

1 **New soil carbon sequestration with nitrogen enrichment: a meta-analysis**

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22

23 **Abstract**

24 *Background and aims*

25 Through agriculture and industry, humans are increasing the deposition and availability of
26 nitrogen (N) in ecosystems worldwide. Carbon isotope tracers provide useful insights into soil
27 C dynamics, as they allow to study soil C pools of different ages. We evaluated to what extent
28 N enrichment affects soil C dynamics in experiments that applied C isotope tracers.

29 *Methods*

30 Using meta-analysis, we synthesized data from 35 published papers. We made a distinction
31 between “new C” and “old C” stocks, *i.e.*, soil C derived from plant C input since the start of
32 the isotopic enrichment, or unlabeled, pre-existing soil C.

33 *Results*

34 Averaged across studies, N addition increased new soil C stocks (+30.3%), total soil C stocks
35 (+6.1%) and soil C input proxies (+30.7%). Although N addition had no overall, average, effect
36 on old soil C stocks and old soil C respiration, old soil C stocks increased with the amount of
37 N added and respiration of old soil C declined. Nitrogen-induced effects on new soil C and soil
38 C input both decreased with the amount of extraneous N added in control treatments.

39 *Conclusion*

40 Although our findings require additional confirmation from long-term field experiments, our
41 analysis provides isotopic evidence that N addition stimulates soil C storage both by increasing
42 soil C input and (at high N rates) by decreasing decomposition of old soil C. Furthermore, we
43 demonstrate that the widely reported saturating response of plant growth to N enrichment also
44 applies to new soil C storage.

45

46 **Introduction**

47 By burning fossil fuel and applying artificial fertilizer to cropland, humans have increased
48 atmospheric N deposition three- to five-fold over the last century (IPCC, 2007; Bouwman et
49 al. 2013; Lamarque et al. 2013). Global N deposition rates are projected to increase by 2.5
50 times or more by the end of this century (Reay et al. 2008), and much of this N will be deposited
51 in terrestrial ecosystems. Because plant growth is typically limited by N availability (LeBauer
52 and Treseder 2008; Bai et al. 2010; Wright 2019) and N limits the CO₂ fertilization on plant
53 biomass (Terrer et al. 2019), increasing N deposition rates are widely expected to stimulate
54 ecosystem C storage (Reay et al. 2008; Janssens et al. 2010; Chen et al. 2018). However, it is
55 still unclear to what extent N additions affect soil C stocks. Soils store about twice as much C
56 as the atmosphere and form a large natural source of CO₂ (Eswaran et al. 1993). Thus, soil C
57 responses to N enrichment could play a key role in determining future trajectories of
58 atmospheric CO₂ concentrations (Dijkstra et al. 2004; Loisel et al. 2019).

59 The size of the soil C pool is determined by the balance between soil C input – mostly
60 from plant litter and roots (*e.g.*, exudates and allocation to mycorrhizal fungi) – and soil C
61 output through the decomposition of soil organic matter (Trumbore 1997; Jastrow et al. 2007).
62 By stimulating plant growth and litter production, N input from anthropogenic sources can
63 increase soil C stocks through the enhancement of C inputs (*e.g.*, Gong et al. 2012; Tian and
64 Niu 2015). However, in some cases N enrichment stimulates soil C input without increasing
65 soil C stocks (Mack et al. 2004; Allison et al. 2010) and *vice versa* (Pregitzer et al. 2008),
66 suggesting that N-induced changes in decomposition (*i.e.*, soil C output) affect soil C storage

67 as well (Janssens et al. 2010). Indeed, N additions can decrease the activity of lignin-modifying
68 enzymes (Chen et al. 2018) and N-induced decreases in soil pH can decrease decomposition
69 rates (e.g. Zhou et al. 2017); both these responses would stimulate soil C storage.

70 Because most N enrichment experiments do not directly measure the fate of newly added
71 plant litter vs. native soil organic matter, they provide limited mechanistic insight in the
72 processes underlying soil C sequestration (Cardon et al. 2001). However, the dynamics of these
73 two different C pools can be studied through isotopic labeling, in which the isotopic signature
74 of soil C inputs differ from pre-existing soil C (from now on referred to as “old soil C”). This
75 approach enables us to determine the amount of soil C derived from old soil C versus “new soil
76 C” (*i.e.*, cumulative plant inputs since labeling began) (Keith et al. 1986; Balesdent et al. 1987).
77 Similarly, by analysing the isotopic composition of total soil CO₂ respiration in labeling
78 experiments, we can quantify the decomposition of old soil C (Rochette et al. 1999).

79 The response of soil C storage to N addition varies with environmental conditions and
80 between ecosystems. For instance, field experiments suggest that N enrichment generally does
81 not alter soil C stocks in grasslands (Lu et al. 2011), but stimulates soil C sequestration in
82 temperate, tropical, subtropical and boreal forests (Janssens et al. 2010; Cusack et al. 2011;
83 Maaroufi et al. 2019). Furthermore, N enrichment reduced litter decomposition in N-rich
84 ecosystems, but not in N-limited ecosystems (Chen et al. 2015). Effects of N also depend on
85 litter quality; whereas N addition typically stimulates the decomposition of labile C, it slows
86 down the decomposition of recalcitrant C (Fog 1988; Talbot and Treseder 2012; Chen et al.
87 2018).

