

1 **Faster turnover of new soil carbon inputs under increased atmospheric CO₂**

2 Running Head: Soil carbon dynamics under elevated CO₂

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28

29 **Abstract**

30 Rising levels of atmospheric CO₂ frequently stimulate plant inputs to soil, but the
31 consequences of these changes for soil carbon (C) dynamics are poorly understood. Plant-
32 derived inputs can accumulate in the soil and become part of the soil C pool (“new soil C”), or
33 accelerate losses of pre-existing (“old”) soil C. The dynamics of the new and old pools will
34 likely differ and alter the long-term fate of soil C, but these separate pools, which can be
35 distinguished through isotopic labeling, have not been considered in past syntheses. Using
36 meta-analysis, we found that while elevated CO₂ (ranging from 550 to 800 parts per million
37 by volume) stimulates the accumulation of new soil C in the short term (< 1 year), these
38 effects do not persist in the longer term (1 - 4 years). Elevated CO₂ does not affect the
39 decomposition or the size of the old soil C pool over either temporal scale. Our results are
40 inconsistent with predictions of conventional soil C models and suggest that elevated CO₂
41 might increase turnover rates of new soil C. Because increased turnover rates of new soil C
42 limit the potential for additional soil C sequestration, the capacity of land ecosystems to slow
43 the rise in atmospheric CO₂ concentrations may be smaller than previously assumed.

44

45 **Introduction**

46 Because soils are one of the largest natural sources of the greenhouse gas CO₂ (Raich &
47 Schlesinger, 1992), they play a crucial role in determining the future trajectory of climate
48 change. Yet, the response of soil C dynamics to future atmospheric conditions remains
49 uncertain. Numerous studies have found that rising CO₂ concentrations stimulate plant growth
50 (Ainsworth & Long, 2005). If the resulting increase in soil C input increases the size of the
51 soil C pool, soils may slow the rise in atmospheric CO₂ concentrations (Thornton *et al.*,
52 2007). However, long-term changes in soil C stocks are determined by the balance between

53 the input of new organic matter to soil pools, and the decomposition of soil organic matter
54 (Hungate *et al.*, 1995). Many CO₂ enrichment experiments do not directly measure C fluxes or
55 the fate of recently added plant detritus vs. soil organic matter that is already present, possibly
56 limiting their predictive power for the response of soil C stocks to rising atmospheric CO₂
57 (Cardon *et al.*, 2001). A recent meta-analysis used a data-model assimilation approach to
58 show that CO₂ enrichment increases decomposition rates of both new plant inputs and soil
59 organic matter (van Groenigen *et al.*, 2014). However, without separate measurements of both
60 these C pools, estimates of decomposition rates could in theory be affected by the structure of
61 the soil C model used to analyze experimental data (Georgiou *et al.*, 2015; van Groenigen *et*
62 *al.*, 2015).

63 The dynamics of different C pools can be assessed through isotopic labeling, in which
64 the isotopic composition of the totality of recently fixed C differs from pre-existing soil C
65 (hereafter “old soil C”). With this approach, we can determine the amount of soil C derived
66 from the cumulative plant inputs since labeling began (i.e., “new soil C”; Keith *et al.*, 1986;
67 Balesdent *et al.*, 1987). A similar approach enables us to determine what fraction of total soil
68 CO₂ respiration is derived from decomposition of old C (Rochette *et al.*, 1999), and these
69 results can be combined to assess the net C storage in an ecosystem (Pendall *et al.*, 2005).
70 Results vary from studies that use isotopic labeling to quantify CO₂ effects on soil C
71 dynamics, making it difficult to infer global responses from individual experiments. A
72 quantitative synthesis of results across a wide range of studies can overcome this problem.
73 Thus, we used meta-analysis (Osenberg *et al.*, 1999) of results from 28 published studies to a)
74 summarize the effect of atmospheric CO₂ enrichment on new and old C stocks in mineral soil,
75 on soil respiration rates and soil C input rates, and to b) explore the factors that shaped the
76 responses to CO₂ enrichment.

77

78

79 **Methods**

80 *Data Collection*

81 We extracted results for soil C content and CO₂ fluxes from atmospheric CO₂ enrichment
82 studies conducted in the field, in growth chambers, or in glass houses. For studies reporting
83 new soil C contents, we also extracted data on soil C input proxies. We used Web of Science
84 (Thompson Reuters) for an exhaustive search of journal articles published before June 2016,
85 using search terms “CO₂” for article title, and “soil AND carbon” and “isotop* OR label*” for
86 article topic. To be included in our dataset, studies had to meet several criteria:

87 1. Studies needed to include at least two CO₂ treatments: ambient (between 350–400 ppmV)
88 and increased (550–800 ppmV).

89 2. Plants and soils needed to have distinctive isotopic composition in each of the treatments.

