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Root biomass responses to elevated CO_2 limit soil C sequestration in managed grasslands

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Abstract

Elevated atmospheric CO_2 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_2 concentrations. However, an increased rate of C flux through the soil compartment as a consequence of elevated CO_2 concentrations has been suggested to limit C sequestration in terrestrial ecosystems, questioning the potential for terrestrial C uptake to mitigate the increasing atmospheric CO_2 concentrations. Our study used data from 69 published studies to investigate whether CO_2 elevation and/or nitrogen fertilization could induce an increased carbon storage in grasslands, and considered the influence of management practices involving biomass removal or irrigation on the elevated CO_2 effects. Our results confirmed a positive effect of elevated CO_2 levels and nitrogen fertilization on plant growth, but revealed that N availability is essential for the increased C influx under elevated CO_2 to propagate into belowground C pools. However, moderate nutrient additions also promoted decomposition processes in elevated CO_2, reducing the potential for increased soil C storage. An important role in the soil carbon response to elevated CO_2 was attributed to the root response, since there was a lower potential for increases in soil C content when root biomass was more responsive to CO_2 elevation. Future elevated CO_2 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_2 concentrations have strongly increased since the pre-industrial era (IPCC, 2007), resulting in the contemporary CO_2 concentration of 380 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

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Berner, 2001). Because of the stimulating effect of these elevated CO_2 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_2 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 Gt C (Lal, 2004), which implies that 4 % of total global emissions of greenhouse gasses could be buffered by grasslands (Soussana and Luscher, 2007).

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 (Soussana 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_2 could overcome increased soil organic matter (SOM) decomposition processes when ecosystems are supplemented with additional N. However, their study focused on both woody and herbaceous systems, and indicated different trends in C sink strength between both system types. In addition, the largest proportion of the elevated CO_2 effect in grasslands tends to be due to improved water relations (Morgan et al., 2004b, 2011; Körner, 2006), which was not found to be a dominant driver for tree stands (Körner, 2006). Moreover, grasslands showed very variable responses of

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belowground biomass, while tree stands consistently displayed intensified belowground metabolism (Körner, 2006).

Because of these functional differences between grasslands and tree stands, and the management component involved in grasslands, we focused on elevated CO_2 effects in grasslands only. More specifically, we investigated the effects of elevated CO_2 concentrations and N fertilization individually, and the influence of N fertilization, biomass removal and irrigation on the CO_2 effects on C pools in grasslands (aboveground, root and microbial biomass, and soil C) using the following hypotheses: (1) elevated CO_2 will stimulate plant production and will increase allocation of C to root compartments due to direct effects on photosynthesis and increasing depletion of nutrients, (2) addition of N will stimulate plant productivity but will leave microbial biomass unaffected due to increasing C limitation of microbes, (3) the combined CO_2 and N treatment will strongly stimulate biomass production and will stimulate soil C storage via larger C inputs, and (4) aboveground biomass removal and irrigation will affect C allocation patterns towards aboveground plant compartments, because of reduced LAI and reduced need for root production.

2 Methods

2.1 Data acquisition

We constructed a database, consisting of results from 69 manipulation experiments in grassland systems exposed to elevated CO_2 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. This resulted in 182 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.

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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_2 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.

We extracted information about 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 analyze our data. The natural logarithm of the response ratio (r = (response to elevated CO_2 or N fertilization)/(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 sample size (Hedges et al., 1999). For bootstrapping, 2500 repetitions were used.

We examined the effect of elevated CO_2 concentrations and fertilization separately (in experiments where single factor CO_2 and combined CO_2 and fertilization treatment effects were reported, we extracted a single factor fertilization treatment response using the control values of both CO_2 treatments), the effect of elevated CO_2 concentration in combination with fertilization, the effects of the type and the amount of N fertilizer added (classification in low and high amounts was based on a background value of

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50 kg N ha^{-1} yr^{-1}, based on projected N deposition values in 2050, Galloway, 2008), and the effects of biomass removal or irrigation on the elevated CO_2 effects.

