



# Effects of elevated CO<sub>2</sub> and N fertilization on plant and soil carbon pools of managed grasslands: a meta-analysis

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**Abstract.** Elevated atmospheric CO<sub>2</sub> levels and increasing nitrogen deposition both stimulate plant production in terrestrial ecosystems. Moreover, nitrogen deposition could alleviate an increasing nitrogen limitation experienced by plants exposed to elevated CO<sub>2</sub> concentrations. However, an increased rate of C flux through the soil compartment as a consequence of elevated CO<sub>2</sub> concentrations has been suggested to limit C sequestration in terrestrial ecosystems, questioning the potential for terrestrial C uptake to mitigate increasing atmospheric CO<sub>2</sub> concentrations. Our study used data from 77 published studies applying elevated CO<sub>2</sub> and/or N fertilization treatment to monitor carbon storage potential in grasslands, and considered the influence of management practices involving biomass removal or irrigation on the elevated CO<sub>2</sub> effects. Our results confirmed a positive effect of elevated CO<sub>2</sub> levels and nitrogen fertilization on plant growth, but revealed that N availability is essential for the increased C influx under elevated CO<sub>2</sub> to propagate into belowground C pools. However, moderate nutrient additions also promoted decomposition processes in elevated CO<sub>2</sub>, reducing the potential for increased soil C storage. An important role was attributed to the CO<sub>2</sub> response of root biomass in soil carbon responses to elevated CO<sub>2</sub>, since there was a lower potential for increases in soil C content when root biomass increased. Future elevated CO<sub>2</sub> concentrations and increasing N deposition might thus increase C storage in plant biomass, but the potential for increased soil C storage is limited.

## 1 Introduction

Atmospheric CO<sub>2</sub> concentrations have strongly increased since the pre-industrial era (IPCC, 2007), resulting in the contemporary CO<sub>2</sub> concentration of about 393 ppm that exceeds all earlier concentrations since the late Tertiary era, when most of the modern plants evolved into their present shapes (Pearson and Palmer, 2000; Crowley and Berner, 2001). Because of the stimulating effect of these elevated CO<sub>2</sub> concentrations on photosynthesis and plant productivity (Nowak et al., 2004; Ainsworth and Long, 2005; Soussana and Luscher, 2007), it has been hypothesized that plants can partly buffer human induced CO<sub>2</sub> emission by sequestering C (Gifford, 1994). Grasslands are estimated to embody more than 10 % of the carbon (C) reservoir of the biosphere (Eswaran et al., 1993; Nosberger et al., 2000), with most C (up to 98 % of the total C) located in their belowground compartment (Hungate et al., 1997). The 3.7 billion ha of the Earth's surface with permanent grasslands have an estimated potential annual C sequestration capacity of 0.01–0.3 GtC (Lal, 2004), which implies that 4 % of total global emissions of greenhouse gasses could be buffered by grasslands (Soussana and Luscher, 2007).

Elevated CO<sub>2</sub> tends to increase C allocation to root compartments (Rogers et al., 1994; Luo et al., 2006) as plants need more resources to sustain the enhanced growth (Bryant et al., 1983). In addition, plants also tend to increase root exudation in elevated CO<sub>2</sub> (Fitter et al., 1997; Drigo et al., 2008; Lukac et al., 2009). As soil organisms tend to be C-limited (Zak et al., 1993; Hu et al., 2006), these C inputs

could fuel the microbial community (Zak et al., 2000; Heath et al., 2005), leading to increased microbial biomass and respiration. However, when the N necessary to convert these C inputs into microbial biomass is lacking (Zak et al., 2000; Heath et al., 2005), these C inputs are mainly respired. Therefore, microbial respiration (Rh) can increase despite the lack of change in microbial biomass. As a consequence, effects of elevated CO<sub>2</sub> on soil C content are unclear because both C inputs and decomposition processes are stimulated, and because the effect on microbial growth and functioning seems to be modulated by N availability.

Because many grasslands are managed for feeding domestic herbivores, either directly through grazing or through forage production, grassland C and N cycles might be affected because a large part of primary production is removed (Sousana et al., 2007). As a consequence, grasslands are often fertilized with nutrients to sustain productivity. In addition, the increased reactive nitrogen (N) deposition caused by the burning of fossil fuels and the use of artificial fertilizers (Davidson, 2009) may affect large areas of the world in the future (Galloway, 2008). Excessive N deposition can negatively influence ecosystem health and species diversity (Aber et al., 1998), but lower concentrations can alleviate the N limitation that plants generally experience in grasslands, thereby stimulating plant production (Lu et al., 2011). In their review, de Graaff et al. (2006) hypothesized that increased plant production in elevated CO<sub>2</sub> could overcome increased soil organic matter (SOM) decomposition processes when ecosystems are supplemented with additional N.

In this study, we used meta-analysis to investigate whether CO<sub>2</sub> elevation and/or nitrogen fertilization is likely to change carbon storage in managed grasslands. More precisely, we analysed effects of elevated CO<sub>2</sub> concentrations and N fertilization (i.e., combined and individually) on above and belowground biomass, microbial biomass and soil C content by quantitatively synthesizing data from 77 studies. More specifically, we used following hypotheses: (1) the single factor elevated CO<sub>2</sub> treatment will stimulate plant production and will increase allocation of C to root compartments, (2) the single factor N fertilization treatment will stimulate plant productivity, but will leave microbial biomass unaffected due to C limitation, (3) the combined CO<sub>2</sub> and N treatment will strongly stimulate above and belowground biomass production, which in turn stimulates soil C storage, and (4) management practices (i.e., aboveground biomass removal, irrigation) will shift C allocation towards aboveground plant compartments and will reduce C inputs to soil compartments.