90 Such differences in isotopic composition were established in one of two ways. First,
91 experiments exploited the difference in C₃ and C₄ plants; the abundance of ¹³C relative to ¹²C
92 is less in plant tissue than in atmospheric CO₂ due to isotope discrimination, with C₄ plants
93 discriminating less than C₃ plants (Farquhar *et al.*, 1989). Thus, growing C₃ plants on soil
94 developed under C₄-vegetation (or vice versa) creates a difference in isotopic signature
95 between plants and soil. Second, some experiments grew plants under an atmosphere with
96 CO₂ that had a different composition from atmospheric CO₂ under natural conditions. This
97 was achieved through ¹³C or ¹⁴C labeling of CO₂ in glass houses, growth chambers or field
98 experiments. In all cases, the contribution of each source to the total soil C pool was
99 calculated using an isotopic mixing model with two end members, i.e. new plant material and
100 old soil C (Keith *et al.*, 1986; Balesdent *et al.*, 1987). Using the same approach, the
101 contribution of old soil C respiration to soil CO₂ efflux was determined as well (Rochette *et*
102 *al.*, 1999). Because root respiration and CO₂ derived from new C input have a similar isotopic
103 signature, isotopic labeling usually cannot distinguish between the contributions of these two

104 sources to soil CO₂ efflux. As such, we did not quantify CO₂ production derived from the
105 decomposition of new soil C.

106 3. Plants needed to be labeled using methods that distributed the isotope among all plant
107 parts. Therefore, we excluded studies that applied a single pulse of ¹⁴C-CO₂ or ¹³C-CO₂ to
108 plants, because this approach results in a distribution of labeled C that does not correspond to
109 the distribution of total C across different plant parts (Kuzyakov & Domanski, 2000).

110 4. Means and sample sizes had to be available for both ambient and increased CO₂ treatments
111 to be included in our dataset. Estimates of variance were tabulated when available but were
112 not required for inclusion in the analysis.

113 We found 31 papers that met our requirements. One study was excluded because no
114 new soil C input was detected in either the control or the increased CO₂ treatment. Another
115 study was excluded because it assumed temporal variation in the old soil C end member; this
116 approach prohibited direct comparisons with new and old C stocks in other studies in our
117 dataset. Finally, one study was excluded because low image resolution prevented extraction of
118 graphical data (see Data S1). Out of the remaining 28 papers, 18 papers reported new soil C
119 stocks; 18 papers reported soil C input proxy data; 14 papers reported old soil C respiration
120 rates; and 7 papers reported old soil C stocks (Table 1).

121 We extracted final observations on soil C contents (only 1 experiment reported soil
122 C data for more than one time point). Although this was not a requirement for a study to be
123 included in our dataset, all soil C measurements in our dataset were from mineral soil layers.
124 We averaged observations of old soil C respiration rates over time. For each study, we also
125 tabulated experimental duration, plant species, and the type of experimental facility that was
126 used to increase CO₂ concentrations. Experiment duration (i.e. the time period during which
127 soil C input was isotopically labeled) varied between 6 days and 4 years (Table 1, Data S2-5).

128 *Soil C input proxies*

129 For each study we choose the proxy that we assumed was most indicative of net primary
130 productivity (NPP), while taking into account the experimental design (Table 1). In studies on
131 newly seeded plants that lasted less than one growing season, the incorporation of
132 aboveground litter in mineral soil was likely to be minimal. In these cases we used standing
133 root biomass, which we assumed was an estimate of belowground NPP. For experiments that
134 determined new soil C in root ingrowth cores (Hoosbeek *et al.*, 2004; Phillips *et al.*, 2012), we
135 used root growth as the proxy. In several longer-term experiments, aboveground biomass was
136 periodically harvested (e.g. van Kessel *et al.*, 2000) or aboveground litter was removed
137 (Cardon *et al.*, 2001; Heath *et al.*, 2005), which minimized the input of aboveground biomass.
138 Because root growth data were not available for these studies, we used standing root biomass
139 as a proxy. For longer-term (1-4 years) experiments without litter removal or biomass
140 harvesting (Olszyk *et al.*, 2003) we used total plant biomass. For all experiments, we only
141 included proxies of C input from the time point closest to the corresponding new-soil C
142 measurements. For all experiments < 1 year, soil C input proxies were measured at the same
143 time as new-soil C stocks.

144

145 *Meta-analysis*

146 We quantified the effect of increased CO₂ on new soil C, soil C input proxies, old C
147 respiration and old soil C by calculating the natural log of the response ratio (*r*), a metric
148 commonly used in meta-analyses (Hedges *et al.*, 1999; Osenberg *et al.*, 2001):

149
$$\ln r = \ln(V_{ic}/V_{ac})$$

150 where V is the value for new soil C, soil C input proxies, old C respiration or old soil C under
151 increased (ic) or ambient (ac) CO₂ conditions. We performed a mixed-effects meta-analysis in
152 R, using the `rma.mv` function in the “metafor” package (Viechtbauer *et al.*, 2010), including
153 "paper" as a random effect (because several papers contributed more than one effect size), and

154 weighting $\ln r$ by the inverse of its variance. We estimated missing variances using the average
155 coefficient of variation across the dataset. To ease interpretation, the results from all our
156 analyses were back-transformed and reported as the percentage change under increased
157 CO_2 $((r - 1) \times 100)$.