The effect of elevated CO_2 concentrations or fertilization were considered statistically significant when zero was not included in the 95 % CI. Differences between categorical variables and linear regressions analyses were considered statistically significant when *P*-values were lower than 0.05. Unweighed linear regressions were performed in Matlab (Version R2007a, MathWorks, Natick, MA, USA). Linear regressions 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_2 and N addition

Aboveground biomass increased under all three treatments (i.e. elevated CO_2, N fertilization and their combination) (Fig. 1). Root biomass decreased when only CO_2 levels were elevated, but increased when nutrients were added, either with or without CO_2 elevation as a co-treatment (Fig. 1, Table 2). Microbial biomass increased in elevated CO_2 concentrations, both with and without fertilization, and showed an opposing trend in response to the single factor fertilization treatment (Fig. 1, Table 2). Soil C content increased in the single factor CO_2 treatment and was unaltered under the other treatments (Fig. 1).

In the combined elevated CO_2 and fertilization treatment, aboveground biomass responded equally strong to different fertilizer types, but was stimulated more when lower doses of N fertilizer were added (Fig. 2, Table 2). In contrast, root biomass responded strongly positively to CO_2 elevation with NPK fertilizer addition, while pure N addition did not affect root biomass (Fig. 2, Table 2). Similar to the aboveground biomass response, root biomass increased more when low doses of N were applied (Fig. 2, Table 2). Microbial biomass and soil C responses to elevated CO_2 were not affected

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differently by different fertilizer types or doses (Table 2), but interestingly they demonstrated increases only when fertilized with pure N fertilizers and at high doses of N addition (Fig. 2). Weighted linear regression analysis also suggested an increase in microbial biomass in elevated CO_2 with higher N fertilization doses (Table 3).

The single factor N fertilization treatment effects on C pools were not significantly different between fertilizer type or dosage (Fig. 2, Table 2), although a trend towards stronger aboveground biomass responses was apparent under NPK fertilization. This trend was confirmed by weighted linear regression analysis (Table 3).

3.2 Other management procedures (biomass removal and irrigation)

Biomass removal or irrigation did not significantly affect CO_2 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 2).

3.3 Carbon allocation shifts

The root-to-shoot ratio (RS) of grasslands decreased in single factor CO_2 and N fertilization treatments, indicating an preferential allocation of C towards aboveground biomass (Fig. 4). The combined CO_2 and N treatment did not change allocation patterns in grasslands (Fig. 4). There was a strong contrast between RS-responses to elevated CO_2 depending on the type of fertilizer added: pure N addition decreased RS, while NPK fertilizers increased RS in elevated CO_2 (Fig. 4, Table 2). Biomass removal and irrigation did not affect the overall RS response to elevated CO_2 (Fig. 4).

4 Discussion

Elevated CO_2 effects were generally in accordance with previous studies indicating increased biomass production, and a tendency to increase soil C content (Fig. 1) (de

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Graaff et al., 2006; Luo et al., 2006; Hungate et al., 2009). However, we found a decrease in root biomass as a consequence of elevated CO_2 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 partly refutes our 1st hypothesis. However, unfertilized systems did not always display increases in root biomass in response to elevated CO_2 (de Graaff et al., 2006), and showed a clear dependence on N additions (van Groenigen et al., 2006).

In addition, several pieces of evidence in this study can help to explain the observed decrease in root biomass under elevated CO_2: firstly, when plants are deprived of their shoots multiple times by harvest, burning or grazing, proportionally more energy has to be allocated to aboveground biomass for repair and regrowth, which could impair root growth by lowering the amount of C available for belowground biomass. Secondly, in irrigated systems, root biomass tended to decrease even more, compared to non-irrigated systems. According to Volk et al. (2000), Bunce (2004) and Morgan et al. (2004b), an increased water use efficiency (WUE) as a consequence of reduced stomatal conductance in elevated CO_2 is the major reason for increased plant biomass in higher atmospheric CO_2 concentrations. Irrigation would reduce the need for an extensive root network, and reduce the advantage based on increased WUE. Therefore, although we did not find significant direct effects of biomass removal or irrigation on C pools, we suggest grassland management might have affected root biomass responses to elevated CO_2. When we excluded all experiments that were irrigated or where biomass was removed, root biomass was no longer significantly decreased by elevated CO_2 (data not shown), offering support for our 4th hypothesis.