88 Because N enrichment studies cover a wide range of ecosystem types and environmental
89 conditions, deriving a global response to N enrichment from individual experiments is
90 challenging. Moreover, high spatial variability in soil C stocks and low replication mean that
91 individual experiments often lack the statistical power to detect changes in soil C dynamics
92 (Hungate et al. 1995). A quantitative synthesis of results across multiple studies can overcome
93 both these problems. Thus, we used meta-analysis to synthesize studies that applied isotopic
94 labeling to evaluate the impacts of N enrichment on new and old soil C. By synthesizing these
95 data, we aimed to identify the main factors determining new and old soil C stocks with N
96 enrichment, thereby increasing mechanistic insight into the processes underlying soil C storage.
97 We hypothesized that N addition stimulates soil C storage both by increasing plant growth and
98 new soil C storage, and by reducing the decomposition of old soil C.

99

100 **Methods**

101 Data collection

102 We used Web of Science (Thompson Reuters) to search for studies published before March
103 2019 that employed an isotopic C tracer to study the effect of N enrichment on soil C dynamics.
104 We used the search terms “nitrogen”, “soil AND carbon” and “isotop* OR label*” for article
105 topic.

106 To be included in our dataset, studies had to meet the following criteria. First, studies had
107 to include at least two N treatments (“control” & “high N”), with N addition rates (kg N ha^{-1}
108 yr^{-1}) for both treatments clearly indicated. Second, to distinguish “new soil C” and “old soil C”,
109 C labeling should be applied to create a difference in the isotopic composition of plants and

110 soils. There are two ways to achieve this goal: (1) growing plants under isotopically labeled
111 CO₂ (*i.e.*, ¹³CO₂ or ¹⁴CO₂); (2) growing C₃ plants on soils that developed under C₄ vegetation,
112 or vice versa. C₃ plants discriminate more strongly against ¹³C than C₄ plants because of
113 differences in the photosynthetic pathway, thereby creating differences in the ¹³C isotopic
114 composition of plant biomass and soil organic matter input (Farquhar et al. 1989). Thus, in
115 both approaches, new soil C derived from plants will differ from native (*i.e.*, unlabeled) soil C.
116 Third, we only considered studies that applied continuous labeling, usually from the first leaf
117 emergence to sampling time, to ensure that the total amount of new soil C could be determined
118 (Kuzyakov and Domanski 2000). We included both pot- and field- experiments in our analysis.
119 In total we found 35 studies that met our requirements (Table 1).

120 From each study we extracted data on new, old, and total soil C, and old soil C respiration
121 when these were reported, for both control and high N treatments (Data S1-S5). For studies
122 reporting new soil C stocks, we also extracted data on soil C input proxies (root biomass, or
123 yield data if root biomass was unavailable), following the approach of van Groenigen et al.
124 (2017). To avoid pseudoreplication, we only included the most recent observations from each
125 study in our dataset. We tabulated means, standard deviation and the number of replicates for
126 both control and high N treatments. Missing standard deviations were estimated from the mean
127 coefficient of variation across the dataset (*e.g.*, van Groenigen et al. 2017).

128 Previous studies suggest that plant growth and total soil C storage with N addition depend
129 on ΔN (*i.e.*, the difference in N addition between the control and high N treatments; *e.g.*,
130 Maaroufi et al. 2015), plant type (*e.g.*, Yue et al. 2016), atmospheric CO₂ concentration (van
131 Groenigen et al. 2006), soil N availability (Chen et al. 2015) and soil pH (Nottingham et al.

132 2015). Similarly, several studies suggest that N fertilizer stimulates plant growth more strongly
133 when it is applied in combination with other nutrients (*e.g.*, Crowther et al. 2019; Elser et al.
134 2007). Thus, we categorized studies based on these experimental conditions. To account for
135 plant type, we made a distinction between studies on woody species and studies on herbaceous
136 species. We made a distinction between studies that added other nutrients together with N, and
137 studies that did not. Atmospheric CO₂ concentration, soil pH and ΔN were included in our
138 analysis as continuous factors. Our dataset included only 5 studies on woody species, limiting
139 the representativeness of the overall treatment effects for this category.

140 We used soil C:N ratios as an indicator of initial soil N availability (*e.g.*, Terrer et al. 2019).
141 Because plant growth responses to N addition show a saturating response (Aber et al. 1998),
142 we also included N addition levels in control treatments as a predictor variable. Some pot
143 studies in our dataset applied N fertilizer homogenously throughout the entire soil column by
144 mixing (*e.g.*, Heath et al. 2005). In these cases, we tabulated N addition levels equivalent to the
145 amount of N added to the 0-20 cm layer. Finally, we tabulated information about labeling type
146 (*i.e.*, C₃-C₄, ¹³C, or ¹⁴C), study method (*i.e.*, pot vs. field), clay content (%) and experimental
147 duration (*i.e.*, the natural log of the number of days since the isotopic label was introduced in
148 the experiment).

149

150

151 Meta-analysis

152 For each study in our dataset, we calculated the response of new soil C stocks, old soil C
153 stocks, total soil C stocks, C input proxies and old C respiration to N enrichment. We expressed

154 treatment effects as the natural log of the response ratio (lnR), a metric commonly used in meta-
155 analysis (Hedges et al. 1999; Osenberg et al. 1999):

$$156 \quad \ln R = \ln \left(\frac{V_h}{V_c} \right)$$

157 with V_h and V_c as the arithmetic mean values of new soil C, old soil C, total soil C, soil C input,
158 and old C respiration in the high N and control treatments, respectively. The variance (var) of
159 lnR was calculated as:

$$160 \quad \text{var} = \frac{SD_h^2}{N_h V_h^2} + \frac{SD_c^2}{N_c V_c^2}$$

161 with N_h and N_c as the replicate numbers for high N and control treatments, respectively.