158 Several factors have been suggested to affect the response of plant growth and soil C
159 dynamics to CO_2 enrichment: 1) type of vegetation (Ainsworth *et al.*, 2005), 2) the CO_2
160 fumigation technology used (De Graaff *et al.*, 2006), 3) experiment duration (Norby *et al.*,
161 2010), 4) soil texture (Procter *et al.*, 2015), 5) age of the vegetation (Körner *et al.*, 2005), and
162 6) N availability (van Groenigen *et al.*, 2006). To test whether these factors affected CO_2
163 responses, we categorized each study based on plant type (that is, woody vs. herb),
164 experimental facility (greenhouse, GH, and growth chamber, GC vs. open top chamber, OTC
165 and free air CO_2 enrichment, FACE), and study duration (< 1 year vs. 1-4 years). We based
166 our cut-off point on expected abrupt changes in soil C input over time; in the first growing
167 season of an experiment isotopically labeled input mostly consists of root exudates and fine
168 root turnover (Norby *et al.*, 1987), whereas in longer studies, dead coarse root material and
169 aboveground litter will contribute as well (Hobbie *et al.*, 2004). One study reported respiration
170 data for more than 1 year. For this study, we time-averaged the short-term and longer-term
171 responses separately, and included them as two separate comparisons in our dataset. For each
172 study we also tabulated the age of vegetation (number of years at the start of the isotopic
173 labeling) and clay content. When studies reported soil texture class but not the exact clay
174 content, we estimated clay content as the mean of the minimum and maximum value of that
175 texture class according to the soil textural triangle
176 (<http://en.wikipedia.org/wiki/File:SoilTextureTriangle.jpg>). In addition, we categorized
177 studies on soil C stocks and respiration rates according to isotopic labeling method and we
178 categorized soil C input studies according to the type of proxy that was used (Table 1).

179 We selected our meta-analytic models using the same approach as Terrer *et al.*
180 (2016). Briefly, we analyzed the data with all possible models that could be constructed using
181 combinations of the experimental factors described above as main effects, using the “glmulti”
182 package in R. The relative importance of the factors was then calculated as the sum of Akaike
183 weights derived for all the models in which the factor occurred.

184 We assessed the effect of N availability using studies that included multiple N levels
185 in a full factorial design, comparing CO₂ responses between high vs. low N treatments. The
186 interaction between CO₂ enrichment and soil N availability was calculated according to
187 Lajeunesse (2011):

$$188 \quad \ln i = \ln r_{+N} - \ln r_{-N}$$

189 with $\ln i$ as the natural log of the interaction term, $\ln r_{+N}$ as $\ln r$ in the high N treatment,
190 and $\ln r_{-N}$ as $\ln r$ in the low N treatment.

191 Models were fitted according to the Knapp and Hartung (2003) method; 95%
192 confidence intervals (CI) of treatment effects were based on critical values from a t-
193 distribution. For all analyses, we inferred an effect of CO₂ if the 95% CI of the mean effect
194 size did not overlap 0. We used a Wald test to determine whether treatment effects were
195 statistically different between study categories.

196

197 **Results**

198 Averaged across the entire data set, elevated CO₂ tended to increase new soil C contents
199 (+14.4%, $P = 0.12$). The effect of elevated CO₂ on new soil C was best predicted by
200 experiment duration and soil texture; the sum of Aikake weights indicate that other predictors
201 were of minor importance (Fig. 1). Based on these results, we calculated treatment effects for
202 short- and longer-term experiments, using experiment duration as the sole moderator in our
203 model. Experimentally elevated CO₂ only stimulated new soil C accumulation in short-term
204 experiments (Fig. 2a and Table S1). The effect of elevated CO₂ on new C also depended on

205 soil texture; treatment effects on new soil C decreased with clay content (Table S1). We found
206 similar results when we analyzed our data using a model that included both moderators (Fig.
207 S1).

208 Within the experiments that measured new soil C, elevated CO₂ increased soil C input
209 proxies by 40.7% ($P < 0.001$), with positive effects both in short- and longer-term
210 experiments (Fig. 2b). The effects of elevated CO₂ on soil C input proxies did not depend on
211 experiment duration or any of the other model predictors (Fig. 2b, Fig. S2). When we limited
212 our analysis to studies conducted in the field (that is, FACE and OTC studies), we found
213 similar results: the effect of elevated CO₂ on new soil C contents in short-term experiments
214 was significantly higher than in longer-term experiments, but elevated CO₂ increased C input
215 proxies regardless of experimental duration (Table S1).

216 The average effect of elevated CO₂ on soil C input in longer-term studies was strongly
217 affected by the data from one study (Cardon *et al.*, 2001) which reported exceptionally strong
218 positive CO₂ effects (178 - 343%, see table S3). Excluding the results from this study from
219 our analysis lowered CO₂ effects on soil C input proxies for longer-term studies to a similar
220 level as those for short-term studies, whereas CO₂ effects on new soil C stocks remained
221 largely unchanged (Fig. S3). Averaged across the entire data set, elevated CO₂ did not affect
222 old soil C respiration ($P = 0.99$) and old soil C stocks ($P = 0.16$). Treatment effects on old soil
223 C respiration and old soil C stocks were not affected by any of the model predictors (Fig. 2cd,
224 Figs. S4-S5).

225 Within studies that included N availability treatments, elevated CO₂ increased the soil
226 C input proxy more strongly at high N levels (Table 2). The effect of elevated CO₂ on old soil
227 C stocks tended to be more positive at high N levels ($P = 0.11$); we found no CO₂ × N
228 interactions for the other response variables.