4.1 Nutrients regulate C allocation responses to elevated CO_2

Elevated CO_2 increased aboveground biomass in all treatments (Figs. 1–3), while root biomass was only significantly stimulated when nutrients were applied (Figs. 1–3). This was reflected in an increased allocation of C to aboveground biomass compartments in the single factor CO_2 treatment (Fig. 4). It was only in the combined CO_2 and

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fertilization treatment that C allocation was balanced between aboveground and root biomass, or even increased towards root biomass depending on the type and amount of fertilizer (Fig. 4). The increased allocation to aboveground biomass could have been a consequence of increased water use efficiency of plants in elevated CO_2 (Morgan et al., 2004b), or a consequence of the typical annual regrowth of grassland biomass to reinstate light capture. However, our results showed that the root biomass response in elevated CO_2 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_2 with addition of pure N fertilizer, while it increased under NPK fertilization in elevated CO_2 (Fig. 4). In addition, in the single factor fertilization treatment, aboveground biomass tended to respond stronger to NPK fertilizers (Fig. 2, Table 2) suggesting 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_2 (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_2 experiments in these grasslands.

4.2 Constructive use of C in microbial biomass

Elevated atmospheric CO_2 concentrations stimulated microbial biomass and soil C content (Fig. 1), confirming earlier work (Zak et al., 2000; Pendall et al., 2004; Hungate et al., 2009; Dieleman et al., 2010). The negative effect of N addition 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). 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). Because root biomass increased in N fertilized experiments (Fig. 1), and microbial biomass was found to further decrease at

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higher N fertilization rates (Fig. 2, Table 3), it seems more likely that the inhibiting effects of N fertilization dominated in the microbial biomass response. 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.

The strong increase in the combined CO_2 and fertilization treatment for microbial biomass (Fig. 1), and the borderline significant difference with the single factor fertilization treatment reaffirms 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 the highest increase in microbial biomass in the combined CO_2 and N fertilization treatment (Fig. 1).

4.3 Soil C storage in grasslands under elevated CO_2

We found a striking similarity between soil C and microbial biomass responses (Figs. 1–2), and opposing trends between microbial biomass and soil C responses on the one hand, and root biomass responses to elevated CO_2 on the other hand (Figs. 1–2). As we show in Fig. 5, elevated CO_2 concentrations without addition of mineral fertilizer reduced root biomass but stimulated microbial biomass. However, aboveground biomass increased, which suggests that the increase in microbial biomass possibly resulted as a consequence of priming, which is more likely to happen in nutrient-poor systems (Hoosbeek et al., 2004). This mechanism might have provided the nitrogen to maintain plant growth. In contrast, when purely N was added to grasslands, root biomass increased and microbial biomass decreased (Fig. 5). In that case, C might have been stored in root tissues with longer turnover times, and hence the C accumulation in plant biomass was not transferred to soil compartments limiting microbial growth.

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In the combined CO_2 and N fertilization treatment, both root biomass and microbial biomass significantly increased in elevated CO_2. The combination of elevated CO_2 and N fertilization resulted in excess C that was allocated to root biomass. In the soil compartment this increased C input in concert with sufficient N availability translated into higher microbial biomass. However, despite the positive effects on microbial and root biomass in the combined treatment, the soil C pool remained unaffected, possibly because of an increased cycling of C in the soil compartments (Körner et al., 2005; Lukac et al., 2009; Dieleman et al., 2010).

We suggest an important role for root biomass and dynamics and their response to nutrients under elevated CO_2 concentrations, based on our findings above (see Figs. 1–2). To further support this, we did not find a correlation between root biomass responses and soil C sequestration in "pure" CO_2 experiments, but found a significant correlation between the root biomass response and the soil C response in elevated CO_2 when realistic amounts of N fertilizer (i.e. max. of 50 kg N ha^{-1} yr^{-1}) were added (Fig. 6, Table 3), suggesting lower potential for increases in soil C content when root biomass becomes more responsive to elevated CO_2. 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_2.

For experiments with higher rates of N fertilization, soil C did tend to increase regardless of root responses (Figs. 2 and 6), 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^{-1} yr^{-1}). Moreover, respiration rates can be reduced when terrestrial systems are fertilized with large amounts of N (Fog, 1988; Janssens et al., 2010). So at high fertilization rates, the inhibiting effects of N fertilizer on decomposition might have overpowered the CO_2 effects on roots, promoting an increasing soil C response in

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elevated CO_2. We thus cannot confirm, nor refute our 3rd hypothesis, as soil C did not increase in combined CO_2 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_2.

4.4 Implications

Both CO_2 elevation and N addition appeared to be limited in their effect by the presence of the other resource: N resp. C. Elevated CO_2 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_2 elevation is lacking for the stimulus to propagate into the soil community. Consequently, as indicated by our results, it is the combination of CO_2 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_2 on aboveground biomass production. However, CO_2 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_2 to propagate into belowground C pools. However, moderate nutrient additions also promoted decomposition processes in elevated CO_2, 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

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terrestrial ecosystems, and determines the potential for increased soil C storage in elevated CO_2. In conclusion, while future elevated CO_2 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_2 emissions is limited.