## 2 Methods

### 2.1 Data acquisition

We constructed a database consisting of results from 77 manipulation experiments in grassland systems exposed to elevated CO<sub>2</sub> concentrations with/without nutrient additions. Here, we focus on aboveground (AB), root (RB) and microbial biomass (MB), root to shoot ratio (RS, calculated where AB and RB were available) and soil C content. Figures and tables within articles were used as a source for data. Aboveground and root biomass were expressed on a dry weight per area basis. Microbial biomass was expressed on a dry weight per unit of soil weight basis, and soil C content was expressed on a dry weight per area, or dry weight per unit of soil weight basis. For soil C content data expressed on an area basis, we assumed that soil density was not affected by elevated CO<sub>2</sub> treatments. This resulted in 192 entries that were used in the meta-analysis. A full description of the experiments and data sources is given in the supplementary Tables A1–A5.

Only studies that reported standard errors and the number of replicates were included in our analysis. We selected studies on grassland systems that were exposed to elevated CO<sub>2</sub> concentrations. Results for different treatments, species, or different locations within one and the same experiment were considered as independent measurements and were included separately in the database. Weighted means were calculated for experiments with data from different years, using the measurement uncertainties of individual years as weighting factor.

We extracted mean annual precipitation (MAP) and mean annual temperature (MAT) data, a description of the amount and type of fertilizer added (independent from the intention of creating a different treatment) and the execution of other management practices (biomass removal or irrigation) from the articles. Whenever this information was lacking, the study was considered as not including fertilization or other management. The extracted information is synthesized in Table 1.

### 2.2 Meta-analysis

MetaWin 2.1 software (Rosenberg et al., 2000) was used to analyse our data. The natural logarithm of the response ratio ( $r = (\text{response to elevated CO}_2 \text{ or N fertilization}) / (\text{response to reference conditions})$ ) was used to define the effect value. By using this metric, the calculation of an effect by percentage was made possible, while this would not have been the case if we were to use Hedges' d-index. In addition, the response ratio is less sensitive to changes in small control groups (Hedges et al., 1999). Confidence intervals (CI) were calculated by using bootstrapping techniques. This method is advantageous when less than 20 studies are used to calculate a CI, since the traditional 95 % CI then tends to underestimate the width of the interval at low

**Table 1.** Information about irrigation, fertilization, management practices and climatic conditions at the sites that were used in the experiments in our analysis. Different letters (a and b) within the fertilizer specifications are used to separate different experiments that were executed on the same site.

Site Name	Irrigation	Fertilization	Fertilizer Type	Fertilizer Amount	Biomass removal	MAP (mm)	MAT (°C)	Reference
Aberdeen	Yes	Yes & No	NaH <sub>2</sub> PO <sub>4</sub> , KNO <sub>3</sub> and NaNO <sub>3</sub>	–	Clipping	–	–	Paterson et al. (2008)
Amsterdam	Yes	Yes	NH <sub>4</sub> NO <sub>3</sub>	47 kg ha <sup>-1</sup> yr <sup>-1</sup>	–	–	–	Hoorens et al. (2003)
Cedar Creek grassland	No	Yes & No	NH <sub>4</sub> NO <sub>3</sub>	(a) 40 kg ha <sup>-1</sup> yr <sup>-1</sup> (b) 100 kg ha <sup>-1</sup> yr <sup>-1</sup>	Burning	679	5.6	Dijkstra et al. (2006); Keeler et al. (2009)
Canberra Phytotron	Yes	Yes	Not specified	22, 67 and 198 kg ha <sup>-1</sup> yr <sup>-1</sup> in different treatments	–	–	–	Lutze et al. (2000)
Duolun	No	Yes	NH <sub>4</sub> NO <sub>3</sub>	100 kg ha <sup>-1</sup> yr <sup>-1</sup>	–	386	2.1	Xia et al. (2009)
French Massif Central	No	No	–	–	–	780	8.7	Bloor et al. (2010)
Gainesville grassland	Yes	Yes	NPK (and Mg and S)	70–80 kg ha <sup>-1</sup> depending on year	–	808	–	Allen et al. (2006)
Ginninderra	Yes	Yes	slow release fertilizer	100 kg ha <sup>-1</sup> yr <sup>-1</sup>	–	–	–	Volder et al. (2007)
Jasper Ridge (FACE)	Yes & No	Yes & No	Ca(NO <sub>3</sub> ) <sub>2</sub>	70 kg ha <sup>-1</sup> yr <sup>-1</sup>	–	677	19.3	Dukes et al. (2005)
Jasper Ridge (OTC)	No	Yes & No	(a) Urea + Osmocote 120 days slow release fertilizer (b) NPK (120 day release fertilizer)	(a) 200 kg ha <sup>-1</sup> (b) low: 30 kg ha <sup>-1</sup> ; high: 200 kg ha <sup>-1</sup>	–	677	19.3	Hungate et al. (1997); Cardon et al. (2001)
Jokioinen	Yes	No	–	–	Mowing	–	–	Kanerva et al. (2008)
Linden-Leihgestern (FACE)	No	Yes	Thomas kali fertilizer and N	4 kg ha <sup>-1</sup> yr <sup>-1</sup>	Cutting	644	9.9	Sonnemann and Wolters (2005)
Manawatu	No	Yes	superphosphate, K <sub>2</sub> SO <sub>4</sub> , MgSO <sub>4</sub> , Cu and Zn	–	–	870	12.9	Ross et al. (2004)
Moor House	No	No	–	–	–	–	–	Fitter et al. (1997)
Näntuna	No	No	–	–	Cutting	527	5.5	Marissink et al. (2002)
NERC	Yes	No	–	–	Herbivory	–	–	Kandeler et al. (1998)
New Zealand (FACE)	No	Yes	superphosphate, KSO <sub>4</sub>	–	Grazing	875	–	Allard et al. (2005)
Niwot Ridge	No	Yes	(a) urea-N as osmocote pellets (b) osmocote pellets (urea-N and P <sub>2</sub> O <sub>5</sub> -P)	250 kg ha <sup>-1</sup> yr <sup>-1</sup> for the first two years, 100 kg ha <sup>-1</sup> yr <sup>-1</sup> thereafter	–	–	–	Bowman et al. (1993); Neff et al. (2002)
OCCAM	No	No	–	–	–	1322	14.3	Kardol et al. (2010)
PHACE	No	No	–	–	–	384	–	Dijkstra et al. (2010)
Swiss Central Alps	No	Yes & No	NPK (3:2:3)	45 kg ha <sup>-1</sup> yr <sup>-1</sup>	–	168	8.4	Niklaus and Korner (1996); Schappi and Korner (1996)
Swiss Jura	No	No	low dose P fertilization (superphosphate)	–	Mowing/ Clipping	1063	–	Leadley et al. (1999); Stocker et al. (1999); Niklaus et al. (2003); Niklaus and Korner (2004)
SwissFACE (Bromus/Carex)	No	No	–	–	–	–	–	van Kleunen et al. (2006)
SwissFACE (Lolium)	No	Yes	NH <sub>4</sub> NO <sub>3</sub>	low: 140 kg ha <sup>-1</sup> yr <sup>-1</sup> ; high: 420 kg ha <sup>-1</sup> yr <sup>-1</sup> in 1993 and 560 kg ha <sup>-1</sup> yr <sup>-1</sup> after 1993	Cutting	–	–	Sowerby et al. (2000); de Graaff et al. (2004); Bazot et al. (2006)
SwissFACE (Trifolium)	No	Yes	NPK, N as NH <sub>4</sub> NO <sub>3</sub> solution	low: 140 kg ha <sup>-1</sup> yr <sup>-1</sup> ; high: 420 kg ha <sup>-1</sup> yr <sup>-1</sup> in 1993 and 560 kg ha <sup>-1</sup> yr <sup>-1</sup> after 1993	Cutting	–	–	de Graaff et al. (2004)
TasFACE	No	No	–	–	–	560	11.6	Pendall et al. (2011)
University of Antwerp	No	No	–	–	–	–	–	unpublished
University of Guelph	Yes	Yes	Hoagland's solution	47 kg ha <sup>-1</sup> yr <sup>-1</sup>	–	–	–	Klironomos et al. (1998)
University of Michigan Biological Station	Yes	No	–	–	–	–	–	Treonis and Lussenhop (1997)
USDA ARS	Yes	No	–	–	–	–	–	Blank and Derner (2004)
USDA Central Plains	No	No	–	–	–	320	–	Morgan et al. (2004b); Pendall and King (2007)