162 Our meta-analytical approach was adopted from van Groenigen et al. (2017). Briefly, we
163 analysed our data using a mixed-effects model with the *rma.mv* function in the R package
164 “metafor” (Viechtbauer 2010). Because several studies contributed more than one effect size
165 (e.g. in multifactorial experiments), we included “study” as a random effect. We weighted lnR
166 by the inverse of its variance. Models were fitted according to Knapp and Hartung (2003); 95%
167 confidence intervals (CI) of treatment effects were based on critical values from a t-distribution.
168 Effect sizes were considered significant if their 95% CI did not include zero. We used a Wald
169 test to statistically evaluate differences in treatment effects among categories. The “glmulti”
170 package was used to identify which of the factors described above best predicted treatment
171 effects of N enrichment, following the same approach as Terrer et al. (2016) and van Groenigen
172 et al. (2017). In short, we analysed our data with all possible models that could be constructed
173 using combinations of the experimental factors. Model selection was based on Akaike
174 Information Criterion corrected for small samples (AICc) as criterion. The relative importance

175 value for a particular predictor was equal to the sum of the Akaike weights (probability that a
176 model is the most plausible model) for the models in which the predictor appears. A cut-off of
177 0.8 was set to differentiate between important and redundant predictors, so that predictors with
178 relative importance near or less than 0.8 are considered unimportant.

179

180 **Results**

181 Averaged across the entire dataset, N addition significantly stimulated soil C input proxies
182 (+30.7%, $p < 0.001$), new soil C stocks (+30.3%, $p < 0.001$), and total soil C stocks (+6.1%, p
183 < 0.001) (Fig. 1). In contrast, N addition did not significantly affect the respiration of old soil
184 C (+0.2%, $p = 0.98$) or old soil C stocks (+1.2%, $p = 0.60$). When we limited our analysis to
185 long-term (i.e., >5 year) studies conducted under field conditions, we found quantitatively
186 similar results for all variables (Fig. S1). Unfortunately, no long-term data were available for
187 old C respiration.

188 Our model selection approach indicated that “Control N” (i.e., N addition level in control
189 treatments) was the most important predictor for N-induced effects on both new soil C stocks
190 and soil C input proxy (Fig. 2ab). Among all the potential models for the response of new soil
191 C stocks to N additions (“lnR_N”), the model lnR_N ~ Control N had the lowest AIC value (Table
192 S1). Of all the potential models for the response of C input to N additions (“lnR_I”), the model
193 lnR_I ~ Control N was the most parsimonious within 2 AIC units (Table S2). Across the entire
194 dataset, lnR_N and lnR_I both decreased with increasing control N levels ($p < 0.05$, Fig. 3ab).
195 Nitrogen-induced changes in soil C input and new soil C stocks were significantly correlated
196 ($p < 0.05$; Fig. S2).

197 The dataset of N effects on old soil C respiration ($\ln R_{OR}$) only contained pot studies on
198 herbaceous species. Therefore, the model selection approach for this dataset did not include
199 plant type or study method as possible predictors. Model selection indicated that “ ΔN ” was the
200 most important predictor for $\ln R_{OR}$ (Fig. 4a). The model $\ln R_{OR} \sim \Delta N$ was the most
201 parsimonious model within 2 AIC units (Table S3); $\ln R_{OR}$ decreased by 0.125 units per 100 kg
202 $N\ ha^{-1}\ yr^{-1}$ change in ΔN ($p < 0.05$; Fig. 5).

203 The response of old soil C stocks to N addition (“ $\ln R_O$ ”) was best predicted by Control N,
204 ΔN and CO_2 concentration (Fig. 4b). Among all the potential models, the model $\ln R_O \sim$ Control
205 N level + ΔN + CO_2 concentration was the most parsimonious model within 2 AIC units (Table
206 S4). Analysing our dataset with this model, the effect of N addition on old soil C stocks
207 increased with ΔN ($p < 0.001$) and atmospheric CO_2 concentrations ($p < 0.001$), but it decreased
208 with control N levels ($p < 0.01$; Fig. 6).

209 The response of total C stocks to N addition (“ $\ln R_T$ ”) was best explained by atmospheric
210 CO_2 concentrations (Fig. 4c). Among all the potential models, “ $\ln R_T \sim CO_2$ concentration” had
211 the lowest AIC value (Table S5). As with old soil C stocks, the effect of N addition increased
212 with atmospheric CO_2 ; $\ln R_T$ increased by 0.012 units per 100 ppm change in atmospheric CO_2
213 concentrations (95% CI: 0.003 - 0.021; $p < 0.01$).

214

215 **Discussion**

216 New soil C plays a key role in soil C dynamics, since its quantity and quality influence soil
217 C decomposition rates (*e.g.*, De Graaff et al. 2010). The positive correlation between N-effects
218 on new soil C stocks and soil C input proxy suggests that, N-induced changes in new soil C

219 stocks are at least partly driven by changes in soil C input. N-effects on soil C input and new
220 soil C stocks both decreased with increasing control N levels. These findings can probably be
221 explained by saturation of the N enrichment effect (Aber et al. 1998). Adding N to N-rich soils
222 often induces shifts in nutrient limitations of plant growth from N to phosphorus (Vitousek et
223 al. 2010; Penuelas et al. 2013; Deng et al. 2017). Under these conditions, further N addition no
224 longer increases plant growth and may even decrease plant growth when competition
225 dominates plant–microbe relationships (Čapek et al. 2018). Furthermore, high N addition rates
226 tend to decrease soil pH (Tian and Niu 2015), thereby causing a loss of base cations and
227 increasing soluble aluminium concentration, both of which negatively affect plant production
228 (Bowman et al. 2008).

