

Soil [N] modulates soil C cycling in CO₂-fumigated tree stands: a meta-analysis

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ABSTRACT

Under elevated atmospheric CO₂ concentrations, soil carbon (C) inputs are typically enhanced, suggesting larger soil C sequestration potential. However, soil C losses also increase and progressive nitrogen (N) limitation to plant growth may reduce the CO₂ effect on soil C inputs with time. We compiled a data set from 131 manipulation experiments, and used meta-analysis to test the hypotheses that: (1) elevated atmospheric CO₂ stimulates soil C inputs more than C losses, resulting in increasing soil C stocks; and (2) that these responses are modulated by N. Our results confirm that elevated CO₂ induces a C allocation shift towards below-ground biomass compartments. However, the increased soil C inputs were offset by increased heterotrophic respiration (Rh), such that soil C content was not affected by elevated CO₂. Soil N concentration strongly interacted with CO₂ fumigation: the effect of elevated CO₂ on fine root biomass and –production and on microbial activity increased with increasing soil N concentration, while the effect on soil C content decreased with increasing soil N concentration. These results suggest that both plant growth and microbial activity responses to elevated CO₂ are modulated by N availability, and that it is essential to account for soil N concentration in C cycling analyses.

Key-words: [CO₂] enrichment; C sequestration; fine root production; microbial respiration; N fertilization; root biomass.

INTRODUCTION

Terrestrial ecosystems play a pivotal role in the global carbon (C) cycle and are believed to sequester 30% of the anthropogenic CO₂ emissions (Canadell *et al.* 2007; IPCC 2007). As the atmospheric CO₂ concentration ([CO₂]) continues to rise, plant productivity and C sequestration may further increase, either directly through enhanced photosynthesis or indirectly via increased water- or nutrient-use efficiency. This fertilizing effect of increasing [CO₂] is well established (Eamus & Jarvis 1989; Ceulemans & Mousseau 1994; Idso & Idso 1994; Wullschleger, Norby & Gunderson 1997; Norby *et al.* 1999, 2002, 2005; Temperton, Millard & Jarvis 2003; Norby & Luo 2004; Ainsworth & Long 2005; Hyvönen *et al.* 2007). However, it has been hypothesized that as nutrients become progressively immobilized in plant biomass and soil organic matter (SOM) pools, nutrient limitations might eventually inhibit CO₂-induced increases in plant productivity, and thus limit a further increase of C sequestration in terrestrial ecosystems (Kramer 1981; Ceulemans & Mousseau 1994; Oren *et al.* 2001; Temperton *et al.* 2003; Luo *et al.* 2004; de Graaff *et al.* 2006). In the short term, priming (a stimulation of microbial growth and activity, and decomposition of older, more recalcitrant organic matter as a consequence of increased labile C inputs (Cheng & Johnson 1998; Kuzyakov 2002; Fontaine *et al.*

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2007) could serve as an alleviating mechanism, and in the longer term, nutrient-poor systems may adjust by steadily redistributing their acquired nitrogen (N) stocks (Rastetter, Agren & Shaver 1997; Cannell & Thornley 1998), but neither mechanism suffices to overcome the progressive N limitation completely.

Coinciding with the rising levels of atmospheric [CO₂], atmospheric deposition of reactive N has also increased over the last century (Denman *et al.* 2007), originating mainly from fossil fuel burning and artificial fertilizer applications (Davidson 2009). Besides a number of negative effects on terrestrial ecosystems, nitrogen fertilization significantly stimulates above-ground biomass production (Ciais *et al.* 2008; Pregitzer *et al.* 2008; de Vries *et al.* 2009; Luysaert *et al.* 2010; Thomas *et al.* 2010), and therefore could increase the amount of plant-derived C entering the soil. While a stronger CO₂ effect on above-ground biomass in N fertilized systems compared to unfertilized ones is a general response (Curtis & Wang 1998; de Graaff *et al.* 2006), this is not the case for the below-ground biomass response (de Graaff *et al.* 2006). In addition, previous meta-analysis did not reveal a clear microbial C or microbial respiration response to N fertilization in CO₂-fumigated tree stands (de Graaff *et al.* 2006).

Thus, altogether, in contrast to our knowledge of above-ground processes, below-ground processes, and the complex interactions between plants and microbes within the coupled carbon and nutrient cycles are still poorly understood, and it is not elucidated yet whether N constrains C cycling or C inputs stimulate N cycling (Luo, Field & Jackson 2006a). To improve projections of changes in below-ground C pools in response to global climate change, Zak *et al.* (2000) stressed that fine root production, longevity and biochemistry should serve as a starting point to resolve microbe-related research, because fine root dynamics and rhizodeposition are likely to exert a stronger influence than above-ground litter inputs. van Groenigen *et al.* (2006) and de Graaff *et al.* (2006) concluded that soil C can increase significantly in elevated CO₂, but only when sufficient amounts of N fertilizer are added. In that case, the increased soil C inputs from plant material could overcome CO₂-induced increases in decomposition, resulting in a net increase of soil C.

