical role in
 193 maintaining the sustainability of ecosystems in a changing world (Phillips et al., 2011; Dijkstra et
 194 al., 2013). Our findings add to a growing body of evidence that eCO₂ has positive effects on plant
 195 biomass, plant N availability, rhizosphere priming, and total SOM decomposition (Wand et al.,
 196 1999; Morgan et al., 2004; Pendall et al., 2004a; Parton et al., 2007; Phillips et al., 2011; Nie et al.,
 197 2013a). However, the direct evidence of priming-related effects on plant N uptake is very rare

198 (Gardenas et al., 2011; Cheng et al., 2014), especially the differences in priming between plant
199 functional types. In this study, we were able to link priming-induced SOM decomposition and plant
200 N uptake by using a dual ^{13}C and ^{15}N isotope labelling technique. This work demonstrated that
201 accelerated SOM decomposition due to priming effects can positively feedback to plant N uptake
202 under eCO_2 (Hu et al., 2001), but the C4 and C3 grasses had different response intensities. Our
203 results suggest that sustained growth responses of grasses to eCO_2 could be maintained by enhanced
204 available N from SOM.

205 4.1. Experimental procedure effects and uncertainty analysis

206 Plants were successfully labelled by ^{13}C -depleted CO_2 in this study (Table 1). There was $>10\%$
207 difference in $\delta^{13}\text{C}$ value between plant tissue and CO_2 from SOM decomposition across all pots. In
208 addition, more depleted $\delta^{13}\text{C}$ value of CO_2 respired from plant roots (mean values C3: -49% ; C4:
209 -39%) than CO_2 from SOM decomposition (-21%) allowed us to accurately quantify the
210 contributions of SOM decomposition and root respiration to total soil respiration, as in previous
211 studies (Cheng and Dijkstra 2007; Dijkstra and Cheng 2007; Zhu and Cheng 2011; Nie et al., 2015).
212 We should note that disturbances due to soil sieving, air-drying, and rewetting could potentially
213 stimulate SOM decomposition and increase release of soil labile C substrates and nutrient
214 availability (Gordon et al., 2008; Nie et al., 2014). In order to minimize these possible disturbance
215 effects, we leached the soil with DI water and then waited for 3 months before starting the
216 experiment. Moreover, this short-term experiment at an early plant life stage may potentially
217 overestimate the rhizosphere priming effects because feedbacks between plants and soils may take a
218 long time (years) to develop.

219 4.2. Increased plant N uptake under eCO₂

220 It is well known that eCO₂ can mediate N cycling at plant-soil interface and plants' N uptake rate
221 (Dijkstra et al., 2008; Dijkstra et al., 2010; Nie et al., 2015). By using the ¹⁵N isotopic method, we
222 infer that eCO₂ induced both C4 and C3 grasses to absorb more N from mineralized SOM relative
223 to original inorganic N (Fig. 1c). In addition, total plant N pools of both grasses were significantly
224 increased by eCO₂ (Fig. 1b), suggesting that eCO₂ increased plant N uptake. Although soil organic
225 N such as free amino acids may be directly absorbed by grasses as a nitrogen source (Schimel and
226 Bennett 2004; Nie et al., 2011a), our previous study suggested that these grasses assimilate little
227 organic N and eCO₂ does not affect plant uptake of organic N in the US Great Plains (Chen et al.,
228 2015). In addition, eCO₂ increased C:N ratio of total biomass of the C3 grass ($P < 0.01$), suggesting
229 a N limitation to some extent. However, there was no difference in C:N ratio of total biomass of the
230 C4 grass between the two CO₂ concentrations. Nevertheless, for both grasses, the positive
231 relationship of plant $\delta^{15}\text{N}$ value and plant N pool indicated that increased N mineralization under
232 eCO₂ can at least partly meet plant N requirement to achieve high biomass (Fig. 2).

233 Our study provides direct evidence that an eCO₂-induced increase in priming effects can stimulate
234 soil microbial decomposition activity, and lead to increased soil N mineralization and plant N
235 uptake. In the same ecosystem as this study, plant rhizodeposition increased from 35 g C m⁻² yr⁻¹ at
236 ambient CO₂ to 83 g C m⁻² yr⁻¹ at eCO₂ over a 4 yrs field experiment (Pendall et al., 2004b). As a
237 result, high C input from plants to soil can stimulate microbial decomposition activity and lead to
238 enhanced plant N availability (Dijkstra et al., 2008; Nie et al., 2014; Nie et al., 2015). In our study,
239 total SOM decomposition significantly increased under both grasses when grown with eCO₂ (Fig.
240 1d). The results are consistent with our field observations and model predictions of increased soil

241 respiratory C loss and microbial decomposition activity under eCO₂ in the ecosystem (Parton et al.,
242 2007; Nie et al., 2013b; Pendall et al., 2013; Nie et al., 2014). Furthermore, we observed enhanced
243 NH₄⁺ and dissolved organic N concentrations in response to eCO₂ at the Prairie Heating and CO₂
244 Enrichment (PHACE) experiment (Carrillo et al. 2012), providing additional evidence for
245 stimulation of N availability owing to SOM decomposition in field soils. Our microcosm study
246 suggests a stimulating role in SOM turnover under eCO₂ (Fig. 3a) that is associated with
247 rhizosphere priming in the C3 grass (Fig. 3b), in agreement with other studies (Cheng et al., 2012;
248 Phillips et al., 2012).