Supplementary material related to this article is available online at: http://www.biogeosciences-discuss.net/9/357/2012/bgd-9-357-2012-supplement.zip.

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Table 1. Information about the ecosystem type and the irrigation, fertilization and management practices 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	Reference
Aberdeen	Yes	Yes & No	NaH_2PO_4, KNO_3 and $NaNO_3$	–	Clipping	(Paterson et al., 2008)
Amsterdam	Yes	Yes	NH_4NO_3	47 kg ha^{-1} yr^{-1}	–	(Hoorens et al., 2003)
Cedar Creek grassland	No	Yes & No	NH_4NO_3	(a) 40 kg ha^{-1} yr^{-1} (b) 100 kg ha^{-1} yr^{-1}	Burning	(Dijkstra et al., 2006; Keeler et al., 2009)
Canberra Phytotron	Yes	Yes	Not specified	22, 67 and 198 kg ha^{-1} yr^{-1} in different treatments		(Lutze et al., 2000)
Duolun	No	Yes	NH_4NO_3	100 kg ha^{-1} yr^{-1}	–	(Xia et al., 2009)
Gainesville grassland	Yes	Yes	NPK (and Mg and S)	70–80 kg ha^{-1} depending on year	–	(Allen et al., 2006)
Ginninderra	Yes	Yes	slow release fertilizer	100 kg ha^{-1} yr^{-1}	–	(Volder et al., 2007)
Jasper Ridge (FACE)	Yes & No	Yes & No	$Ca(NO_3)_2$	70 kg ha^{-1} yr^{-1}	–	(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^{-1} (b) low: 30 kg ha^{-1}; high: 200 kg ha^{-1}	–	(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^{-1} yr^{-1}	Cutting	(Sonnemann and Wolters, 2005)
Manawatu	No	Yes	superphosphate, K_2SO_4, $MgSO_4$, Cu and Zn	–	–	(Ross et al., 2004)
Moor House	No	No	–	–	–	(Fitter et al., 1997)
Nåntuna	No	No	–	–	Cutting	(Marissink et al., 2002)
NERC	Yes	No	–	–	Herbivory	(Kandeler et al., 1998)
New Zealand (FACE)	No	Yes	superphosphate, KSO_4	–	Grazing	(Allard et al., 2005)
Niwot Ridge	No	Yes	(a) urea-N as osmocote pellets (b) osmocote pellets (urea-N and P_2O_5-P)	250 kg ha^{-1} yr^{-1} for the first two years, 100 kg ha^{-1} yr^{-1} thereafter	–	(Bowman et al., 1993; Neff et al., 2002)
PHACE	No	No	–	–	–	(Dijkstra et al., 2010)
Swiss Central Alps	No	Yes & No	NPK (3 : 2 : 3)	45 kg ha^{-1} yr^{-1}	–	(Niklaus and Korner, 1996; Schappi and Korner, 1996)
Swiss Jura	No	No	low dose P fertilization (superphosphate)	–	Mowing/Clipping	(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_4NO_3	low: 140 kg ha^{-1} yr^{-1}; high: 420 kg ha^{-1} yr^{-1} in 1993 and 560 kg ha^{-1} yr^{-1} after 1993	Cutting	(Sowerby et al., 2000; de Graaff et al., 2004; Bazot et al., 2006)

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Table 1. Continued.

Site Name	Irrigation	Fertilization	Fertilizer Type	Fertilizer Amount	Biomass removal	Reference
SwissFACE (Trifolium)	No	Yes	NPK, N as NH_4NO_3 solution	low: 140 kg ha^{-1} yr^{-1}; high: 420 kg ha^{-1} yr^{-1} in 1993 and 560 kg ha^{-1} yr^{-1} after 1993	Cutting	(de Graaff et al., 2004)
TasFACE	No	No	–	–	–	(Pendall et al., 2011)
University of Antwerp	No	No	–	–	–	unpublished
University of Guelph	Yes	Yes	Hoagland's solution	47 kg ha^{-1} yr^{-1}	–	(Klironomos et al., 1998)
Univerisity of Michigan Biological Station	Yes	No	–	–	–	(Treonis and Lussenhop, 1997)
USDA ARS	Yes	No	–	–	–	(Blank and Derner, 2004)
USDA Central Plains	No	No	–	–	–	(Morgan et al., 2004a; Pendall and King, 2007)