Using meta-analysis, we investigated how soil C inputs, C pools and C losses are affected by elevated atmospheric [CO₂]. More specifically, we aimed to: (1) test the hypothesis that elevated atmospheric stimulates soil C inputs more than C losses, resulting in increasing soil C stocks; and (2) evaluate how N can modulate these responses.

Although some of the N fertilization effects in elevated CO₂ studies that were found in previous studies are small or not existing, they do not necessarily imply that nutrient availability only plays a minor role, because unfertilized soils may in fact be nutrient rich, while nitrogen or even NPK-fertilized soils may be characterized by limitations of other nutrients. Therefore, in addition to the comparison of CO₂ effects between fertilized and unfertilized experiments, we also compared the CO₂ responses in poor and richer systems along a gradient of soil [N].

MATERIALS AND METHODS

Data acquisition

We focus on tree stands only because many grassland sites are managed by grazing or through forage production, which plays a key role in the C balance as a large part of the primary production is removed (Soussana *et al.* 2007). Therefore, grasslands are often fertilized to sustain productivity, which is likely to modify their responses to CO₂ fumigation.

Most of the data included in the analysis were extracted from figures and tables in published papers, although some data were not published in peer reviewed literature, but obtained directly from researchers. We collected data on above-ground biomass, fine and total root biomass, fine root production, root-to-shoot ratio, above-ground litterfall, microbial biomass C, heterotrophic respiration (Rh), soil CO₂ efflux, net N mineralization and soil C content from 32 sites, resulting in 279 entries for the meta-analysis (Supporting Information Table S1). General information about the sites is given in Table 1.

We included CO₂-enriched studies, using free air carbon enrichment (FACE) or open top chamber (OTC) technology, where roots could proliferate freely (i.e. pot or growth chamber studies were not included). Experimental conditions were summarized by a number of variables (Table 2). Studies were categorized as fertilized when any N-based fertilizer was added during the experiment, or unfertilized when no fertilizer was added. Experiments were classified as irrigated when water was added, and not irrigated when no water was added during the experiment. Some studies also included other manipulations (e.g. temperature, ozone, different soil types or used multiple species in the same experiment). Results from different treatments, plant species, soils or measurement protocols within the same experiment were considered independent measurements. Sampling methods are described in Supporting Information Table S1. We refer to the manuscripts cited in the tables and appendices for detailed methodologies in the specific experiments.

Note the difference between fertilized and 'high soil N concentration'. Although both annotations can be interpreted as nutrient rich, we tested their effects with different analyses: categorical analysis (fertilized versus unfertilized) for the former, and continuous (regression with soil [N] as variable) for the latter one.

Meta-analysis¹

Data were analysed with meta-analytical techniques using MetaWin 2.1 software (Rosenberg, Adams & Gurevitch 2000). In conventional meta-analysis, each individual observation is weighted by the reciprocal of the

¹References used in the meta-analysis (see Supporting Information for full references).