249 4.3. C4 vs. C3 grasses

250 Differential responses of C4 and C3 grasses to environmental changes would affect productivity
251 and diversity of the Great Plains prairie (Collatz et al., 1998; Morgan et al., 2001; LeCain et al.,
252 2006). Many studies demonstrated a stronger response of plant productivity of C3 grass to eCO₂
253 than that of C4 grass because C3 grass has increased CO₂ uptake rates coupled with greater stomatal
254 resistance that reduces water loss under eCO₂ (Wand et al., 1999; Morgan et al., 2004). In our study,
255 the C3 grass appeared to benefit more from the increase in plant N uptake under eCO₂ than the C4
256 grass because CO₂-induced increase in plant C/N pool sizes and δ¹⁵N value were much stronger in
257 the C3 grass than the C4 grass (Fig. 1). In the same ecosystem as this study, for example, eCO₂ had
258 larger stimulating effects on fine roots and functional traits of the C3 grass than that of the C4 grass,
259 possibly leading to increased competitive ability of the C3 grass (LeCain et al., 2006). Our previous
260 studies suggest that fine root production is positively related to new plant C inputs (Pendall et al.,
261 2004a; Pendall et al., 2004b; Carrillo et al., 2011) and that C3 root biomass increases with eCO₂
262 (Nelson et al., 2015). As a result, rhizosphere priming effects on SOM decomposition could play a

263 more important role in N availability of the C3 grass in comparison with the C4 grass. Future
264 studies are required to demonstrate whether these results can be extended to other C3 and C4
265 grasses.

266

267 **5. Conclusions**

268 Overall, the findings of this study suggest that eCO₂ increased plant N uptake through accelerated
269 SOM decomposition, but the C3 grass was more dependent on ¹⁵N acquired from SOM by priming
270 than was the C4 grass, suggesting rhizosphere priming effects on SOM decomposition could play a
271 more important role in N availability of the C3 grass in comparison with the C4 grass. Further
272 assessment is needed to extend these findings to field experiments and to validate the long-term
273 effects of rhizosphere priming on plant N availability.

274

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282

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417

418 **Figure captions**

419 Fig.1. Total plant C pool (a), total plant N pool (b), and $\delta^{15}\text{N}$ value of whole plant (c). The $\delta^{15}\text{N}$
420 values of inorganic and organic N of initial soil were 445.7 ± 12.5 and $587.5 \pm 10.8\text{‰}$,
421 respectively ($n = 8$; $t\text{-statistic} < 0.0001$). Error bars show standard error of the mean ($n = 5$).
422 Treatments followed by the same letters are not significantly different between treatments at P
423 < 0.05 .

424 Fig.2. Linear relationships of plant $\delta^{15}\text{N}$ values with plant N pools.

425 Fig.3. Total SOM decomposition (a) and rhizosphere priming of SOM decomposition (b). Error
426 bars show standard error of the mean ($n = 5$). Treatments followed by the same letters are not
427 significantly different between treatments at $P < 0.05$.

428 Fig.4. Linear relationships of plant $\delta^{15}\text{N}$ value with rhizosphere priming of SOM decomposition and
429 total SOM decomposition.

Table 1 $\delta^{13}\text{C}$ values of plant tissue, CO_2 respired from roots grown in soil organic matter-free sand, soil organic matter without plants ($n = 6$ at ambient and elevated CO_2 , note that slightly depleted $\delta^{13}\text{C}$ value respired from unplanted pots at eCO_2 resulted from diffusion of more depleted CO_2 into the soil), and the soil respiration from planted pots. The $\delta^{13}\text{C}$ value of SOM was $-20.4 \pm 0.6\text{‰}$ and inorganic C was not present.

Plant functional type	CO_2	Plant tissue $\delta^{13}\text{C}$	se	Root CO_2 $\delta^{13}\text{C}$	se	SOM CO_2 $\delta^{13}\text{C}$	se	Soil Resp CO_2 $\delta^{13}\text{C}$	se
C3	a CO_2	-44.52	0.82	-47.8	0.68	-20.2	1.76	-26.4	0.34
C4	a CO_2	-30.48	0.11	-36.1	0.82	-20.2	1.76	-23	0.68
C3	e CO_2	-48.48	0.61	-50.4	0.6	-22.4	0.49	-27.6	1.46
C4	e CO_2	-36.16	0.35	-41.9	0.44	-22.4	0.49	-26.3	0.59

Fig.1.

Fig.2.

Fig.3.

Fig.4.