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Table 2. Overview of the *P*-values for the meta-analytical comparison between the responses of grassland C pools to different treatments. Results shown for: (1) CO_2 elevation and fertilization treatments (C = elevated CO_2, CF = elevated CO_2 with fertilization, F = fertilization); (2) different fertilization specifications when CO_2 is elevated (type: fertilizer consisting of N only or of NPK; amount: low when less than 50 kgN ha^{-1} yr^{-1} is applied and high when more is applied) and (3) other management procedures when CO_2 is elevated (biomass removal and irrigation). The parameters considered are: aboveground plant 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	MB	Soil C
C vs. F	0.4682	**0.0044**	0.8169	**0.0128**	0.086
C vs. CF	0.6269	**0.0008**	0.1859	0.4346	0.7017
F vs. CF	0.9676	0.3255	0.1811	0.0716	0.5274
CO_2 + N type	0.9736	**0.0016**	**0.0012**	0.4262	0.6809
CO_2 + N amount	**0.0172**	0.2491	0.1919	**0.0336**	0.2019
N, N type	0.1076	0.6006	**0.0344**	–	0.8477
N, N amount	0.5674	0.4702	0.1795	0.3419	–
CO_2 + biomass removal	0.7889	0.0744	–	0.7093	–
CO_2 + irrigation	0.2603	0.0776	0.99	0.926	0.3503

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Table 3. Meta-analysis results for linear regression analysis between amount of N fertilization and effects on C pools, and the relationship between biomass responses and soil C responses to elevated CO_2. Indicated are the P-values for regressions with aboveground biomass (AB), root biomass (RB), microbial biomass (MB) and soil C (soil C), the amount of datapoints (n) and the slopes of the regressions. Regressions are considered statistically significant at $P < 0.05$ (**bold**).

N dosage	P-value	n	slope
In elevated CO_2			
AB	0.5196	16	−0.0053
RB	0.9891	15	−0.0001
MB	**0.0314**	7	0.014
soil C	0.8884	11	0.0007
In single factor N fertilization			
AB	**0.0417**	11	0.0267
RB	0.833	13	0.001
MB	**0.0183**	4	−0.0455
soil C	0.1117	8	0.0091
Soil C response			
Pure C	P-value	n	slope
AB	0.9004	8	−0.0269
RB	0.8183	6	0.0295
MB	0.9751	4	−0.0049
Pure C and Cf ($< 50\,\mathrm{kgN\,ha^{-1}\,yr^{-1}}$)			
AB	0.6008	11	−0.0948
RB	**0.0411**	9	−0.0866
MB	0.9269	5	−0.0139
C + Cf + CF ($> 50\,\mathrm{kgN\,ha^{-1}\,yr^{-1}}$)			
AB	0.4392	15	−0.135
RB	0.1205	13	−0.0557
MB	0.9853	7	−0.0028