Table 1. General information for the sites used in the meta-analysis

Site	Treatment	Species	Soil [N] (g N kg ⁻¹ soil)	Latitude	Longitude	MAP (mm)	MAT (°C)
Bily Kriz	CO ₂	<i>Picea abies</i>	–	49.5 N	18.53 E	1400	5
Birmensdorf (calcareous)	CO ₂ × fertilization × soil	<i>P. abies</i> , <i>Fagus sylvatica</i>	0.76	47.35 N	8.43 E	691	10
Birmensdorf (acidic)	CO ₂ × fertilization × soil	<i>P. abies</i> , <i>F. sylvatica</i>	0.512	47.35 N	8.43 E	691	10
Christchurch	CO ₂	<i>Pinus radiata</i>	0.1485	43.53 N	172.7 E	616	12.2
DukeFACE	CO ₂	<i>Pinus taeda</i>	0.79	35.95 N	79.15 W	1140	15.5
DukeFACE	CO ₂ × fertilization	<i>P. taeda</i>	0.79	35.95 N	79.15 W	1140	15.5
EUROFACE	CO ₂ × fertilization	<i>Populus alba</i> , <i>Populus nigra</i> , <i>Populus × euramericana</i>	1.2	42.37 N	11.8 E	700	14.1
FACTS II FACE	CO ₂ × ozone	<i>Populus tremuloides</i> , <i>Betula papyrifera</i> , <i>Acer saccharum</i>	1.2	49.67 N	89.57 W	833	11.5
Forestry Commission	CO ₂ × water	<i>Fraxinus excelsior</i> , <i>Pinus sylvestris</i> , <i>Quercus petraea</i>	–	35.9 N	84.33 W	1378	14.3
Glencorse	CO ₂	<i>Betula pendula</i>	–	55.52 N	3.2 W	850	8.2
Glendevon	CO ₂ × fertilization	<i>Alnus glutinosa</i> , <i>B. pendula</i> , <i>Picea sitchensis</i> , <i>P. sylvestris</i>	–	56.2 N	4 W	1416	6.8
Mekrijarvi Research Station	CO ₂ × warming	<i>P. sylvestris</i>	–	62.78 N	30.95 E	667	2.1
Merrit Island	CO ₂	<i>Quercus myrtilifolia</i> , <i>Quercus geminata</i> , <i>Quercus chapmanii</i>	0.1	28.63 N	80.7 W	1310	–
Montalto di Castro	CO ₂	<i>Quercus ilex</i> forest	1.1	42.37 N	11.53 E	612	15
Oak Ridge FACE	CO ₂	<i>Liquidambar styraciflua</i>	1.12	35.9 N	84.33 W	1371	13.9
Oak Ridge OTC (maple)	CO ₂ × warming	<i>A. saccharum</i> , <i>Acer rubrum</i>	–	35.9 N	84.33 W	1378	14.3
Oak Ridge OTC (oak)	CO ₂	<i>Quercus alba</i>	–	35.9 N	84.33 W	1378	14.3
Oak Ridge OTC (yellow-poplar)	CO ₂	<i>Liriodendron tulipifera</i>	–	35.9 N	84.33 W	1378	14.3
POPFACE	CO ₂	<i>P. alba</i> , <i>P. nigra</i> , <i>P. euramericana</i>	1.2	42.37 N	11.8 E	700	14.1
Suonenjoki	CO ₂	<i>B. pendula</i> ROTH 4 and 80	0.46	62.65 N	27.05 E	579	3.59
Swiss Treeline FACE	CO ₂	<i>Larix decidua</i> , <i>Pinus uncinata</i>	–	46.8 N	9.83 E	–	–
UA OTC	CO ₂	<i>P. sylvestris</i>	1.2	51.17 N	4.38 E	770	12
UMBS – Alder	CO ₂	<i>A. glutinosa</i>	0.183	45.57 N	84.67 W	–	–
UMBS – Aspen (eur.)	CO ₂ × fertilization	<i>Populus × euramericana</i>	–	45.57 N	84.67 W	–	–
UMBS – Aspen (grand.)	CO ₂	<i>Populus grandidentata</i>	0.079	45.55 N	84.78 W	–	–
UMBS – Aspen (trem.)	CO ₂ × fertilization	<i>P. tremuloides</i>	0.97 (Rich soil), 0.21 (poor soil)	45.57 N	84.67 W	–	–
UMBS – Aspen (trem.2)	CO ₂ × fertilization	<i>P. tremuloides</i>	0.97 (Rich soil), 0.21 (poor soil)	45.57 N	84.67 W	–	–
UMBS – Maple	CO ₂ × fertilization	<i>A. saccharum</i>	0.97 (Rich soil), 0.21 (poor soil)	45.57 N	84.67 W	–	–
USDA – Orange	CO ₂	<i>Citrus aurantium</i>	–	33.43 N	112.07 W	–	–
USDA Placerville	CO ₂ × fertilization	<i>Pinus ponderosa</i>	0.856	38.73 N	120.8 W	1000	18
Vielsalm	CO ₂ × fertilization	<i>P. abies</i>	–	50.28 N	5.92 E	972	7.5
WebFACE	CO ₂	Mixed deciduous forest	–	47.47 N	7.5 E	990	–

MAP, mean annual precipitation; MAT, mean annual temperature.

Table 2. Treatment conditions at the experimental sites

Site	CO ₂ increase ($\mu\text{mol mol}^{-1}$)	Fumigation type	Fertilization	Irrigation	Age at start (years)
Bily Kriz	350	OTC	NF	NI	13
Birmensdorf (calcareous)	200	OTC	F and NF	I	2
Birmensdorf (acidic)	200	OTC	F and NF	I	2
Christchurch	292	OTC	F	I	Seedlings
DukeFACE	200	FACE	NF	NI	13
DukeFACE	200	FACE	F and NF	NI	22
EUROFACE	180	FACE	F and NF	I	Cuttings (3 years SRC)
FACTS II FACE	180	FACE	NF	NI	1
Forestry Commission	300	OTC	NF	I and NI	1
Glencorse	350	OTC	NF	NI	Seedlings
Glendevon	350	OTC	F and NF	I	1 or 2
Mekrijarvi Research Station	200	OTC	NF	NI	20–30
Merrit Island	350	OTC	NF	NI	Post-burn (3 months)
Montalto di Castro	350	OTC	NF	NI	30
Oak Ridge FACE	180	FACE	NF	NI	10
Oak Ridge OTC (maple)	300	OTC	NF	NI	1
Oak Ridge OTC (oak)	300	OTC	NF	NI	1
Oak Ridge OTC (yellow-poplar)	300	OTC	NF	NI	1
POPFACE	180	FACE	NF	I	Cuttings (3 years SRC)
Suonenjoki	360	OTC	F	I	7
Swiss Treeline FACE	180	FACE	NF	NI	29
UA OTC	400	OTC	NF	NI	1
UMBS – alder	345	OTC	NF	NI	Cuttings
UMBS – aspen (eur.)	345	OTC	F and NF	I	Cuttings
UMBS – aspen (grand.)	350	OTC	NF	I	Cuttings
UMBS – aspen (trem.)	350	OTC	F and NF	I	Cuttings
UMBS – aspen (trem.2)	200	OTC	F and NF	NI	Cuttings
UMBS – maple	200	OTC	F and NF	NI	Cuttings
USDA – orange	300	OTC	F	I	3
USDA Placerville	350	OTC	F and NF	I	3
Vielsalm	350	OTC	F and NF	I	11
WebFACE	160	FACE	NF	NI	Mature