229 Because N addition promotes plant growth more strongly in N-poor ecosystems (*e.g.*, Chen
230 et al. 2015), we expected N additions to increase new C stocks and plant growth more strongly
231 in experiments with high soil C:N ratios. Yet, treatment effects did not depend on soil C:N ratio
232 for studies in our dataset. One possible explanation for this result is that soil disturbance
233 distorted the relation between soil C:N ratio and soil N availability. Experiments in our analysis
234 inherently involve some level of soil disturbance, such as replacing vegetation (*i.e.*, by using
235 soil that developed under vegetation with a different photosynthetic pathway than that of the
236 experimental vegetation) and transferring soil from the field to pots. Furthermore, some
237 experiments in agricultural systems applied disturbance during the experiment in the form of
238 tillage operations. When soil disturbance breaks up aggregates, physically protected soil
239 organic matter becomes available to microbes, stimulating organic matter decomposition and
240 increasing nutrient availability (*e.g.*, Kristensen et al. 2000). Thus, soil disturbance may have

241 contributed to the relatively high variation in treatment effects on new soil C and soil C input
242 in our dataset.

243 The central role of control N levels in determining the potential of N-induced new soil C
244 storage has important implications for soils both in agricultural and natural ecosystems. Soil C
245 sequestration in agricultural systems has been widely suggested as a main mechanism to reduce
246 anthropogenic CO₂ emissions and slow down climate change (*e.g.*, Minasny et al. 2017). Our
247 results suggest that N additions to agricultural soils that previously received little or no
248 additional N could contribute to these efforts. In contrast, further N additions to agricultural
249 systems that already receive substantial amounts of fertilizer N are unlikely to stimulate soil C
250 storage. Soil emissions of N₂O strongly increase when fertilizer N rates exceed crop N uptake
251 (*e.g.*, van Groenigen et al. 2010), suggesting that further N additions in these systems might
252 even work counterproductive in terms of greenhouse gas mitigation. Less intensively managed
253 ecosystems on the other hand typically receive low amounts of N input, mostly through
254 atmospheric N deposition. Thus, our results suggest that future increases in atmospheric N
255 deposition will lead to net soil C sequestration in these systems.

256 Although N addition on average did not affect old soil C contents or old C respiration,
257 $\ln R_O$ increased and $\ln R_{OR}$ decreased with ΔN . We speculate that these results reflect the various
258 mechanisms through which N additions can affect old C respiration. N additions can stimulate
259 decomposition of native SOM indirectly by increasing plant growth and labile C inputs which
260 act as substrate for soil microbes (*e.g.* Paterson et al. 2008). On the other hand, N additions can
261 have direct negative effects on lignin-modifying enzymes (Chen et al. 2018), and N-induced
262 decreases in soil pH may decrease decomposition rates (*e.g.* Zhou et al. 2017). In the absence

263 of plants, N additions typically decrease microbial respiration (Fog, 1988; Janssens et al. 2010),
264 suggesting that negative effects of N additions on soil microbial activity dominate when N rates
265 exceed plant N uptake, i.e. at high ΔN . This interpretation is consistent with several studies
266 indicating that N additions decrease soil respiration more strongly at high N addition rates (e.g.
267 Janssens et al. 2010).

268 The positive relation between atmospheric CO_2 concentration and $\ln R_O$ corroborates
269 studies suggesting that N enrichment reduces old soil C decomposition under elevated CO_2
270 (e.g. Cardon et al. 2001; Cheng and Johnson, 1998). It is also consistent with a recent meta-
271 analysis showing that N additions tend to increase old C stocks under elevated CO_2 (van
272 Groenigen et al. 2017). This result might be explained by N additions reducing CO_2 -induced
273 priming of soil organic matter by alleviating N limitation of plant growth. Indeed, several
274 studies show that elevated CO_2 stimulates decomposition of old soil organic matter, thereby
275 releasing N to support plant productivity (e.g. Cheng et al. 1999; Langley et al. 2009). CO_2 -
276 induced priming occurs mostly in N limited ecosystems (Dijkstra et al. 2013; Terrer et al. 2018),
277 possibly explaining why N addition reduces CO_2 -induced decomposition of old C.

278 Three limitations of our analysis must be noted. Most importantly, our dataset includes
279 relatively few long-term studies under field conditions. For instance, the longest experiment on
280 woody species lasted 6 years, a relatively short period to measure treatment effects on long-
281 living plants. Unfortunately, the subset of long-term field studies is too small for our model
282 selection approach. However, the few long-term field studies that directly tested the impact of
283 important model predictors largely support our findings. For instance, Wilts et al. (2004) found
284 that in a 29 yr old experiment, average new soil C stocks increased with N addition, and $\ln R_N$

285 decreased with increased control N levels. In a study with relatively high control N levels (140
286 kg N ha⁻¹yr⁻¹) and high ΔN values (420 kg N ha⁻¹ yr⁻¹), van Groenigen et al. (2003) found that
287 N addition tended to decrease new soil C stocks and increase old C stocks. However, individual
288 studies are limited by high spatial variability in soil C stocks and the effects described above
289 were not significant. Clearly, more long-term studies are needed to determine whether the
290 factors affecting N-induced soil C storage change over time. Spatial variability in soil C stocks
291 can be reduced by planting communities on homogenized soils (e.g. Cardon et al. 2001; van
292 Kessel et al. 2000), and statistical sensitivity to detect treatment effects might also be improved
293 by increasing the difference in isotopic signature between newly fixed and old C pools (Ogle
294 and Pendall 2015), or by combining isotopic labeling with physical soil fractionation
295 techniques (e.g., Dijkstra et al. 2004).