229

230 **Discussion**

231 Our results show that elevated CO₂ did not affect new soil C contents in longer-term
232 experiments. At the same time, our finding that elevated CO₂ increased soil C input proxies
233 both in short- and longer-term experiments indicate that CO₂ enrichment stimulated soil C
234 input regardless of experiment duration. Increased soil C input with no concomitant increase
235 in new soil C storage can only be explained by increased decomposition rates. Thus, our
236 results strongly suggest that faster decomposition of new C under increased CO₂ negated the
237 higher soil C input rates, thereby limiting the potential for longer-term soil C storage.
238 Experiments included in our dataset show that elevated CO₂ also increases soil C input
239 proxies other than the ones used in our analysis, such as litter production (Gielen *et al.*, 2005),
240 NPP (McCarthy *et al.*, 2010), photosynthetic rate (Heath *et al.*, 2005) and fine root turnover
241 (Lukac *et al.*, 2003; Trueman & Gonzalez-Meler, 2005) both in the short- and longer term.
242 Similarly, a recent meta-analysis shows that elevated CO₂ increases fine root production and
243 litter fall regardless of experimental duration (Dieleman *et al.*, 2010). Thus, several lines of
244 evidence suggest continued positive effects of elevated CO₂ on soil C input. This provides
245 further support for our interpretation that the lack of an effect of elevated CO₂ on new soil C
246 accumulation is not due to decreasing treatment effects on soil C input over time, but rather to
247 an increase in decomposition rates under elevated CO₂.

248 Our finding that new soil C is unresponsive to elevated CO₂ - despite increased C
249 input to soil - is inconsistent with the idea that more rapid C turnover through soil is an
250 artifact of the model structure used to infer rates of soil C turnover (Georgiou *et al.*, 2015; van
251 Groenigen *et al.*, 2015). Rather, finding that elevated CO₂ increased C input to soil with no
252 effect on the size of the new soil C pool supports the interpretation that elevated CO₂
253 increases the turnover rate of new soil C (Phillips *et al.*, 2012; van Groenigen *et al.*, 2014).

254 Why does increased atmospheric CO₂ stimulate the decomposition of new soil C?
255 Rising levels of atmospheric CO₂ increase the supply of labile C root exudates (Phillips *et al.*,
256 2011) and the release of labile C by mycorrhizae (Cheng *et al.*, 2012), which can stimulate the

257 decomposition of plant litter by saprotrophs (Phillips *et al.*, 2012; De Graaff *et al.*, 2010). This
258 explanation is consistent with direct measurements of higher *in situ* litter decomposition rates
259 with increased atmospheric CO₂ compared to ambient CO₂ (Cotrufo *et al.*, 2005; Cheng *et al.*,
260 2012; Carrillo *et al.*, 2014) and with non-girdled trees compared to girdled trees (Subke *et al.*,
261 2004). Furthermore, increased CO₂ can improve the efficiency of water use by plants, which
262 reduces soil water loss through transpiration and increases soil water content (Field *et al.*,
263 1995; van Groenigen *et al.*, 2011). This response stimulates decomposition rates in
264 ecosystems where low water availability constrains the activity of soil microbes and their
265 access to substrate (Hungate *et al.*, 1997; Pendall *et al.*, 2003). We note that this latter
266 mechanism will only have a limited impact in experiments where irrigation minimizes the
267 effects of elevated CO₂ on soil moisture contents.

268 Our analysis suggests that increased turnover of new C could be a general response to
269 atmospheric CO₂ enrichment. Nonetheless, increased CO₂ stimulated new C accumulation in
270 the short-term. This positive treatment effects on new soil C in experiments < 1 year might
271 reflect an adjustment period, where microbial activity and decomposition rates did not fully
272 respond following a step increase in soil C input rates under elevated CO₂. The change in
273 composition of soil C input over time may have played a role as well. In short-term
274 experiments, plant inputs to soil will consist mostly of root exudates (Norby *et al.*, 1987); the
275 positive effect of CO₂ on new soil C in these experiments likely reflects increased root
276 exudation. Over time, isotopically labelled root litter, mycorrhizal tissue and leaves contribute
277 to soil C input as well (Hobbie *et al.*, 2004). Indeed, increased CO₂ has been shown to
278 stimulate the decomposition of these types of plant input (Cheng, 1999; Cheng *et al.*, 2012;
279 Phillips *et al.*, 2012).

280 Our findings of faster decomposition rates with increased CO₂ are corroborated by
281 studies that did not include an isotopic C label. For instance, increased CO₂ has been shown to
282 increase the ability of microbes to decompose soil organic matter (Nie *et al.*, 2013), and to

283 stimulate the activity of enzymes associated with decomposition of both recalcitrant (Carney
284 *et al.*, 2007) and labile soil organic matter (Kelley *et al.*, 2011). However, it should be noted
285 that our analysis only pertains to mineral soils; to the best of our knowledge, no study has
286 reported CO₂ responses of old and new C in organic layers. This is important, because
287 experimentally elevated CO₂ can increase litter fall and stimulate C accumulation in forest
288 floors, thereby forming a minor additional C sink (Drake *et al.*, 2011).