F, fertilized; FACE, free-air CO₂ enrichment; I, irrigated; NF, not fertilized; NI, not irrigated; OTC, open top chamber.

mixed-model variance (Hedges, Gurevitch & Curtis 1999). We used standard deviation (SD) values reported in the individual studies, or calculated the SD from the standard error and the number of replicates (number of FACE rings or OTCs). Studies that did not report standard error or deviation were not included in the database. The natural log of the response ratio (r = response in treatment plots/response in untreated plots) was used as metric in the analyses, and is reported as the percentage change in elevated CO₂. The use of the natural logarithm instead of the Hedges d-index has the advantage of linearizing the metric, thereby being less sensitive to changes in a small control group.

A mixed model was used to assess the overall treatment effect of CO₂ enrichment, and the influence of fertilizer addition and soil N concentration. We also tested for differences between irrigation treatments, seasonal growth strategy (deciduous or evergreen trees), fumigation type used, amount of CO₂ increase and duration of the treatments. When several years of data were reported for one experiment, we calculated a weighted mean, using the reciprocal of the measurement variance.

If the number of studies used to calculate a mean and confidence interval (CI) is lower than 20, the CI can be too narrow (Hedges *et al.* 1999). Therefore, we used the CI based on resampling methods for the assessment of statistical differences (2500 iterations). Confidence limits based on bootstrapping tests are wider than standard confidence limits, implying that resampling estimates are more conservative (Adams, Gurevitch & Rosenberg 1997). If the calculated 95% CI did not overlap with zero, a significant response to elevated CO₂ was accepted. Significant differences between groups (=categorical analyses for treatment comparisons, different seasonal strategies and fumigation technologies) were identified on the basis of the within- and between-group heterogeneity. Significant differences are reported at $P < 0.05$. Analyses with continuous variables (soil N concentration, and amount and duration of CO₂ increase) were performed when the number of studies was larger than 10. Both a weighted regression using MetaWin as an unweighted regression [using Matlab 7.4.0.287 (R2007a) (MathWorks, Natick, MA, USA)] were performed and used for comparison. Significant correlations were reported at $P < 0.05$.

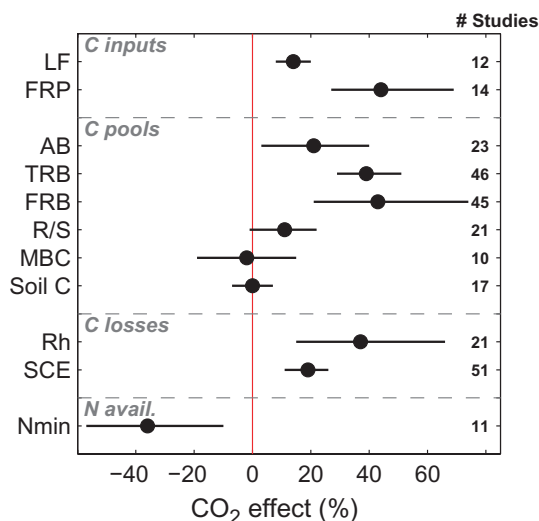


Figure 1. Overall CO₂ effects on soil C inputs, C pools, C losses and N availability. The effects on litterfall (LF), fine root production (FRP), above-ground biomass (AB), total root biomass (TRB), fine root biomass (FRB), root-to-shoot ratio (R/S), microbial biomass C (MBC), soil C content (soilC), heterotrophic respiration (Rh), soil CO₂ efflux (SCE) and net N mineralization (Nmin) are indicated as percentage response to elevated CO₂. Overall means and confidence intervals (CIs) are given, which means a significant CO₂ effect is apparent when the zero line is not crossed. The number of studies used for the analysis is indicated above the *x*-axis.

RESULTS

Soil C inputs

Above-ground litterfall and fine root production responded to elevated CO₂ with an increase of 14 and 44%, respectively (Fig. 1; Table 3).

Both parameters were not affected differently by elevated CO₂ in fertilized and unfertilized plots (Table 4). The CO₂ effect on fine root production was positively related to soil N concentration, for both the weighted meta-analysis regression (Table 4) as for the unweighted regression (Fig. 2a). No significant effect of amount or duration of the CO₂ increase in the treated plots was observed (Table 4).