296 Second, our dataset only includes studies evaluating the effect of N enrichment as a single
297 factor. However, terrestrial ecosystems are currently exposed to multiple types of
298 environmental change, and these changes are likely to interact. For instance, rising levels of
299 atmospheric CO₂ stimulate plant growth and soil C storage, especially when combined with N
300 additions (van Groenigen et al. 2006; Terrer et al. 2018). Thus, under future atmospheric CO₂
301 concentrations, N saturation of plant growth and soil C storage may occur at higher N rates
302 than under current CO₂ levels.

303 Finally, our analysis does not include any studies conducted in the tropics. Because plant
304 productivity in the tropics is usually limited by both N and P, plants growth responses to N
305 enrichment may saturate at relatively low levels (Wright 2019). Thus, the potential for N-
306 induced C sequestration might be smaller in tropical soils than in temperate soils. Together,

307 these three limitations underline the need for more long-term, multi-factor global change
308 experiments on a wider range of ecosystems to study the fate of future soil C stocks.

309 In summary, our analysis indicates that the response of soil C dynamics to N addition
310 depends on numerous environmental factors and varies strongly between experiments.
311 However, our results suggest that N enrichment increases new soil C stocks substantially.
312 Nitrogen-induced increases in new soil C sequestration are strongest in ecosystems receiving
313 low amounts of N, indicating that the positive effect of N deposition on soil C storage likely
314 diminishes with continuous N enrichment. Furthermore, we found that decomposition of old
315 soil C decreased at high N addition levels. Thus, we provide isotopic evidence that N addition
316 stimulates soil C storage both by increasing soil C input and by decreasing decomposition rates.
317 These results suggest that to improve prediction of future soil C storage with N enrichment,
318 changes in soil C input and decomposition should both be considered, along with temporal
319 changes in soil N status.

320

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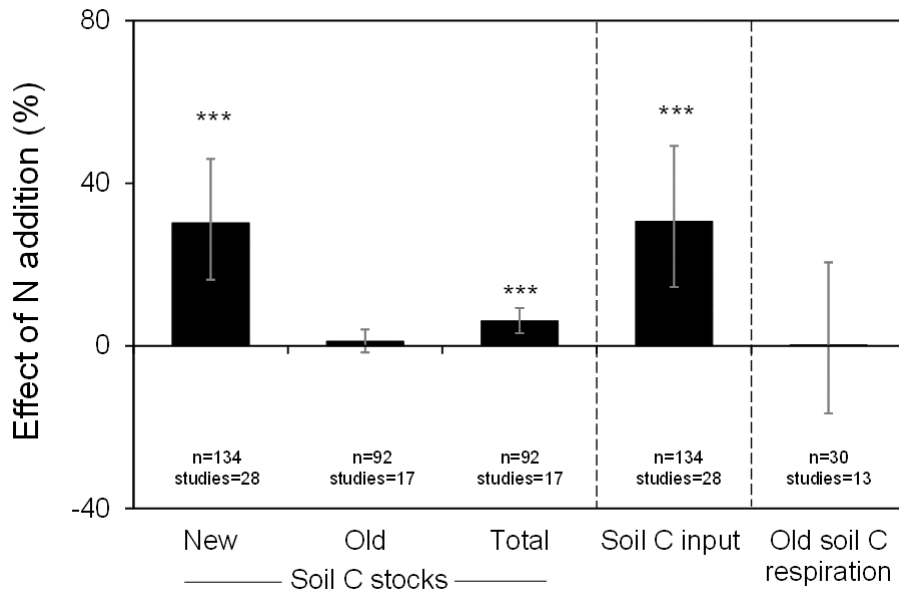
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577

578 **Figures**

579



580

581 **Fig. 1** Results of a meta-analysis on the responses of new soil C stocks, old soil C stocks, total
582 soil C stocks, soil C input proxies and old C respiration to N addition. The number of
583 observations (n) and total number of independent studies included in each analysis are
584 displayed below each bar. Error bars indicate 95% confidence intervals. ** and *** indicate
585 significance at $p < 0.01$ and $p < 0.001$, respectively.