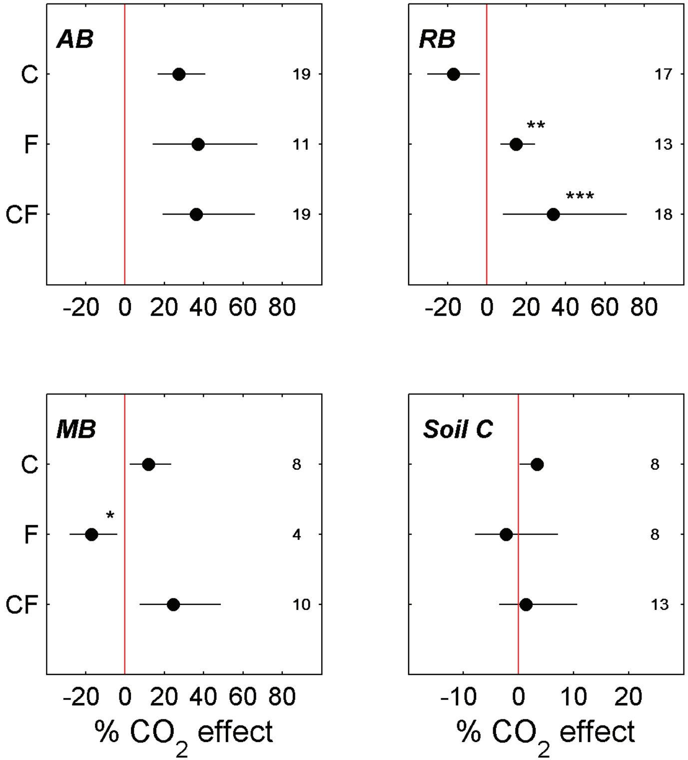

Fig. 1. Responses of grassland C pools to three different treatments: CO_2 elevation (C), fertilization (F) and the combination of CO_2 elevation and fertilization (CF). Responses are shown as percentage increase and 95 % confidence intervals (CI) for aboveground biomass (AB), root biomass (RB), microbial biomass (MB), and soil C content (Soil C). Treatment responses were considered statistically significant when zero was not included in the 95 % CI. Statistically significant differences with the single factor CO_2 treatment are indicated by: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

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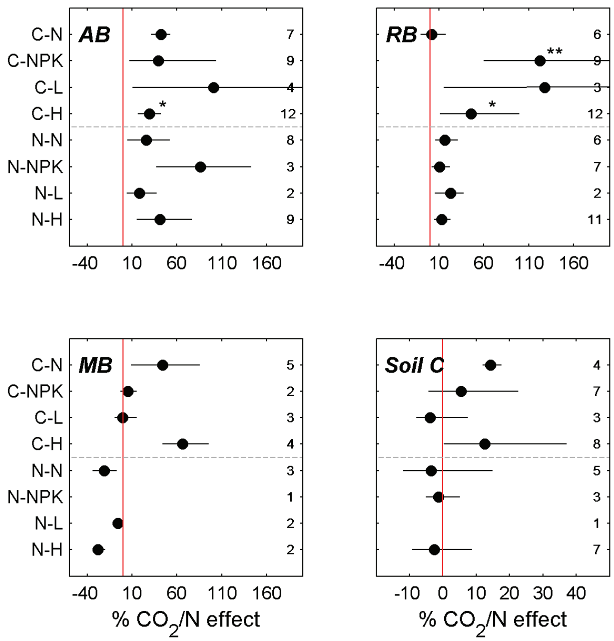

Fig. 2. CO_2 and N fertilization responses of grassland C pools to different N fertilizer type and intensity: CO_2 elevation with pure N fertilizer (C-N), CO_2 elevation with NPK fertilizer (C-NPK), CO_2 elevation with low N fertilizer application (C-L, less than 50 kgN ha^{-1} yr^{-1}) and CO_2 elevation with high N fertilizer application (C-H, more than 50 kgN ha^{-1} yr^{-1}), N fertilization with pure N fertilizer (N-N), N fertilization with NPK fertilizer (N-NPK), N fertilization with low N fertilizer application (N-L, less than 50 kgN ha^{-1} yr^{-1}) and N fertilization with high N fertilizer application (N-H, more than 50 kgN ha^{-1} yr^{-1}). Responses are shown as percentage increase and 95 % confidence intervals (CI) for aboveground biomass (AB), root biomass (RB), microbial biomass (MB), and soil C content (Soil C). 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: * $P < 0.05$; ** $P < 0.01$.

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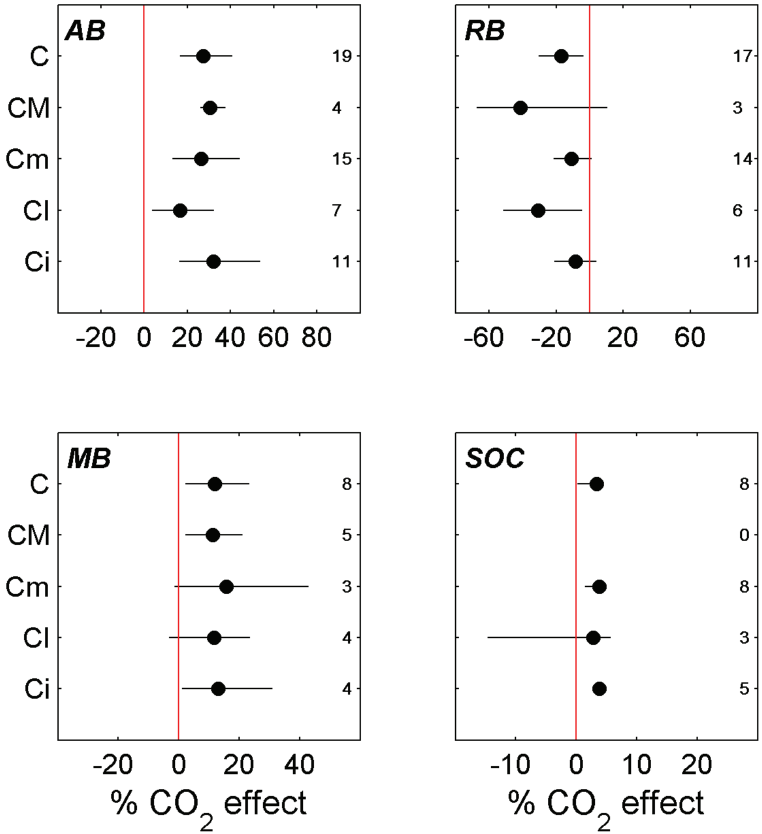