C pools

Above-ground biomass, and total and fine root biomass responded positively to elevated CO₂ with a 21, 39 and 43% increase, respectively (Fig. 1; Table 3). This did not result in a statistically significant increase in root-to-shoot ratio in CO₂-fumigated studies, although there was a clear positive trend (Fig. 1; Table 3). Microbial biomass C and soil C were not significantly affected by elevated CO₂ (Fig. 1; Table 3).

There was no significant difference in CO₂ response between fertilized and unfertilized plots for any of the studied C pools (Table 4). However, above-ground biomass and soil C were significantly stimulated by elevated CO₂ in

the N fertilized studies, but not in the unfertilized experiments (Table 3). The CO₂ effect on fine root biomass was positively related to soil N concentration, for both the weighted meta-analysis regression (Table 4) as for the unweighted regression (Fig. 2b). The CO₂ effect on soil C content demonstrated a negative relationship with increasing soil N concentration in both regressions (Fig. 2c; Table 4).

There was no significant effect of amount or duration of the CO₂ increase in the treated plots (Table 4).

Soil C losses and net N mineralization

Rh and soil CO₂ efflux increased by 37 and 19%, respectively (Fig. 1; Table 3). Net N mineralization decreased by 36% in elevated CO₂ (Fig. 1; Table 3).

There was no significant difference in CO₂ response between fertilized and unfertilized plots for any of the studied parameters (Table 4). Both Rh and net N mineralization were significantly affected by elevated CO₂ only in the unfertilized experiments (Table 3). Increasing soil N concentration had a positive effect on the CO₂ response of Rh according to the meta-analysis regression (Table 4), while this relationship was borderline insignificant for the unweighted regression (Fig. 2d).

The CO₂ effect on microbial respiration increased with treatment duration (Table 4). None of the other parameters exhibited significant relationships with the amount of elevated CO₂ added, nor with the duration of the CO₂ treatments (Table 4).

Table 3. Elevated CO₂ effects on above-ground biomass, litterfall, total root biomass, fine root biomass, fine root production, root-to-shoot ratio (R/S), microbial biomass, heterotrophic respiration (Rh), soil CO₂ efflux, net N mineralization and soil C, indicated as percentage response to elevated CO₂

	Overall	Fertilized	Not fertilized
C inputs			
Litterfall	14	20	12
Fine root production	44	19	52
C pools			
Above-ground biomass	21	30	19
Root biomass	39	50	38
Fine root biomass	43	36	46
R/S	16	5	19
Microbial biomass	-2	2	-5
Soil C	0	14	-5
C losses			
Rh	37	27	44
Soil CO ₂ efflux	19	24	17
N availability			
Net N mineralization	-36	2	-43

Numbers in bold italics indicate statistically significant CO₂ effects. The CO₂ effect is considered significant when 0 is not included in the confidence interval (CI).

	Fertilization	CO ₂ increase	Duration	Soil N
C inputs				
Litterfall	0.38	0.29	0.07	0.95
Fine root production	0.22	0.13	0.28	<0.001
C pools				
Above-ground biomass	0.69	0.18	0.89	0.5
Root biomass	0.4	0.47	0.87	0.27
Fine root biomass	0.63	0.48	0.17	<0.001
R/S	0.4	0.63	–	0.43
Microbial biomass	0.82	0.55	0.58	–
Soil C	0.12	0.39	0.79	0.02
C losses				
Heterotrophic respiration (Rh)	0.54	0.06	0.045	0.02
Soil CO ₂ efflux	0.44	0.06	0.09	0.85
N availability				
Net N mineralization	0.25	0.11	0.1	–

Table 4. *P* values for the meta-analytical comparisons of CO₂ effects in different experimental treatments or conditions

Categorical comparison is conducted for fertilization (fertilized versus not fertilized). A continuous regression analysis was performed using the amount and duration of CO₂ increase and the soil N concentration as explanatory variables. Numbers in bold italics indicate statistically significant differences between categories or significant correlations. Differences are significant at $P < 0.05$.

DISCUSSION

Overall CO₂ responses

The observed overall CO₂-induced stimulation of above- and below-ground biomass and production agrees well with previous experimental findings (Rogers, Runion & Krupa 1994; Curtis & Wang 1998; Pendall *et al.* 2004; de Graaff *et al.* 2006). Interestingly, elevated CO₂ increased fine and total root biomass and production in all possible experimental

conditions addressed in this analysis (Table 3), while this was not the case for the above-ground biomass response to elevated CO₂. Together with the positive trend in the root-to-shoot ratio, this provides a strong signal for a C allocation shift towards below-ground biomass compartments in CO₂-fumigated systems. This is a common response in an elevated CO₂ world (Rogers *et al.* 1994; Luo, Hui & Zhang 2006b) as plants need more resources to sustain the enhanced growth (Bryant, Chapin & Klein 1983).