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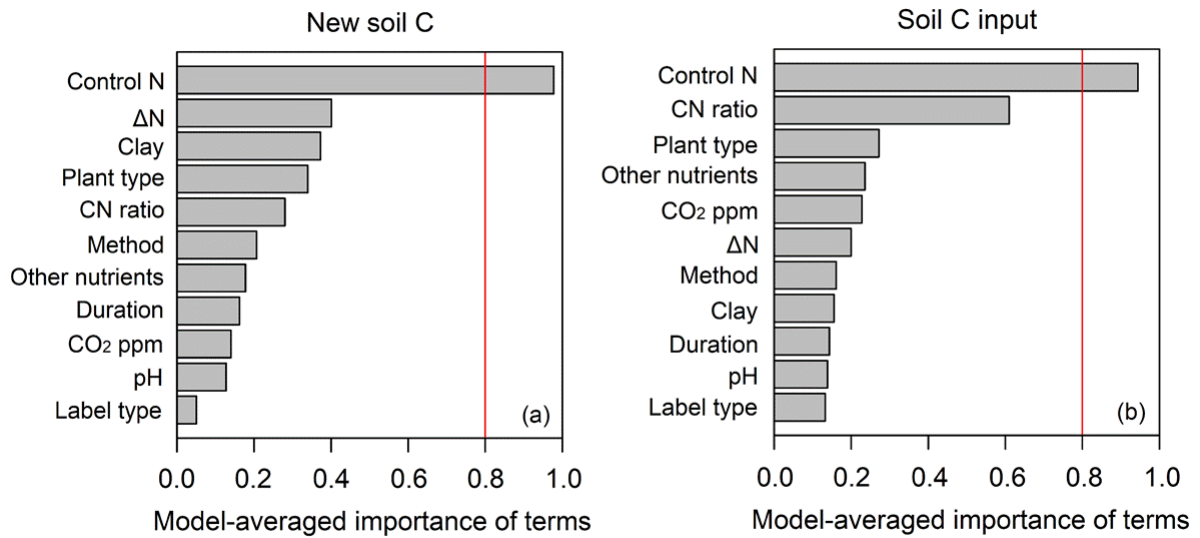
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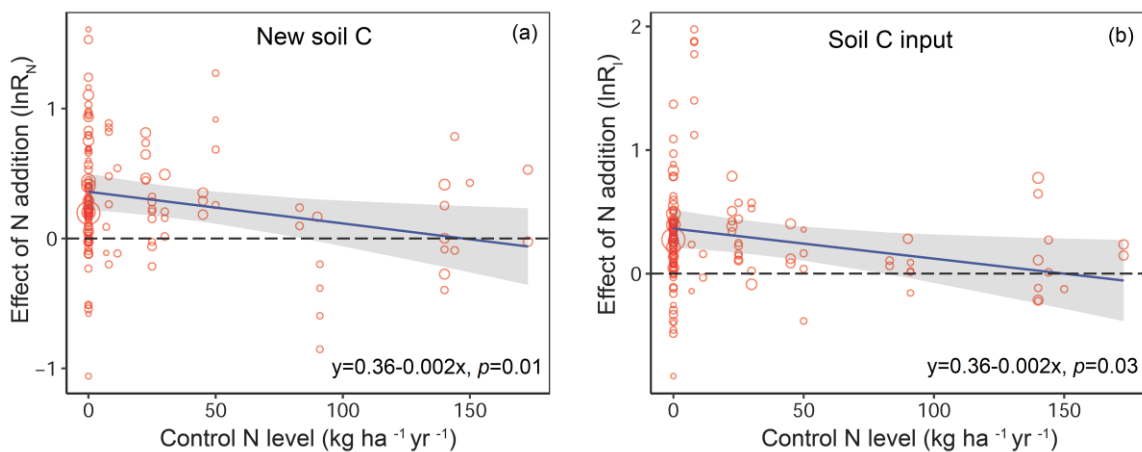
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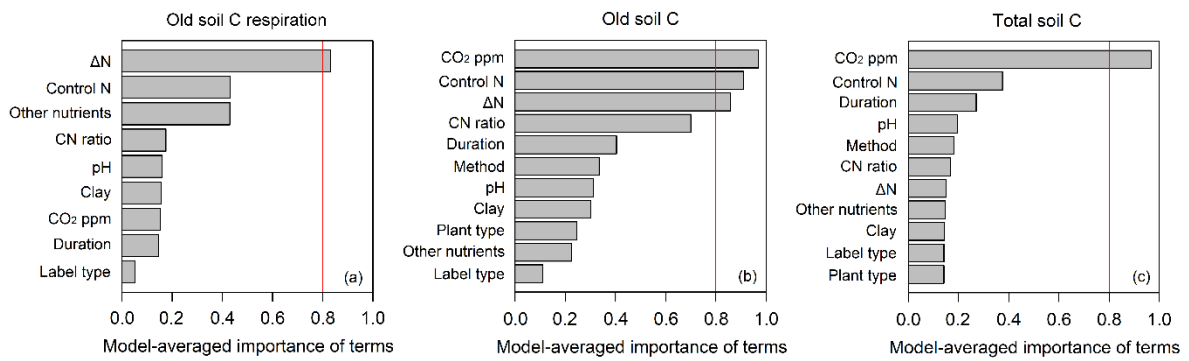
594 **Fig. 2** Model-averaged importance of the predictors of the N-effect on new soil C stocks (a)
 595 and soil C input proxies (b). The importance is based on the sum of Akaike weights derived
 596 from model selection using AICc (Akaike’s Information Criteria corrected for small samples)

597 See Fig. 1 for the number of observations and independent studies used in each analysis.



598

599 **Fig. 3** The relationship between control N levels and treatment effects ($\ln R_N$) on new soil C
 600 stocks (a) and the relationship between control N levels and treatment effects ($\ln R_I$) on soil C
 601 input (b). Symbol size represents the weight of each observation in our meta-analysis. See Fig.
 602 1 for the number of observations and independent studies used in each analysis.