Fig. 3. The CO_2 effect in experiments with (CM) or without (Cm) biomass removal, and irrigated (CI) and non-irrigated (Ci) experiments, compared to the full CO_2 dataset (C). Responses are shown as percentage increase and 95 % confidence intervals (CI) for aboveground biomass (AB), root biomass (RB), microbial biomass (MB), and soil C content (Soil C). Responses were considered statistically significant when zero was not included in the 95 % CI.

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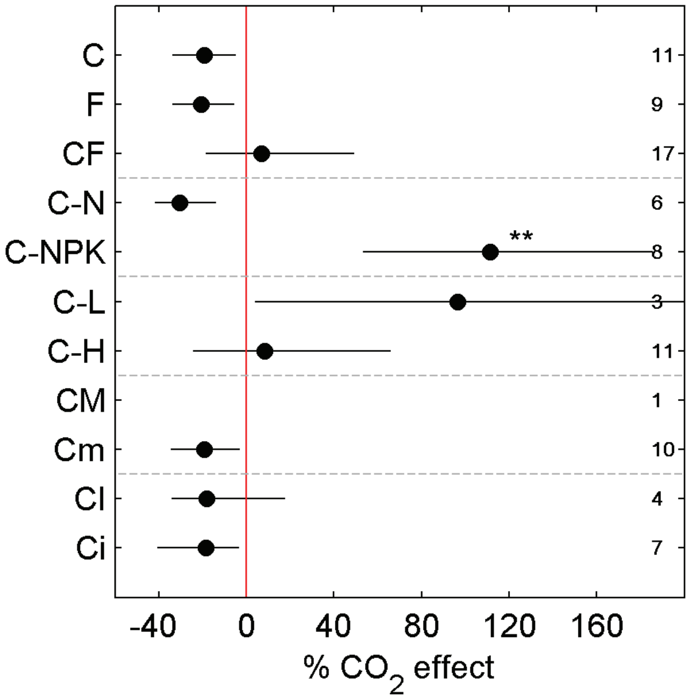

Fig. 4. Effects on the root-to-shoot ratio (RS) in grasslands in elevated CO_2 (C), nitrogen fertilization (F), combined elevated CO_2 and N fertilization (CF), elevated CO_2 with pure N fertilizer (C-N), CO_2 elevation with NPK fertilizer (C-NPK), CO_2 elevation with low N fertilizer application (C-L, less than $50\,kgN\,ha^{-1}\,yr^{-1}$), CO_2 elevation with high N fertilizer application (C-H, more than $50\,kgN\,ha^{-1}\,yr^{-1}$), elevated CO_2 with (CM) or without (Cm) biomass removal, and elevated CO_2 in irrigated (CI) and non-irrigated (Ci) experiments. Responses are shown as percentage increase and 95% confidence intervals (CI), and were considered statistically significant when zero was not included in the 95 % CI. Statistically significant differences between fertilizer type are indicated by: ** $P < 0.01$.