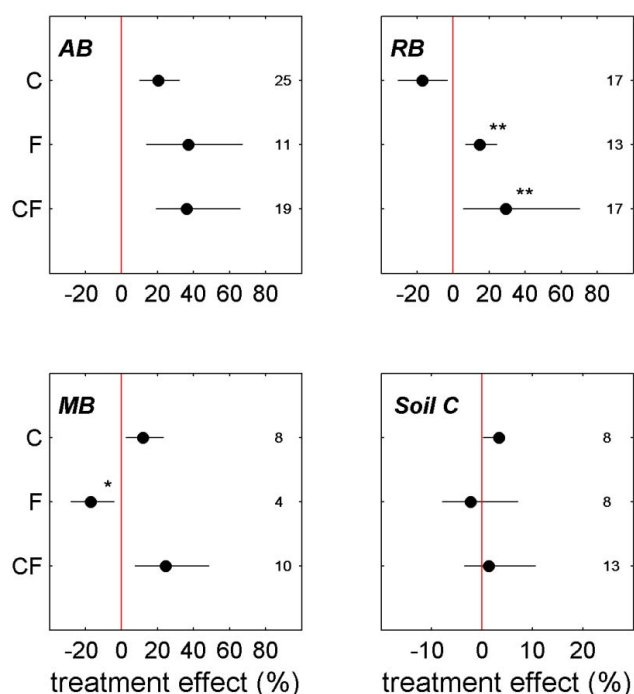
sample size (Hedges et al., 1999). For bootstrapping, 2500 repetitions were used.

In categorical analyses, we examined the effect of elevated CO<sub>2</sub> concentrations and fertilization separately (in experiments where single factor CO<sub>2</sub> and combined CO<sub>2</sub> and fer-

tilization treatment effects were reported, we extracted a single factor fertilization treatment response using the control values of both CO<sub>2</sub> treatments), the effect of elevated CO<sub>2</sub> concentration in combination with fertilization, the effects of the type and the amount of N fertilizer added (classification

**Table 2.** Treatment codes used in the manuscript to describe different CO<sub>2</sub> or N treatment combinations.

Treatment code	Description
C	Elevated CO <sub>2</sub> treatment without any nutrient addition
CF	Elevated CO <sub>2</sub> treatment with nutrient addition (amount of N addition not specified)
F	N fertilization treatment (amount of N addition not specified)
CF-L	Elevated CO <sub>2</sub> treatment with N fertilization <50 kgN ha <sup>-2</sup> yr <sup>-1</sup> (low N)
CF-H	Elevated CO <sub>2</sub> treatment with N fertilization >50 kgN ha <sup>-2</sup> yr <sup>-1</sup> (high N)
CF-N	Elevated CO <sub>2</sub> treatment with pure N fertilization
CF-NPK	Elevated CO <sub>2</sub> treatment with NPK fertilization
C-M	Elevated CO <sub>2</sub> treatment with biomass removal component
C-m	Elevated CO <sub>2</sub> treatment without biomass removal component
C-I	Elevated CO <sub>2</sub> treatment with irrigation treatment
C-i	Elevated CO <sub>2</sub> treatment without irrigation treatment
F-L	N fertilization treatment with N fertilization <50 kgN ha <sup>-2</sup> yr <sup>-1</sup> (low N)
F-H	N fertilization treatment with N fertilization >50 kgN ha <sup>-2</sup> yr <sup>-1</sup> (high N)
F-N	N fertilization treatment with pure N fertilization
F-NPK	N fertilization treatment with NPK fertilization



**Fig. 1.** Responses of grassland C pools to three different treatments: CO<sub>2</sub> elevation (C), N fertilization (F) and the combination of CO<sub>2</sub> elevation and N fertilization (CF). Responses are shown as percentage increase of aboveground biomass (AB), root biomass (RB), microbial biomass (MB), and soil C content (Soil C), and 95 % confidence intervals (CI). Treatment responses were considered statistically significant when zero was not included in the 95 % CI. Statistically significant differences compared to the single factor CO<sub>2</sub> treatment are indicated by: \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .

in low and high amounts was based on a background value of 50 kg N ha<sup>-1</sup> yr<sup>-1</sup>, based on projected N deposition values in 2050, Galloway, 2008), and the effects of biomass removal or irrigation on the elevated CO<sub>2</sub> effect (see Table 2 for treatment codes used in figures and tables). The relationship of elevated CO<sub>2</sub> effects with MAP, MAT, treatment duration and intensity were analysed with weighted regression analysis.