289 A recent synthesis of data from a much larger set of mostly longer-term CO₂
290 experiments (n=53, average experiment duration of 6.8 years) that used a mass balance
291 approach to estimate changes in soil C dynamics found that elevated CO₂ increases the
292 decomposition of both new and old soil C (van Groenigen *et al.*, 2014). Our new findings
293 confirm those earlier results for the new, but not the old, soil C pool. The lack of a significant
294 treatment effect on old C respiration might be due to low statistical power; the small sample
295 size (n=8 for experiments 1-4 years) and high variance associated with the respiration of old
296 soil C (Fig. 2c, Table S1) limit our ability to detect treatment effects. The large variation in
297 treatment effects may be caused by among-system variation in the recalcitrance and physical
298 protection of the old soil C. Moreover, old soil C stocks are large compared to new soil C
299 stocks and they are characterized by high spatial variability, making it difficult to detect
300 changes in pool size (Hungate *et al.*, 1995). The impact of spatial variability may be reduced
301 through long-term experiments involving planted communities on homogenized soils. Large
302 differences in isotopic signatures between recently fixed C and old C may improve sensitivity
303 as well (Ogle & Pendall, 2015). Clearly, additional studies are needed to identify the soil
304 properties determining the turnover of old soil C under increased CO₂.

305 We do not know what caused the negative correlation between clay content and the
306 effect of elevated CO₂ on new soil C stocks. This result seems counter-intuitive, as clay
307 minerals are generally expected to promote soil C accumulation (Six *et al.*, 2002). One
308 possible explanation is that the soil disturbance inherent to all experiments in our data set

309 released previously physically protected C. Experiments that trace soil C input under both
310 ambient and elevated CO₂ conditions involve continuous isotopic labelling of CO₂ (which can
311 be achieved in greenhouses), or replacing vegetation (i.e. by using soil that developed under
312 vegetation with a different photosynthetic pathway than that of the experimental vegetation).
313 As such, all these experiments required a substantial amount of soil disturbance. Undisturbed
314 clay soils contain relatively large amounts of physically protected C (Six *et al.*, 2002). When
315 soil disturbance breaks up soil aggregates, much of this C becomes available to microbes
316 (Hassink *et al.*, 1993). Thus, disturbed clay soils have relatively large and active microbial
317 communities that might be better adapted to decompose the increased amount of soil C input
318 under elevated CO₂ than soils with low clay contents. Alternatively, clay content may
319 correlate with soil properties that were not considered in this analysis (because they weren't
320 always reported) but that may affect decomposition rates (e.g. nutrient availability, soil
321 moisture).

322 Elevated CO₂ stimulated soil C input proxies more strongly under high than under low
323 N inputs, but this response did not result in additional new soil C storage. These results are
324 consistent with a recent study showing that N additions increase decomposition of new soil C
325 input (Chen *et al.*, 2014). Nonetheless, several studies found that N additions stimulate total
326 soil C storage under elevated CO₂ (e.g. Hungate *et al.*, 2009; Luo *et al.*, 2006, van Groenigen
327 *et al.*, 2006). In combination with our finding that N addition does not stimulate new soil C
328 storage under elevated CO₂, this suggests that N addition stimulates net soil C storage by
329 reducing old soil C decomposition (e.g. Cheng & Johnson, 1998; Cardon *et al.*, 2001). This
330 explanation is consistent with our finding that high N additions tended to increase old C
331 stocks under elevated CO₂. However, because this result is based on a small dataset (n=11)
332 and is only marginally significant, it requires additional experimental work to be tested more
333 thoroughly.

334 Two important limitations of our analysis must be noted. First, the experiments in our
335 dataset only lasted 4 years at the most, whereas soil C storage is a process that occurs on
336 decadal timescales. Elevated CO₂ can increase the input of new C into slowly cycling or
337 passive C pools (Jastrow *et al.*, 2005; Iversen *et al.*, 2011), a response that could stimulate
338 new soil C storage over time frames longer than the spans of most experiments. As such, we
339 can only speculate about the extent to which our results are representative for responses on
340 longer time scales. However, a recent global synthesis of soil ¹⁴C data shows that current soil
341 C models actually overestimate the incorporation of new C in soil with rising CO₂
342 concentrations (He *et al.*, 2016), suggesting that our finding of increased turnover rates also
343 may apply to longer time scales in real-world ecosystems.

344 Second, our dataset does not include field experiments in undisturbed natural
345 ecosystems, or systems with a continuous management history. However, our findings are
346 supported by longer-term studies in both continuously managed and natural ecosystems. For
347 instance, Marhan *et al.* (2010) combined soil ¹³C data with inverse modelling to show that 5
348 years of elevated CO₂ increased the decomposition rate of both old and new soil C in cropland
349 by increasing soil moisture contents. Longer-term CO₂ enrichment studies on natural
350 ecosystems often include an isotopic C tracer in the high CO₂ treatment only. Several of these
351 studies found that new C is predominantly allocated to soil C pools with high turnover rates.
352 For instance, Taneva *et al.* (2006) found in a *Pinus taeda* plantation that after 8 years of
353 elevated atmospheric CO₂, the majority of soil-respired CO₂ was derived from pools with a
354 turnover rate of less than 35 days. Importantly, meta-analyses suggest that on average,
355 increased plant growth under elevated CO₂ does not result in additional soil C storage unless
356 nutrients are also added (e.g. De Graaff *et al.*, 2006; van Groenigen *et al.*, 2006). Together,
357 these results strongly suggests that our finding of increased decomposition rates is
358 transferrable to a wide range of ecosystems.