Increased above- and below-ground litterfall in elevated CO₂ enhances the soil C input. As soil organisms tend to be C limited (Zak *et al.* 1993; Hu *et al.* 2006), one would expect an increase in microbial biomass C as a consequence. However, this is not observed. Although microbes probably profit from the improved C availability initially, their biomass turns over relatively quickly (Heath *et al.* 2005; Lukac *et al.* 2009), possibly in part because of enhanced grazing by other soil organisms in elevated CO₂ (Zak *et al.* 2000). Moreover, the higher N immobilization in the increasing plant biomass (Luo *et al.* 2004) may impose a concomitant N limitation of microbial growth (Hu *et al.* 2001, 2006). We hypothesize that N limitation is a more plausible explanation for the lack of response of microbial biomass to elevated CO₂. Indeed, microbial biomass did not increase in spite of the increased plant C inputs. Moreover, the overall response of net N mineralization (a measure for the available inorganic N in the soil) to elevated CO₂ was negative, and net N mineralization decreased even more in elevated CO₂, when only the unfertilized experiments were included. As larger quantities of C entering the soil normally result in more N uptake, even in N-limited systems (Finzi *et al.* 2007), our results thus suggest that elevated CO₂ makes trees more efficient in immobilizing N, and that microbial growth likely becomes N limited in elevated CO₂, at least where N availability is not very high.

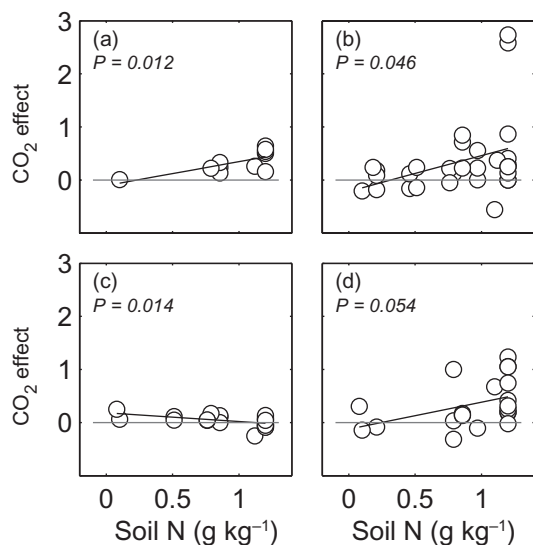


Figure 2. Unweighted regressions relating individual CO₂ responses (y-axis) to soil N concentration (x-axis). Responses of fine root production (a), fine root biomass (b), soil C content (c) and heterotrophic respiration (Rh) (d) are depicted as the log response ratio [$\ln(\text{elevated CO}_2/\text{ambient CO}_2)$]. Soil [N] is given in g N kg⁻¹ soil. Differences are significant at $P < 0.05$.

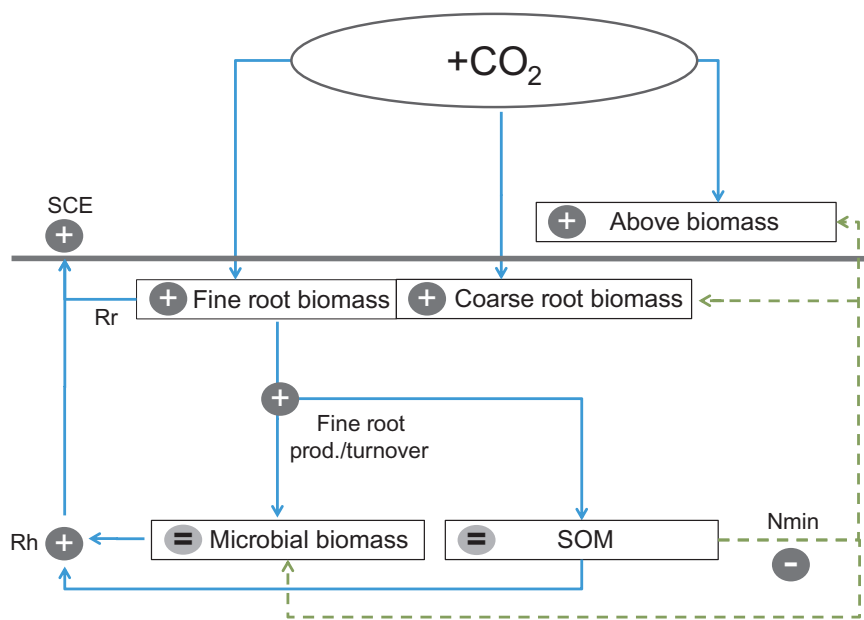


Figure 3. Conceptual representation of the overall CO₂ effects on C cycling in tree stands. Pools are given in boxes; fluxes are given in lines. Blue lines are C-related processes; green dashed lines are N related. Black circles indicate a statistically significant CO₂ effect; grey circles indicate a statistically non-significant trend. Results are considered statistically significant at $P < 0.05$. SOM, soil organic matter, Nmin, net N mineralization; Rh, microbial respiration; Rr, root respiration; SCE, soil CO₂ efflux. Elevated CO₂ induces a C allocation shift towards below-ground biomass, where the increased C inputs (fine root production/turnover) increase the CO₂ response of microbial respiration (Rh), leaving net change in soil C unaffected in elevated CO₂. A strong negative CO₂ response of net N mineralization indicates a lower N availability in elevated CO₂.