The effect of elevated CO<sub>2</sub> concentrations or fertilization were considered statistically significant when zero was not included in the 95 % CI. Differences between categorical variables and linear regression analyses were considered statistically significant when  $P$ -values were lower than 0.05.

### 3 Results

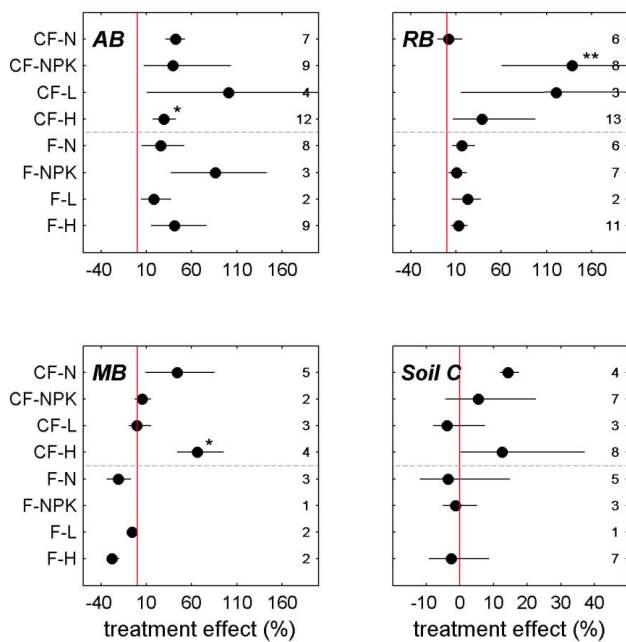
#### 3.1 Single factor and combined treatment effects of elevated CO<sub>2</sub> and N addition

Aboveground biomass increased under all three treatments (i.e., elevated CO<sub>2</sub> (+20 %), N fertilization (+37 %) and their combination (+36 %)) (Fig. 1). Root biomass decreased when only CO<sub>2</sub> levels were elevated (-17 %), but increased when nutrients were added, either with (+29 %) or without CO<sub>2</sub> elevation (+15 %) as a co-treatment (Fig. 1, Table 3). Microbial biomass increased in elevated CO<sub>2</sub> concentrations, both with (+24 %) and without fertilization (+12 %), and showed an opposing trend in response to the single factor fertilization treatment (-17 %) (Fig. 1, Table 3). Soil C content increased in the single factor CO<sub>2</sub> treatment (+3 %) and was unaltered under the other treatments (Fig. 1).

In the combined elevated CO<sub>2</sub> and fertilization treatment, aboveground biomass responded similarly to different fertilizer types, but was stimulated significantly more when lower

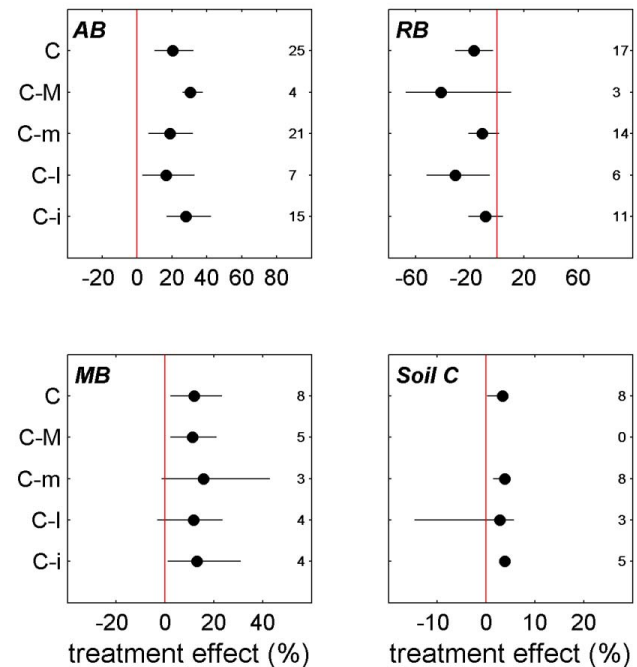
**Table 3.** Summary of the meta-analytical comparison between the responses of grassland C pools to different treatments. Results shown for: (1) CO<sub>2</sub> elevation and N fertilization treatments, (2) different N fertilization specifications with or without CO<sub>2</sub> elevation (type or amount of fertilizer) and (3) other management procedures when CO<sub>2</sub> is elevated (biomass removal and irrigation). The parameters considered are: aboveground biomass (AB), root biomass (RB), root-to-shoot ratio (RS), microbial biomass (MB) and soil C content (Soil C). Differences between responses for a parameter were considered statistically significant when *P* < 0.05 (**bold**).