359 Conventional soil C models assume that decomposition rates (k) are not directly
360 affected by rising CO₂ levels (Friedlingstein *et al.*, 2006; Luo *et al.*, 2016). However, our
361 results (and those of other recent syntheses, e.g. van Groenigen *et al.*, 2014) indicate that k
362 might increase under elevated CO₂. This inconsistency between models and real-world
363 responses can potentially be avoided when models explicitly represent the relation between
364 microbial dynamics and decomposition rates and the interactions between various C
365 pools. Indeed, microbe-centered models (i.e., models in which decomposition is determined
366 by the size and activity of the microbial biomass, both of which are modeled explicitly)
367 predict less new soil C accumulation following an increase in atmospheric CO₂ than
368 conventional models (Wieder *et al.*, 2015; Wutzler *et al.*, 2013; Sulman *et al.*, 2014).

369 This meta-analysis, synthesizing results across 28 studies, suggests that enhanced
370 turnover rates of new soil C with increased atmospheric CO₂ might be common. Therefore,
371 future assessments of terrestrial feedbacks to climate change should consider the effects of
372 increased atmospheric CO₂ on microbial processes such as soil C turnover.

373

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380

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612 **Supporting Information**

613 Additional Supporting information may be found in the online version of this article:

614 **Fig. S1.** Effect of atmospheric CO₂ enrichment on new soil C contents, adjusted for
615 differences in clay content between studies.

616 **Fig. S2.** Model-averaged importance of the predictors of the CO₂ fertilization effect on soil C
617 input proxies.

618 **Fig. S3.** Effect of atmospheric CO₂ enrichment on new soil C contents and soil C input
619 proxies, excluding the data from Cardon *et al.* (2001).

620 **Fig. S4.** Model-averaged importance of the predictors of the CO₂ fertilization effect on old
621 soil C respiration.

622 **Fig. S5.** Model-averaged importance of the predictors of the CO₂ fertilization effect on old
623 soil C contents.

624 **Table S1.** Summary of the results of the meta-analysis on the response of new old soil C, soil
625 C input proxies, old C respiration and old soil C stocks to atmospheric CO₂ enrichment.

626 **Data S1.** Full references to the three studies that were excluded from our meta-analysis.

627 **Data S2.** New C stocks and experimental conditions for all studies included in our meta-
628 analysis.

629 **Data S3.** Soil C input proxies and experimental conditions for all studies included in our
630 meta-analysis.

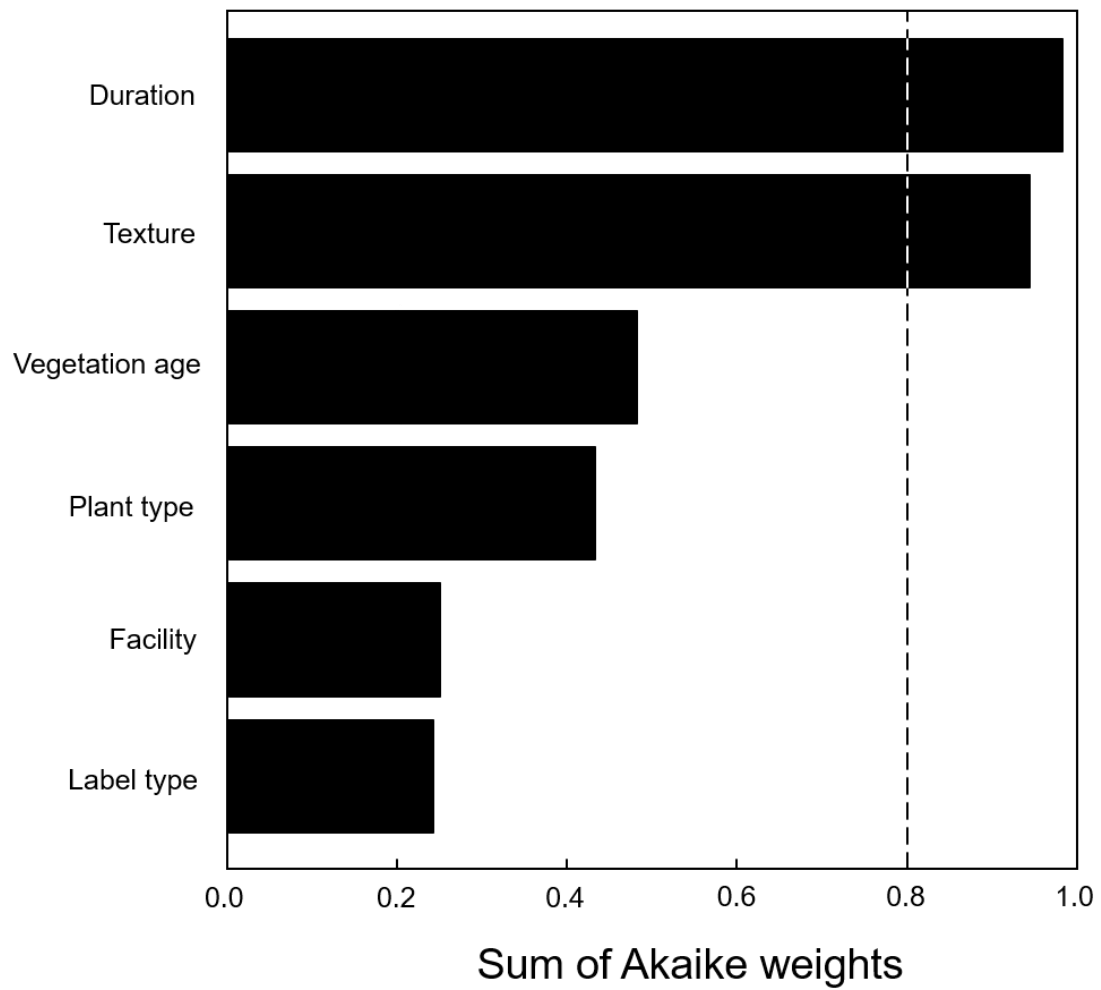
631 **Data S4.** Respiration of old soil C and experimental conditions for all studies included in our
632 meta-analysis.