603

604 **Fig. 4** Model-averaged importance of the predictors of the N-effect on old soil C respiration (a)

605 old soil C stocks (b) and total soil C stocks (c). The importance is based on the sum of Akaike

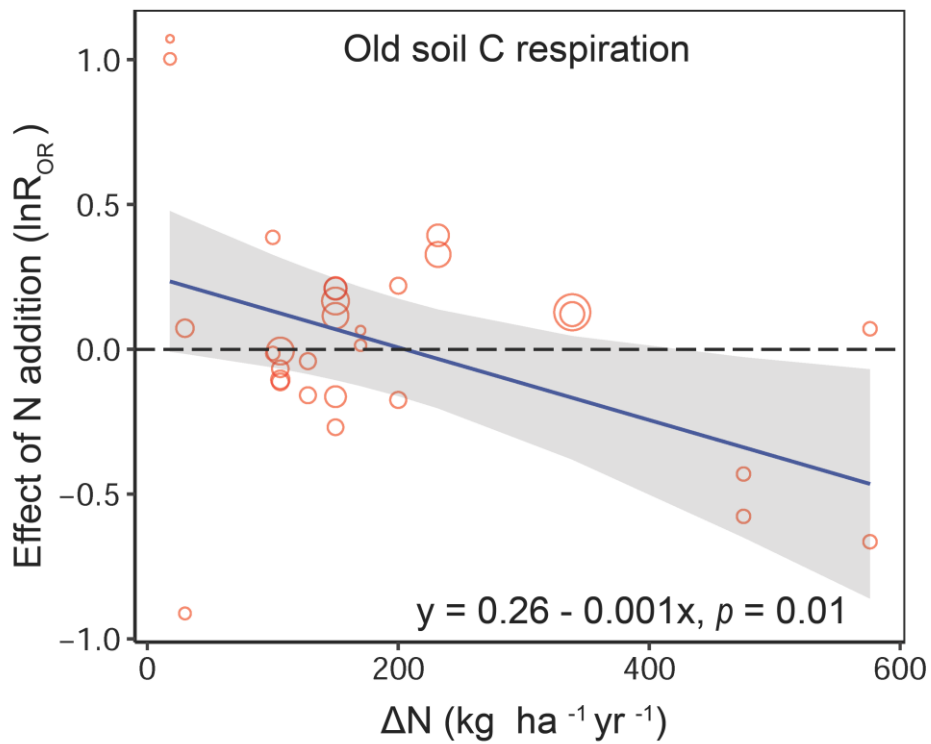
606 weights derived from model selection using AICc (Akaike’s Information Criteria corrected for

607 small samples). See Fig. 1 for the number of observations and independent studies used in each

608 analysis.

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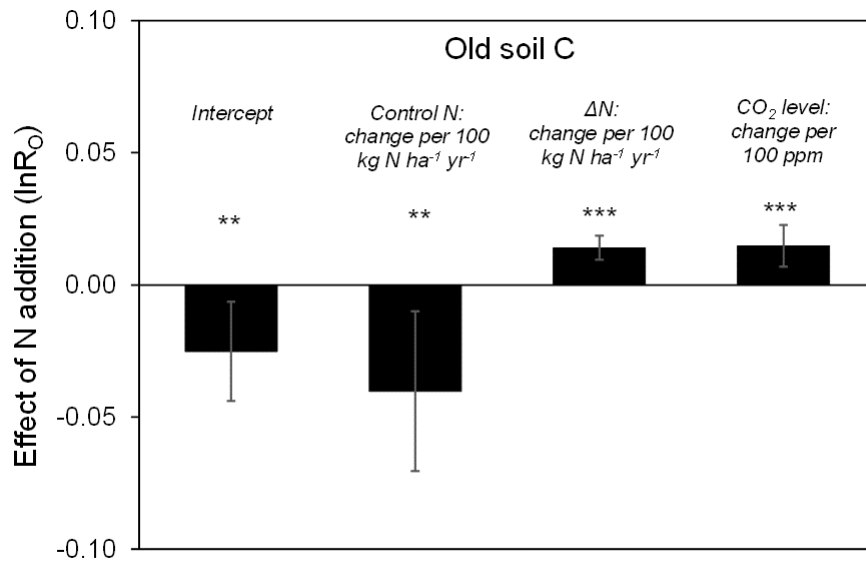
612 **Fig. 5** The relationship between N addition levels (ΔN) and treatment effects on old soil C
 613 respiration ($\ln R_{OR}$). Symbol size represents the weight of each observation in our meta-analysis.

614 The analysis is based on 30 observations, derived from 13 independent studies.

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619 **Fig. 6** Results of a meta-analysis on the response of old soil C to N addition (lnR₀), based on
 620 a model that includes Control N levels, ΔN and atmospheric CO₂ level as moderators. Intercept
 621 represents the lnR₀ for Control N and ΔN at 0 kg N ha⁻¹ yr⁻¹ and atmospheric CO₂ level at 400
 622 ppm. The analysis is based on 92 observations, derived from 17 independent studies. Error bars
 623 indicate 95% confidence intervals. **,*** indicate significance at $p < 0.01$ and $p < 0.001$,
 624 respectively.