	AB	RB	RS	MBC	Soil C
(1) C vs. F	0.2115	<b>0.0060</b>	0.4042	<b>0.0128</b>	0.086
C vs. CF	0.2511	<b>0.0028</b>	0.2827	0.4346	0.7017
F vs. CF	0.9628	0.4974	0.1759	0.0716	0.5274
(2) CF-N vs. CF-NPK	0.9736	<b>0.0036</b>	<b>0.0012</b>	0.4262	0.6809
CF-L vs. CF-H	<b>0.0168</b>	0.2431	0.1811	<b>0.0336</b>	0.2019
F-N vs. F-NPK	0.1076	0.6006	<b>0.0344</b>	—	0.8477
F-L vs. F-H	0.5674	0.4702	0.1795	0.3419	—
(3) C-M vs. C-m	0.4958	0.0672	—	0.7093	—
C-I vs. C-i	0.3227	0.0696	0.3663	0.926	0.3503



**Fig. 2.** CO<sub>2</sub> and N fertilization responses of grassland C pools to different N fertilizer types (CF-N and CF-NPK) and intensities (CF-L and CF-H). Responses are shown as percentage increase of aboveground biomass (AB), root biomass (RB), microbial biomass (MB) and soil C content (Soil C), and 95 % confidence intervals (CI). Treatment responses were considered statistically significant when zero was not included in the 95 % CI. Statistically significant differences between fertilizer type or intensity are indicated by: \* *P* < 0.05; \*\* *P* < 0.01.

doses of N fertilizer were added (Fig. 2, Table 3). In contrast to the aboveground biomass response, root biomass responded strongly positively to CO<sub>2</sub> elevation with NPK fertilizer addition, while pure N addition did not affect root biomass (Fig. 2, Table 3). The microbial biomass response



**Fig. 3.** The CO<sub>2</sub> effect in experiments with (C-M) or without (C-m) aboveground biomass removal, and in irrigated (C-I) and non-irrigated (C-i) experiments, compared to the full CO<sub>2</sub> dataset (C). Responses are shown as percentage increase of aboveground biomass (AB), root biomass (RB), microbial biomass (MB) and soil C content (Soil C). Responses were considered statistically significant when zero was not included in the 95 % CI.

to elevated CO<sub>2</sub> was significantly higher under high N fertilization rates, compared to low fertilization rates (Fig. 2, Table 3). Weighted linear regression analysis also suggested an increase in microbial biomass in elevated CO<sub>2</sub> with higher N fertilization doses (Table 4). Soil C responses to elevated

**Table 4.** Summary of the meta-analytical regression analysis between the responses of grassland C pools to increasing amounts of N fertilization as a single factor (F) or in combination with elevated CO<sub>2</sub> (CF). The parameters considered are: aboveground biomass (AB), root biomass (RB), root-to-shoot ratio (RS), microbial biomass (MB) and soil C content (Soil C). *P*-values, sign of the regression slopes, and number of data points used are given. Linear regressions were considered statistically significant when *P* < 0.05 (**bold**).

Treatment	Response variable	P-value	slope	<i>n</i>
CF	AB	0.5196	–	16
	RB	0.9891	–	15
	RS	0.9083	–	14
	MB	<b>0.0314</b>	+	7
	Soil C	0.8884	+	11
F	AB	<b>0.0417</b>	+	11
	RB	0.833	+	13
	RS	0.3142	–	13
	MB	<b>0.0183</b>	–	4
	Soil C	0.1117	+	8

CO<sub>2</sub> were not affected differently by different fertilizer types or doses (Fig. 2, Table 3).

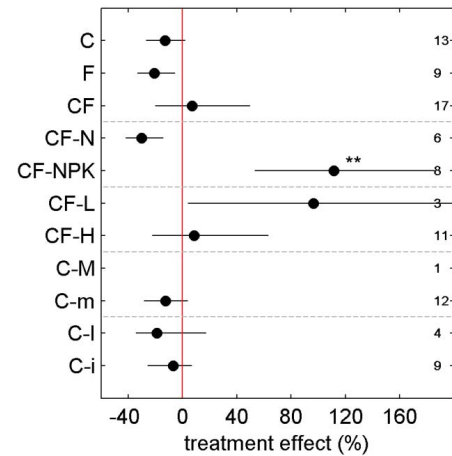
For the majority of the C pools, the single factor N fertilization treatment effects were not significantly different between fertilizer type or dosage (Fig. 2, Table 3), although a trend towards stronger aboveground biomass responses was apparent under NPK fertilization (Fig. 2, Table 3). Biomass removal or irrigation did not significantly affect CO<sub>2</sub> responses, although root biomass showed a stronger trend towards a decrease in systems where aboveground biomass was removed or systems that were irrigated (Fig. 3, Table 3).

### 3.2 Carbon allocation shifts

The root-to-shoot ratio (RS) of grasslands tended to decrease in the single factor CO<sub>2</sub> treatment (–13%), and significantly decreased in the single factor N fertilization treatment (–21%), indicating an preferential allocation of C towards aboveground biomass (Fig. 4). The combined CO<sub>2</sub> and N treatment did not change allocation patterns in grasslands (Fig. 4). There was a strong contrast between RS-responses to elevated CO<sub>2</sub> depending on the type of fertilizer added: pure N addition decreased RS (–30%), while NPK fertilizers increased RS in elevated CO<sub>2</sub> (+112%) (Fig. 4, Table 3). Biomass removal and irrigation did not affect the overall RS response to elevated CO<sub>2</sub> (Fig. 4).

### 3.3 Relation to climatic variables and treatment duration and intensity

The aboveground biomass response to elevated CO<sub>2</sub> concentrations was not related to treatment intensity or duration,



**Fig. 4.** CO<sub>2</sub> and N fertilization responses of the root-to-shoot ratio (RS) to different N fertilizer types (CF-N and CF-NPK) and intensities (CF-L and CF-H), and in experiments with (C-M) or without (C-m) biomass removal, and in irrigated (C-I) and non-irrigated (C-i) experiments. Responses are shown as percentage increase and were considered statistically significant when zero was not included in the 95% CI. Statistically significant differences between fertilizer types are indicated by: \*\* *P* < 0.01.

and did not show any dependence on air temperature (Table 5). In contrast, aboveground biomass responses tended to be smaller on sites with larger annual precipitation amounts, especially where no fertilizer was added to the experiments (Table 5).

Root biomass responses were greater at higher CO<sub>2</sub> elevation (Table 5), and tended to be larger in studies with longer duration of the CO<sub>2</sub> treatments, although the latter effect completely disappeared when N fertilizer was added to the experiments (Table 5). Root responses were not related to precipitation amounts, but showed a stronger CO<sub>2</sub> response in warmer sites that received N fertilizer (Table 5).