633 **Data S4.** Old C stocks and experimental conditions for all studies included in our meta-
634 analysis.

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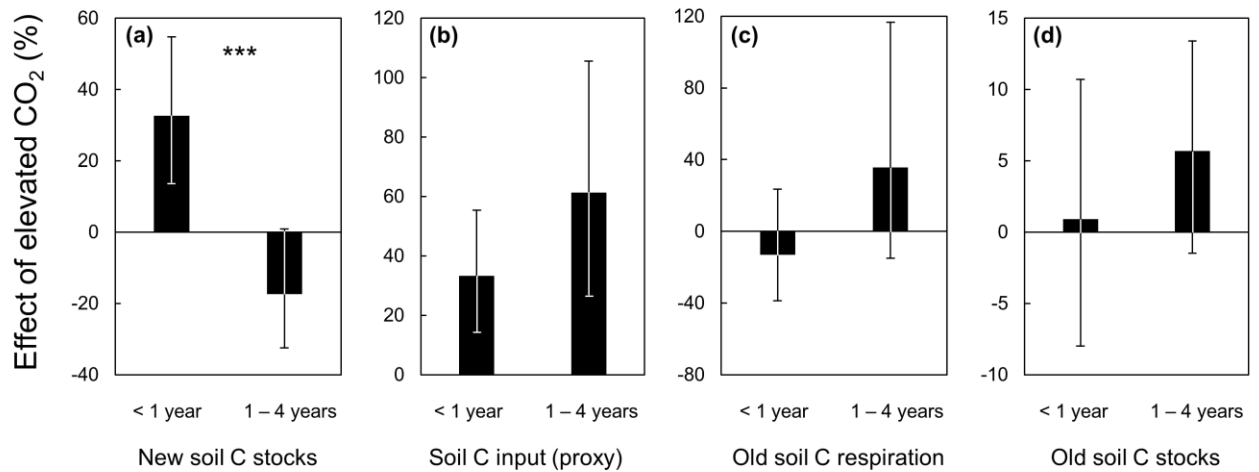
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640 **Fig. 1** Model-averaged importance of the predictors of the CO₂ enrichment effect on new
641 soil C stocks. The importance is based on the sum of Akaike weights derived from model
642 selection using AICc (Akaike's Information Criteria corrected for small samples). Cut-off
643 is set at 0.8 (dashed line) to differentiate important from non-essential predictors.

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646 **Fig. 2** Results of a meta-analysis on the response of new soil C stocks, soil C input proxies,
 647 old soil C respiration and old soil C stocks to increased levels of atmospheric CO₂ for short (<
 648 1 year) and longer-term (1-4 years) studies. (a) Change in new soil C stocks for short-term
 649 studies (n=32) and longer-term studies (n=24); (b) Change in soil C input proxies for short-
 650 term (n=32) and longer-term studies (n=24); (c) Change in respiration of old soil C for short-
 651 term (n=21) and longer-term studies (n=8); (d) Change in old C stocks for short-term studies
 652 (n=10) and longer-term studies (n=24). Error bars indicate 95% confidence intervals. ***
 653 indicates treatment responses that are significantly different between study categories at $P <$
 654 0.001.

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656
657**Table 1** Overview of CO₂ enrichment experiments included in our meta-analysis; responses that were reported in each study are indicated by '•'.