625

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

Reference	System/species	Plant type	Duration in years ^a	Label ^b	New C	C input proxy ^c	Old C resp.	Old C
Allmaras et al. 2004	<i>Zea mays</i>	Herbaceous	13	C ₃ -C ₄	•	• (Y)		•
Bicharanloo et al. 2019	<i>Triticum aestivum</i>	Herbaceous	0.33	¹³ C	•	• (RB)		•
Billes et al. 1993	<i>Triticum aestivum</i>	Herbaceous	0.08	¹⁴ C	•	• (RB)		
Bushby et al. 1992	<i>Panicum maximum</i>	Herbaceous	0.24	¹⁴ C	•	• (RB)		
Butterly et al. 2015	<i>Triticum aestivum</i> / <i>Pisum sativum</i>	Herbaceous	0.46	¹³ C	•	• (RB)		
Cardon et al. 2001	California grassland	Herbaceous	1.84	C ₃ -C ₄	•	• (RB)	•	•
Carrillo et al. 2014	<i>Bouteloua gracilis</i>	Herbaceous	0.08	¹³ C			•	
Cheng and Johnson 1998	<i>Triticum aestivum</i>	Herbaceous	0.08	C ₃ -C ₄			•	
Cotrufo and Gorissen 1997	<i>Lolium perenne</i> / <i>Agrostis capillaris</i> / <i>Festuca ovina</i>	Herbaceous	0.15	¹⁴ C	•	• (RB)		
Dijkstra et al. 2004	<i>Agropyron repens</i> / <i>Bromus inermis</i> / <i>Koeleria cristata</i> / <i>Poa pratensis</i> / <i>Achillea</i> <i>millefolium</i> / <i>Anemone cylindrica</i> / <i>Asclepias tuberosa</i> / <i>Solidago rigida</i> / <i>Amorpha canescens</i> / <i>Lespedeza capitata</i> / <i>Lupinus perennis</i> / <i>Petalostemum</i> <i>villosum</i>	Herbaceous	5	¹³ C	•	• (RL)		•
Ge et al. 2015	<i>Oryza sativa</i>	Herbaceous	0.09	¹³ C	•	• (RB)		
Ge et al. 2017	<i>Oryza sativa</i>	Herbaceous	0.05	¹³ C	•	• (RB)		
Gong et al. 2012	<i>Zea mays</i>	Herbaceous	0.24	C ₃ -C ₄	•	• (RB)	•	•
Hagedorn et al. 2003	<i>Fagus sylvatica</i> / <i>Picea abies</i>	Woody	4	¹³ C	•	• (RB)		•
Haile-Mariam et al. 2000	<i>Pinus ponderosa</i>	Woody	0.51	¹³ C	•	• (RB)		

Heath et al. 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>	Woody	1.23	C ₃ -C ₄	•	• (RB)	•
Hofmann et al. 2009	<i>Zea mays</i>	Herbaceous	36	C ₃ -C ₄	•	• (Y)	•
Hungate et al. 1997	California grassland	Herbaceous	1.51	¹³ C			•
Kazanski 2017	<i>Bromus inermis</i> / <i>Agropyron repens</i>	Herbaceous	0.29	C ₃ -C ₄	•	• (RB)	• •
Liljeroth et al. 1990	<i>Triticum aestivum</i>	Herbaceous	0.15	¹⁴ C	•	• (RB)	•
Liljeroth et al. 1994	<i>Triticum aestivum</i> / <i>Zea mays</i>	Herbaceous	0.16	¹⁴ C	•	• (RB)	•
Paterson et al. 2008	<i>Lolium perenne</i>	Herbaceous	0.18	¹³ C			•
Philips et al. 2012	<i>Pinus taeda</i>	Woody	1	¹³ C	•	• (RG)	•
Silveira et al. 2013 Liu et al. 2017	<i>Paspalum notatum</i> / <i>Cynodon dactylon</i>	Herbaceous	2	C ₃ -C ₄	•	• (RB)	•
Van der Krift et al. 2001	<i>Festuca ovina</i> / <i>Anthoxanthum odoratum</i> / <i>Festuca rubra</i> / <i>Holcus lanatus</i>	Herbaceous	0.19	¹⁴ C	•	• (RB)	
Van Ginkel et al. 1997	<i>Lolium perenne</i>	Herbaceous	0.21	¹⁴ C	•	• (RB)	•
Van Kessel et al. 2000	<i>Lolium perenne</i>	Herbaceous	4	C ₃ -C ₄	•	• (RB)	•
Van Groenigen et al. 2003 Hebeisen et al. 1997	<i>Lolium perenne</i> / <i>Trifolium repens</i>	Herbaceous	9	¹³ C	•	• (RB)	•
Ventura et al. 2019	<i>Populus x canadensis</i> Mönch	Woody	2	C ₃ -C ₄	•	• (BNPP)	
Wilts et al. 2004	<i>Zea mays</i>	Herbaceous	30	C ₃ -C ₄	•	• (S)	•
Xu et al. 2018	<i>Triticum aestivum</i> / <i>Lupinus albus</i>	Herbaceous	0.14	C ₃ -C ₄ / ¹³ C			•
Zhang et al. 2012	<i>Zea mays</i>	Herbaceous	49	C ₃ -C ₄	•	• (Y)	•
Zhou et al. 2020	<i>Lycopersicon esculentum</i>	Herbaceous	0.31	¹³ C	•	• (RB)	• •

627 ^a Number of years during which the soil in the study received isotopically labeled C input.

628 ^b ¹⁴C = isotopic labeling by ¹⁴C-CO₂; ¹³C = isotopic labeling by ¹³C-CO₂; C₃-C₄ = isotopic labeling by using a shift in C₃ vs. C₄ vegetation.

629 ° RB= root biomass, RG = root growth, RL= root litter, Y = yield, BNPP = belowground net primary productivity, S= stover.