Responses of the root-to-shoot ratio to elevated CO<sub>2</sub> were generally not affected by different climatic conditions (Table 5). RS responses did become smaller with longer treatment duration, indicating a gradual increase in C allocation to shoots (Table 5). In contrast, increasing treatment intensity elicited gradually larger RS responses, indicating more C allocation to roots (Table 5). Whereas the relation to treatment duration holds for both fertilized and unfertilized CO<sub>2</sub> experiments, the relation to treatment intensity is only statistically significant for N fertilized CO<sub>2</sub> experiments (Table 5).

Microbial biomass and soil C responses were generally not affected by either climatic differences or increasing treatment intensity or duration (Table 5). However, we did find a statistically significant increase in soil C responses to elevated CO<sub>2</sub> with increasing treatment duration for N fertilized experiments (Table 5).

**Table 5.** Summary of the meta-analytical regression analysis between the responses of grassland C pools to CO<sub>2</sub> elevation with (CF) or without (C) fertilization treatments, and mean annual temperature (MAT), mean annual precipitation (MAP), and duration and intensity of the CO<sub>2</sub> treatment. The parameters considered are: aboveground biomass (AB), root biomass (RB), root-to-shoot ratio (RS), microbial biomass (MB) and soil C content (Soil C). *P*-values, sign of the regression slopes, and number of data points used are given. Linear regressions were considered statistically significant when *P* < 0.05 (**bold**).

Response variable	Treatment	Tested factor	P-value	Slope sign	<i>n</i>	Response variable	Treatment	Tested factor	P-value	Slope sign	<i>n</i>	
AB	CF	MAT	0.846	+	25	MB	CF	MAT	0.453	+	7	
		MAP	0.114	–	30			MAP	0.935	–	9	
		CO <sub>2</sub> Duration	0.328	–	44			CO <sub>2</sub> Duration	0.695	–	18	
		CO <sub>2</sub> Intensity	0.898	+	44			CO <sub>2</sub> Intensity	0.403	+	18	
	C	MAT	0.193	–	20		C	MAT	0.721	+	5	
		MAP	<b>0.027</b>	–	16			MAP	0.562	+	5	
		CO <sub>2</sub> Duration	0.153	–	25			CO <sub>2</sub> Duration	0.823	–	8	
		CO <sub>2</sub> Intensity	0.852	–	25			CO <sub>2</sub> Intensity	0.128	+	8	
RB	CF	MAT	<b>0.044</b>	+	19	Soil C	CF	MAT	0.805	–	15	
		MAP	0.811	+	23			MAP	0.849	+	11	
		CO <sub>2</sub> Duration	0.825	–	34			CO <sub>2</sub> Duration	<b>0.027</b>	+	21	
		CO <sub>2</sub> Intensity	< <b>0.001</b>	+	34			CO <sub>2</sub> Intensity	0.058	+	21	
	C	MAT	0.816	+	10		C	MAT	0.864	+	6	
		MAP	0.751	–	12			MAP	0.962	+	5	
		CO <sub>2</sub> Duration	0.061	+	17			CO <sub>2</sub> Duration	0.358	+	8	
		CO <sub>2</sub> Intensity	<b>0.004</b>	+	17			CO <sub>2</sub> Intensity	0.681	+	8	
RS	CF	MAT	0.803	+	20			MAT	0.803	+	20	
		MAP	0.247	–	24			MAP	0.247	–	24	
		CO <sub>2</sub> Duration	< <b>0.001</b>	–	30			CO <sub>2</sub> Duration	< <b>0.001</b>	–	30	
		CO <sub>2</sub> Intensity	< <b>0.001</b>	+	30			CO <sub>2</sub> Intensity	< <b>0.001</b>	+	30	
	C	MAT	0.558	+	10				MAT	0.558	+	10
		MAP	0.232	–	12				MAP	0.232	–	12
		CO <sub>2</sub> Duration	<b>0.024</b>	–	13				CO <sub>2</sub> Duration	<b>0.024</b>	–	13
		CO <sub>2</sub> Intensity	0.298	+	13				CO <sub>2</sub> Intensity	0.298	+	13

#### 4 Discussion

Elevated CO<sub>2</sub> effects were generally in accordance with previous studies, indicating increased biomass production, an increased microbial biomass and a tendency for small increases or no changes in soil C content (Fig. 1) (de Graaff et al., 2006; Luo et al., 2006; Hungate et al., 2009). However, considering the CO<sub>2</sub> treatment as a single factor we found a decrease in root biomass as a consequence of elevated CO<sub>2</sub> concentrations, which is in sharp contrast to most other studies (Rogers et al., 1994; Curtis and Wang, 1998; Pendall et al., 2004; de Graaff et al., 2006) and refutes our first hypothesis of an increased C allocation to root compartments.

Interestingly, when excluding experiments that were irrigated or where aboveground biomass was removed, root biomass was no longer significantly decreased by elevated CO<sub>2</sub> (data not shown). This can be explained by the functional equilibrium hypothesis, suggesting optimal distribution of plant resources for plant growth (Bloom et al., 1985), and offers support to our hypothesis that plants deprived of their shoots by harvest, burning or grazing, allocate proportionally more energy to aboveground biomass for repair and regrowth. In turn, this would impair root growth by lowering the amount of C available for belowground biomass.

Other findings further demonstrated the regulating role of water availability in plant responses to elevated CO<sub>2</sub>: root biomass tended to decrease with irrigation compared to non-irrigated systems (Fig. 3, Table 3), root biomass responses to elevated CO<sub>2</sub> increased in warmer sites (Table 5), and aboveground biomass responses reduced at sites with higher precipitation rates (Table 5). This is in accordance with Volk et al. (2000), Bunce (2004) and Morgan et al. (2004a), all indicating that an increased water use efficiency (WUE) as a consequence of reduced stomatal conductance in elevated CO<sub>2</sub> is the major reason for increased plant biomass in higher atmospheric CO<sub>2</sub> concentrations. Our data here indeed suggest that, as a result of increased WUE plants do not necessarily need an extensive root network.