Reference	System/species	Duration in years ^a	Label ^b	Facility ^c	New C	C input proxy ^d	Old C resp.	Old C
Billes <i>et al.</i> , 1993	<i>Triticum aestivum</i>	0.08	C-14	GC	•	• (RB)		
Butterly <i>et al.</i> , 2015	<i>Triticum aestivum</i> / <i>Pisum sativum</i>	0.27	C-13	FACE	•	• (RB)		
Cardon <i>et al.</i> , 2001	California grassland	1.8	C ₃ /C ₄	OTC	•	• (RB)	•	•
Carrillo <i>et al.</i> , 2014	<i>Bouteloua gracilis</i>	0.18	C-13	GC			•	
Carrillo <i>et al.</i> , 2016	<i>Bouteloua gracilis</i> / <i>Pascopyrum smithii</i>	0.18	C-13	GC	•	• (RB)	•	•
Cheng & Johnson, 1998	<i>Triticum aestivum</i>	0.08	C ₃ /C ₄	GC			•	
Cheng <i>et al.</i> , 2000	<i>Helianthus annuus</i>	0.15	C ₃ /C ₄	GC	•	• (RB)	•	
Cotrufo & Gorissen, 1997	<i>Lolium perenne</i> / <i>Agrostis capillaris</i> <i>Festuca ovina</i>	0.15	C-14	GC	•	• (RB)		
Heath <i>et al.</i> , 2005	<i>Fagus sylvatica</i> / <i>Quercus rober</i> <i>Carpinus betulus</i> / <i>Betula pendula</i> <i>Abies alba</i> / <i>Pinus sylvestris</i>	1.3	C ₃ /C ₄	GH	•	• (RB)		•
Hobbie <i>et al.</i> , 2004	<i>Pseudotsuga mensiezii</i>	4.0	C-13	OTC	•			•
Hoosbeek <i>et al.</i> , 2004	<i>Populus alba</i> <i>Populus euramericana</i> <i>Populus nigra</i>	0.67	C ₃ /C ₄	FACE	•			•
Hungate <i>et al.</i> , 1997	California grassland	1.5	C-13	FACE			•	
Ineson <i>et al.</i> , 1996	<i>Betula pendula</i>	0.5	C ₃ /C ₄	FACE	•	• (RB)		
Kuikman <i>et al.</i> , 1991	<i>Triticum aestivum</i>	0.13	C-14	GC	•	• (RB)	•	
Lin <i>et al.</i> , 1999	<i>Pseudotsuga mensiezii</i>	1.3	C-13	OTC			•	
Lukac <i>et al.</i> , 2003	<i>Poplar plantation</i>	0.67	C ₃ /C ₄	FACE		• (RG)		
Martens <i>et al.</i> , 2009	<i>Triticum aestivum</i>	0.12	C-14	FACE	•	• (RB)		
Nie <i>et al.</i> , 2015	<i>Bouteloua gracilis</i>	0.08	C-13	GC	•	• (RB)	•	
Nie & Pendall, 2016	<i>Bouteloua gracilis</i> / <i>Hesperostipa comata</i>	0.06	C-13	GC			•	
Olszyk <i>et al.</i> , 2003	<i>Pseudotsuga mensiezii</i>	4.0	C-13	OTC		• (TB)		
Paterson <i>et al.</i> , 2008	<i>Lolium perenne</i>	0.18	C-13	GC			•	
Pendall <i>et al.</i> , 2003	Colorado grassland	2.6	C ₃ /C ₄	FACE			•	
Phillips <i>et al.</i> , 2012	<i>Pinus taeda</i>	1	C-13 ^e	FACE	•	• (RG)		•
Rouhier <i>et al.</i> , 1996	<i>Castanea sativa</i>	0.02	C-14	GC	•	• (RB)		
Trueman & Gonzalez-Meler, 2005	<i>Populus deltoids</i>	4.0	C-13	GH			•	
Van Ginkel <i>et al.</i> , 1997	<i>Lolium perenne</i>	0.12	C-14	GC	•	• (RB)	•	
Van Ginkel <i>et al.</i> , 2000	<i>Lolium perenne</i>	0.23	C-14	GC	•	• (RB)		
Van Kessel <i>et al.</i> , 2000	<i>Lolium perenne</i> / <i>Trifolium repens</i>	4.0	C ₃ /C ₄	FACE	•	• (RB)		•

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662^a Number of years during which the soil in the study received isotopically labeled C input.^b C-14 = isotopic labelling by ¹⁴C-CO₂; C-13 = isotopic labelling by ¹³C-CO₂; C₃/C₄ = isotopic labelling by using a shift in C₃ vs. C₄ vegetation.^c FACE = Free Air Carbon dioxide Enrichment; GC= Growth Chamber; GH = Greenhouse; OTC=Open Top Chamber.^d RB= root biomass, TB= total biomass, RG = root growth.^e This study created a difference in isotopic signature between old soil C and new soil C input by switching soils between ambient and elevated CO₂ treatments.

663 **Table 2** Effect of elevated CO₂ for low and high N addition treatments, and the CO₂ × N
 664 interaction term in CO₂ × N factorial experiments for all response variables included in our
 665 analysis.

Response variable	CO ₂ effect at low N (%)			CO ₂ effect at high N (%)			CO ₂ × N interaction (%)			n
	95% CI			95% CI			95% CI			
	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	
New soil C stocks	-11.7	-31.2	13.3	-2.3	-24.0	25.5	6.7	-12.2	29.8	18
Soil C input (proxy)	43.8	10.2	87.8	60.0	22.2	109.4	13.4	1.2	27.1	18
Old soil C respiration	-5.2	-46.7	68.8	-5.3	-45.8	65.4	-3.0	-48.5	82.9	6
Old soil C stocks	5.5	-4.4	16.3	7.6	-2.4	18.5	2.7	-0.8	6.3	11

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