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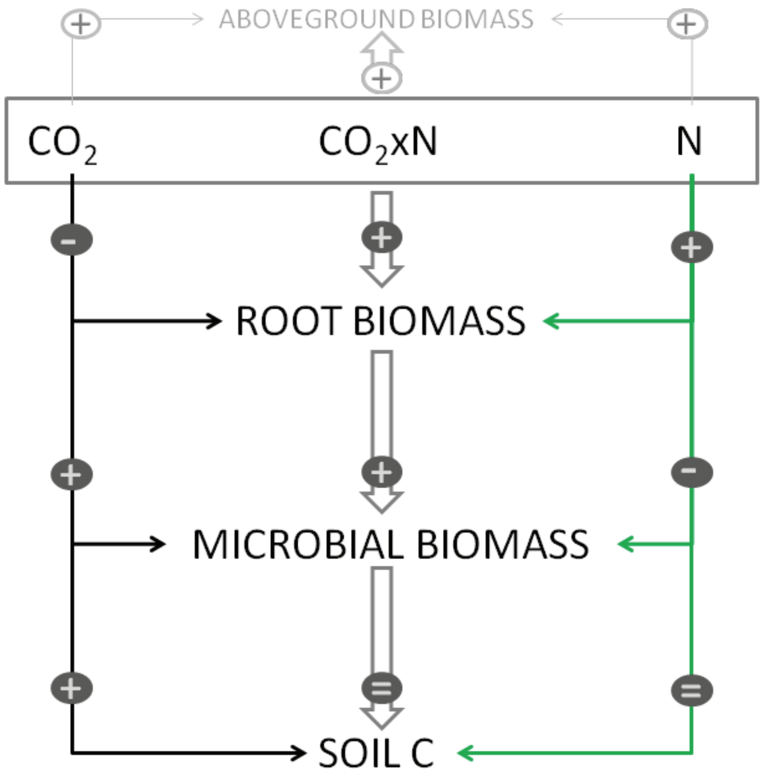

Fig. 5. Synthesis of elevated CO_2 effect in grasslands. When no N fertilizer was added, elevated CO_2 stimulated aboveground biomass, but reduced root biomass. An increased root death as a consequence might have served as substrate for microbes and a C input for soil C pools. When only N fertilizer 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_2 were fertilized with N ($CO_2 \times N$), C storage was largest and both root biomass and microbial biomass were stimulated. Increased cycling of C left soil C pools unaffected.

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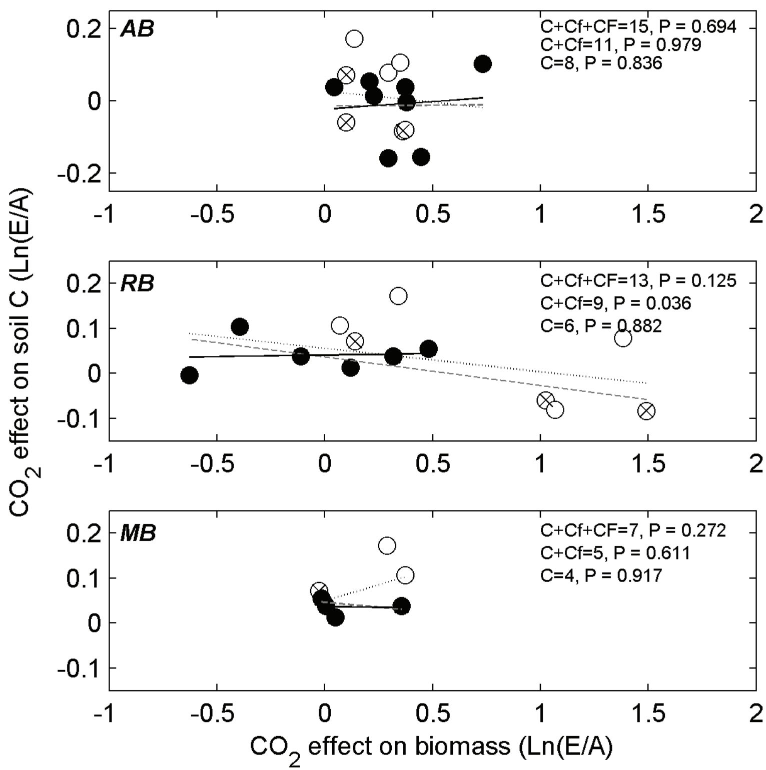

Fig. 6. Relationships between the CO_2 response of soil C content and aboveground biomass (AB), root biomass (RB) and microbial biomass (MB). Data shown are "pure" CO_2 experiments (black circles, C), elevated CO_2 experiments with moderate N additions ($< 50\,kg\,N\,ha^{-1}\,yr^{-1}$) (crossed circles, Cf), and elevated CO_2 experiments with high N additions ($> 50\,kg\,N\,ha^{-1}\,yr^{-1}$) (white circles, CF). The number of data points, the P-value for the regressions and the R^2-value for all regressions are indicated. Regressions are considered statistically significant at $P < 0.05$.

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