Our observed increase in microbial respiration is counterintuitive, considering the lack of microbial biomass response in elevated CO₂ studies. However, besides the increase of biomass C inputs in the soil, plants also tend to increase root exudation in elevated CO₂ (Fitter *et al.* 1997; Drigo, Kowalchuk & van Veen 2008; Lukac *et al.* 2009). This labile C input could fuel the microbial community (Zak *et al.* 2000; Heath *et al.* 2005), but is mainly respired because the N necessary to convert these C inputs into microbial biomass is lacking. Therefore, Rh can increase despite the lack of change in microbial biomass. Another possible mechanism is a shift in microbial community composition towards a more fungal-dominated community, which is less N demanding (Hu *et al.* 2001; Zhang *et al.* 2005; Carney *et al.* 2007). This shift may occur, but would still be expected to increase microbial biomass, albeit less pronounced. Both mechanisms could play a role in explaining the positive response of Rh in elevated CO₂, but data are lacking to test which of these mechanisms is more important.

As both plant litter production and Rh in CO₂-fumigated experiments increase to a similar extent, the lack of response of soil C is not unexpected (Fig. 3). Similar results for forests were already reported by de Graaff *et al.* (2006), who reported a positive response of soil C only for grasslands. Thus, any increase in C accumulation in tree stands subjected to elevated CO₂ will likely be confined to increased woody biomass production.

Influence of N fertilization

We did not find any significant differences in the response of above-ground biomass and soil C storage to elevated CO₂ between fertilized and unfertilized studies, which is in contrast to earlier studies (de Graaff *et al.* 2006; van Groenigen *et al.* 2006; Luo *et al.* 2006a). However, while both above-ground biomass and soil C are significantly stimulated by

elevated CO₂ in the fertilized studies, they are not in the unfertilized ones, suggesting that the lack of a statistically significant difference might be caused by low statistical power because of a smaller data set (in this analysis, only tree stands were considered). Our focus on tree stands only may also explain part of the difference, because forests and grasslands have very different C use patterns (Schulze *et al.* 2009).

van Groenigen *et al.* (2006) and de Graaff *et al.* (2006) indicated that soil C accumulation is significantly enhanced in elevated CO₂ only when sufficient amounts of N were added. They hypothesize that as nitrogen fertilization enhances plant productivity, it therefore could increase the amount of C entering the soil, resulting in a net increase of soil C. The reason for this would be that the stimulated plant production in fertilized stands would overcome the increased decomposition in elevated CO₂. While this hypothesis also fits our results, we suggest a prominent role for fine root dynamics as soil C inputs (Zak *et al.* 2000). As de Graaff *et al.* (2006) suggested, we found a stronger CO₂ response of woody biomass compartments in the fertilized stands. However, while the fine root biomass and production response to elevated CO₂ was significantly affected in both fertilized and unfertilized tree stands, the CO₂ response is larger in the unfertilized ones. Indeed, N fertilization is known to generally stimulate woody biomass increase, without affecting soil C inputs (Pregitzer *et al.* 2008). At the same time, we observed a strongly increased Rh response to elevated CO₂ in the unfertilized stands, while the CO₂ effect is not significant in the fertilized stands. Our results therefore suggest that, because of an increased soil exploration in unfertilized stands, fine root dynamics and rhizodeposition will be more pronounced, and will serve as a direct substrate for the microbial community. As a consequence, we see an increased soil C storage in the fertilized stands subject to elevated CO₂, while a negative trend is apparent in the unfertilized stands.

Aside from this C input-related feedback, retarded rates of SOM decomposition in N-fertilized systems are common (Fog 1988; Berg & Matzner 1997), which could also contribute to an increased soil C storage in tree stands (Janssens *et al.* submitted). The inhibitory effects of N fertilization on SOM decomposition can be obscured in CO₂-fumigated experiments (Janssens *et al.* 2010), as soil C inputs typically increase under CO₂-fumigated systems (DeLucia *et al.* 1999; Pendall *et al.* 2004; Subke, Inglima & Cotrufo 2006; Liu *et al.* 2007; Soussana & Luscher 2007), and CO₂ elevation stimulates root exudation and rhizodeposition, all of which affect microbial activity (Norby, O'Neill & Wullschleger 1995; Canadell, Pitelka & Ingram 1996; Lipson, Wilson & Oechel 2005). This could also explain why N fertilization only stimulates soil C accumulation in elevated CO₂ when very large amounts of N are applied (van Groenigen *et al.* 2006).