Because water availability is such an important factor in the elevated CO<sub>2</sub> effect, a more detailed study of effectively available soil water to the plant would be informative. In this respect, an analysis accounting for different soil textures in the studies included in this analysis (e.g., CO<sub>2</sub> effect along the sandy-clayey soil continuum) could test whether the magnitude of the CO<sub>2</sub> effect would be larger in drought-prone soils (i.e., sandy soils) compared to soils that easily retain water. Future studies would, therefore, need to report soil textures in their site description, which at this point was only available for a limited amount of study sites.

#### 4.1 Non-nitrogen nutrients regulate root responses to elevated CO<sub>2</sub>

In contrast with unfertilized systems, fertilized systems displayed an increase in root biomass in response to elevated CO<sub>2</sub> (see also de Graaff et al., 2006), indicating a clear dependence on nutrient additions (see also van Groenigen et al., 2006). Our results showed that the root biomass response in elevated CO<sub>2</sub> was unaffected when pure N fertilizers were added, but increased strongly when NPK fertilizers were added (Fig. 2) and that RS decreased in elevated CO<sub>2</sub> with addition of pure N fertilizer, while it increased under NPK fertilization in elevated CO<sub>2</sub> (Fig. 4). In addition, in the single factor fertilization treatment, aboveground biomass tended to respond more strongly to NPK fertilizers (Fig. 2, Table 3). These findings all suggest a progressive limitation by nutrients other than N. As it has been shown before that N-fixing plant species in particular can become limited by non-nitrogen nutrients in elevated CO<sub>2</sub> (van Groenigen et al., 2006), it seems likely that non-nitrogen nutrients might play an important role in regulating the C allocation patterns in the elevated CO<sub>2</sub> experiments in these grasslands.

#### 4.2 Constructive use of C in microbial biomass

The increase in the single factor CO<sub>2</sub> and the combined CO<sub>2</sub> and fertilization treatment for microbial biomass (Fig. 1), confirms the general C limitation of microbial communities. Microbes use C compounds as their main source for energy and are, therefore, often C-limited (Zak et al., 1993; Demoling et al., 2007). However, microbes need N to be able to accumulate C into their biomass (Niklaus and Korner, 1996), so in absence of N, microbes use the energy they obtain from decomposing easily degradable C-compounds to decompose N-richer compounds, which can result in higher respiration rates while microbial biomass remains constant. Therefore, as expected, we found a slightly higher increase in microbial biomass in the combined CO<sub>2</sub> and N fertilization treatment compared to the single factor elevated CO<sub>2</sub> treatment (Fig. 1).

Further, we found that microbial biomass positively correlated to increasing amounts of N fertilization in elevated CO<sub>2</sub>, while it was negatively correlated to increasing amounts of N fertilization without CO<sub>2</sub> (Table 4). The negative effect of the single factor N fertilization treatment on microbial biomass is also in accordance with previous work (Treseder, 2008; Janssens et al., 2010), and our 2nd hypothesis, suggesting microbes either became more C limited under N fertilization, or deteriorating soil conditions and chemical stabilization of SOM inhibited microbial growth (DeForest et al., 2004; Treseder, 2008; Janssens et al., 2010). Because root biomass increased in N fertilized experiments (Fig. 1) – suggesting more labile C inputs – and microbial biomass was found to further decrease at higher N fertilization rates (Fig. 2, Table 4), it seems more likely

**Table 6.** Summary of the meta-analytical regression analysis between the responses of aboveground (AB), root (RB) and microbial biomass (MB) to elevated CO<sub>2</sub> and the soil C response to elevated CO<sub>2</sub>. *P*-values, sign of the regression slopes, and number of data points used are given. Linear regressions were considered statistically significant when *P* < 0.05 (**bold**).

Treatment	Response variable	P-value	slope	n
C	AB	0.9004	–	8
	RB	0.8183	+	6
	MB	0.9751	–	4
CF (Fertilization <50 kgN ha <sup>-2</sup> yr <sup>-1</sup> )	AB	0.6008	–	11
	RB	<b>0.0411</b>	–	9
	MB	0.9269	–	5
CF (all fertilization dosages)	AB	0.4392	–	15
	RB	0.1205	–	13
	MB	0.9853	–	7

that the inhibiting effects of N fertilization dominated in the microbial biomass response.

#### 4.3 Soil C storage in grasslands under elevated CO<sub>2</sub>

While microbial biomass increased in elevated CO<sub>2</sub>, its lifespan is relatively short (Zak et al., 2000; Heath et al., 2005). Moreover, while root biomass production generally increases under elevated CO<sub>2</sub>, an increased root turnover (Lukac et al., 2009) can also result in an unchanged standing root biomass under elevated CO<sub>2</sub> (as found in this study) with much of the root production being converted to necromass. This increased microbial and root biomass turnover would produce a considerable amount of C inputs into the soil that could stimulate microbial activity (Dieleman et al., 2010), and possibly prime older soil C pools (for a definition of priming, see Cheng and Jonhson, 1998; Fontaine et al., 2007; Kuzyakov, 2002). At the same time, elevated CO<sub>2</sub> also stimulates root respiration (Lukac et al., 2009). As such, a multitude of effects can stimulate CO<sub>2</sub> release from the soil, and can explain why an increased root and microbial biomass can result in an unchanged soil C pool under elevated CO<sub>2</sub>.