Relationship with soil N concentration

Soil N concentration was significantly correlated to responses of fine root biomass and –production, microbial respiration and soil C to elevated CO₂ (Table 4). Figure 2 illustrates that roots and Rh show only minor responses when soil N is low, which is to be expected, as the elevated CO₂-induced growth stimulation cannot be sustained without sufficient available N. In N-rich soil, however, we see that elevated CO₂ strongly increases plant productivity, which affects below-ground C cycling through a stimulation of both C inputs and losses. In N-rich soils, this accelerated C cycling under elevated CO₂ even results in a negative effect of elevated CO₂ on soil C storage with increasing soil N concentration (Table 4; Fig. 2). Overall, these results confirm that the CO₂ effect on soil C inputs is the driving factor in soil C cycling, and can be modulated by N. However, these effects of soil N on the elevated CO₂ responses in our analysis differ from the approach where N fertilization is used as a measure for soil N availability in tree systems. Therefore, responses to elevated CO₂ and interactions with N are summarized in Box 1.

Box 1. Interactive effects of elevated CO₂ and N on C cycling in tree stands.

N fertilization and soil N concentration are two different ways to approach N availability, yet interact differently with elevated CO₂ (see grey coloured part in the table). These contrasting effects are mainly a function of the direct availability of the N. In fertilized stands, the N is added in mineral form, while in N-rich systems, the N is still embedded in organic molecules or bound to the soil matrix. CO₂-fumigated tree stands will respond to both conditions in a different way, starting with fine root dynamics. In N-fertilized stands, the readily available N reduces the need for soil exploration by fine roots, and the associated reduction in rhizodeposition decreases

the stimulation of Rh in elevated CO₂. This decreased decomposition response in N-fertilized tree stands provides a larger potential for soil C accumulation. When tree stands are not fertilized, the larger demand for N in elevated CO₂ elicits an increased soil exploration by fine roots. Therefore, only in the N-rich systems, plants can sustain the increased growth responses in elevated CO₂. In response to the exacerbated fine root dynamics in elevated CO₂, the Rh response to elevated CO₂ increases, resulting in a decreasing soil C response to elevated CO₂ in systems with high soil N concentrations.

	Fertilized versus unfertilized experiments	High soil [N] versus low soil [N]
C inputs		
Litterfall	↑	–
Fine root production	↓	↑
C pools		
Above-ground biomass	↑	–
Root biomass	↑	–
Fine root biomass	↓	↑
Microbial C	↑	–
Soil C	↑	↓
C losses		
Heterotrophic respiration	↓	↑
Soil CO ₂ efflux	↑	–

Note: Arrows indicate the direction of the effect of N on the CO₂ responses of soil C inputs, C pools and C losses.

Our findings indicate that an approach where treatments are simply opposed is a convenient way for statistical analysis, but fails to cover important information that is often not accounted for. Unfertilized sites can be nutrient rich, and therefore partly miss the expected fertilization treatment response (e.g. POPFACE or EUROFACE experiments). Similarly, it is normal that nutrient-poor sites that are fertilized demonstrate the largest relative responses (e.g. the Birmensdorf experiments). This could be one of the reasons why we do not always find a clear effect of fertilization treatments on soil C cycling in elevated CO₂. The response to CO₂ is an issue of definition, time and resource supply (Körner 2006). Based on our results, we suggest that more attention should be given to proper descriptions and reporting of experimental characteristics and soil properties in manipulation experiments. Parameters such as soil nutrient or water availability, or stand leaf area index (LAI), age and management are often not described properly, although they play a very important role in regulating plant responses to elevated CO₂ and would make evaluating tree stand responses more accurate. Therefore, we underline that a better understanding of terrestrial ecosystem responses to global change could be obtained from better or more standardized reporting of experimental conditions.

Conclusion

Our results confirm the important role of fine root dynamics in soil C cycling in elevated CO₂, as the increased fine root activity induced an acceleration of SOM decomposition processes. At the same time, N availability can limit plant growth responses and can therefore influence soil C cycling responses in elevated CO₂. While we failed to indicate differences between N-fertilized and -unfertilized tree stands in elevated CO₂, we clearly showed that soil N concentration can modulate soil C cycling. In elevated CO₂, fine root biomass and production, and Rh all increase with increasing soil N, while soil C decreases with total soil N concentration, regardless of N fertilization. We can therefore conclude that soil C cycling rates and soil C sequestration potential in elevated CO₂ will be influenced by initial soil properties and fertility, because (woody) plant growth responses to elevated CO₂ are dependent on N availability, while below-ground responses are more dependent on changing soil C availability.

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SUPPORTING INFORMATION

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

Table S1. Information stored in the database for individual sites and experiments. Different tables include site information, sampling methodologies reported in the individual studies and the amount of data used to calculate the meta-analysis entries.

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