Based on the findings in this study, we suggest root dynamics and their response to nutrients under elevated CO<sub>2</sub> play an important role in the effect of elevated CO<sub>2</sub> on soil C storage in these grasslands (see Figs. 1–2). We did not find a correlation between root biomass responses and soil C sequestration in unfertilized elevated CO<sub>2</sub> experiments, but found a significant correlation between the root biomass response and the soil C response in elevated CO<sub>2</sub> when realistic amounts of N fertilizer (i.e., max. of 50 kg N ha<sup>-1</sup> yr<sup>-1</sup>) were

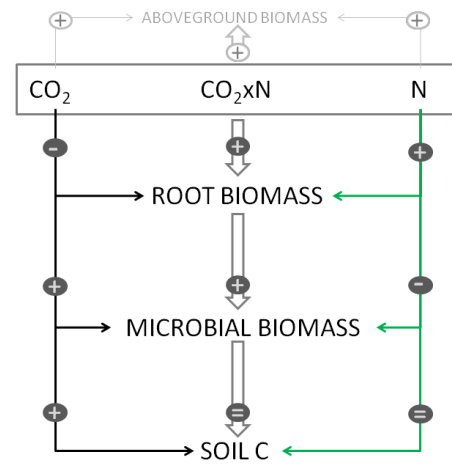


added (Table 6), suggesting lower potential for increases in soil C content when root biomass becomes more responsive to elevated CO<sub>2</sub>. In this case, the C inserted in the soil matrix by root exudation or root turnover might promote more rapid cycling of C inputs into the soil. In support of our findings, Cardon et al. (2001) showed that microbes in nutrient-poor environments are forced to decompose older soil organic matter for N supply, but when excess C is available in nutrient-rich situations, the newly sequestered C inputs into the soil become preferential C substrates for microbial decomposition in elevated CO<sub>2</sub>.

N additions mainly stimulate C sequestration in long-lived biomass compartments (Pregitzer et al., 2008) and, hence, the amount of C being incorporated into the soil matrix might have been limited (Lu et al., 2011), thereby limiting the stimulation of microbial respiration. The larger amount of C being stored in longer-lived biomass might also explain why soil C content was not significantly affected, because C was retained in biomass and not added to the soil matrix. To support this, we found an increasingly positive effect on soil C content with CO<sub>2</sub> treatment duration when fertilized with N (Table 5), and for experiments with higher rates of N fertilization, soil C did tend to increase regardless of root responses (Fig. 2). These results are in accordance with Van Groenigen et al. (2006), who reported that soil C only increased at high rates of N fertilization (>30 kg N ha<sup>-1</sup> yr<sup>-1</sup>). Moreover, respiration rates can be reduced when terrestrial systems are fertilized with large amounts of N through reduced microbial biomass and/or negative effects on decomposing enzyme functioning (Fog, 1988; Janssens et al., 2010). So at high fertilization rates, the inhibiting effects of N fertilizer on decomposition might have overpowered the CO<sub>2</sub> effects on roots (Table 6), promoting an increasing soil C response in elevated CO<sub>2</sub>. We thus cannot confirm, nor refute our 3rd hypothesis, as soil C did not increase in combined CO<sub>2</sub> and fertilization manipulation. Instead, we propose that the soil C response will be determined by the nutrient-dependant root biomass response and the associated feedbacks to soil C decomposition in elevated CO<sub>2</sub>.

#### 4.4 Implications

When no N fertilizer was added, elevated CO<sub>2</sub> stimulated aboveground biomass, but reduced root biomass in grasslands. An increased root death as a consequence might have served as substrate for microbes and a C input for soil C pools (Fig. 5). When only N fertilizer was added, both aboveground and root biomass were stimulated, but microbial biomass decreased, suggesting C limitation or chemical inhibition of microbial communities. In addition, C storage in plant biomass limited the C inputs into soil C pools (Fig. 5). When grasslands in elevated CO<sub>2</sub> were fertilized with N, C storage was largest and both root biomass and microbial biomass were stimulated. However, increased cycling of C left soil C pools unaffected (Fig. 5).



**Fig. 5.** Synthesis of elevated CO<sub>2</sub> effect in grasslands. When no N fertilizer was added, elevated CO<sub>2</sub> stimulated aboveground biomass, but reduced root biomass. An increased root death as a consequence might have served as substrate for microbes and a C input for soil C pools. When only N fertilizer was added, both aboveground and root biomass were stimulated, but microbial biomass was decreased, suggesting C limitation or chemical inhibition of microbial communities. When grasslands in elevated CO<sub>2</sub> were fertilized with N (CO<sub>2</sub>xN), C storage was largest and both root biomass and microbial biomass were stimulated. Increased cycling of C left soil C pools unaffected.

Both CO<sub>2</sub> elevation and N addition thus appeared to be limited in their effect by the presence of the other resource: N respectively C. Elevated CO<sub>2</sub> concentrations stimulated plant productivity, but in a less powerful way compared to when N was added. The excess C that plants thus acquired was transferred to the soil microbial community, where an increased rhizodeposition might have alleviated the C limitation of soil microorganisms.

Addition of nitrogen only, on the other hand, created a strong plant growth response. However, the excess C that is provided by CO<sub>2</sub> elevation is lacking for the stimulus to propagate into the soil community. Consequently, as indicated by our results, it is the combination of CO<sub>2</sub> elevation and N addition that increased the C pool of plant biomass and that stimulated the soil community.

#### 5 Conclusions

In grasslands, different management strategies did not affect the overall stimulating effect of elevated CO<sub>2</sub> on aboveground biomass production. However, CO<sub>2</sub> elevation only increased root biomass significantly when aboveground biomass production was optimized (i.e., when N fertilization was applied). We have shown here that, while other nutrients might become important in the future, N availability is essential for the increased C influx under elevated CO<sub>2</sub> to

propagate into belowground C pools. However, moderate nutrient additions also promoted decomposition processes in elevated CO<sub>2</sub>, reducing the potential for increased soil C storage. The close relationship between root dynamics and soil C storage is a crucial link in plant-soil interactions in terrestrial ecosystems, and determines the potential for increased soil C storage in elevated CO<sub>2</sub>. In conclusion, while future elevated CO<sub>2</sub> concentrations and increasing N deposition might increase C storage in plant biomass, increases in soil C storage are small. Because most of the biomass in non-forest ecosystems is short-lived, we suggest the capacity of grasslands to buffer human CO<sub>2</sub> emissions is limited.

**Supplementary material related to this article is available online at: <http://www.biogeosciences.net/9/2247/2012/bg-9-2247-2012-supplement.zip>.**

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