

REVIEW

Simple additive effects are rare: a quantitative review of plant biomass and soil process responses to combined manipulations of CO₂ and temperature

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Abstract

In recent years, increased awareness of the potential interactions between rising atmospheric CO₂ concentrations ([CO₂]) and temperature has illustrated the importance of multifactorial ecosystem manipulation experiments for validating Earth System models. To address the urgent need for increased understanding of responses in multifactorial experiments, this article synthesizes how ecosystem productivity and soil processes respond to combined warming and [CO₂] manipulation, and compares it with those obtained in single factor [CO₂] and temperature manipulation experiments. Across all combined elevated [CO₂] and warming experiments, biomass production and soil respiration were typically enhanced. Responses to the combined treatment were more similar to those in the [CO₂]-only treatment than to those in the warming-only treatment. In contrast to warming-only experiments, both the combined and the [CO₂]-only treatments elicited larger stimulation of fine root biomass than of aboveground biomass, consistently stimulated soil respiration, and decreased foliar nitrogen (N) concentration. Nonetheless, mineral N availability declined less in the combined treatment than in the [CO₂]-only treatment, possibly due to the warming-induced acceleration of decomposition, implying that progressive nitrogen limitation (PNL) may not occur as commonly as anticipated from single factor [CO₂] treatment studies. Responses of total plant biomass, especially of aboveground biomass, revealed antagonistic interactions between elevated [CO₂] and warming, i.e. the response to the combined treatment was usually less-than-additive. This implies that productivity projections might be overestimated when models are parameterized based on single factor responses. Our results highlight the need for more (and especially more long-term) multifactor manipulation experiments. Because single factor CO₂ responses often

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dominated over warming responses in the combined treatments, our results also suggest that projected responses to future global warming in Earth System models should not be parameterized using single factor warming experiments.

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Introduction

Increasing concentrations of greenhouse gases in the atmosphere are expected to increase global surface temperatures in the coming decades. The latest IPCC report projects a warming of 0.2 °C per decade in the next two decades, and of 0.6–4 °C by the end of the 21st century (relative to observations in 1980–1999). At the same time increased variability and regional changes in precipitation patterns are very likely and the atmospheric CO₂ concentration [CO₂] may continue to rise, up to 490–1260 ppm by the end of this century (Ipcc, 2007). These climatic and atmospheric changes influence ecosystem functioning, whereby uptake or emission of greenhouse gases constitute an important climate-controlling feedback mechanism (Friedlingstein *et al.*, 2006; Van Groenigen *et al.*, 2011).

An important approach to increasing our understanding of the effects of global changes on terrestrial ecosystems is manipulation of these driving variables in ecosystem experiments (e.g. Beier *et al.*, 2004; Rustad, 2008). Apart from providing enhanced understanding of ecosystem responses to global change, the data provided by these manipulation experiments can help to parameterize and evaluate Earth System models, as well as ecosystem models that predict future ecosystem functioning and ecosystem services. Because multifactor manipulation experiments test the anticipated changes in climate and [CO₂] simultaneously as opposed to single factor manipulative experiments, these can be expected to yield more relevant benchmarking data (e.g. Shaw *et al.*, 2002; Dukes *et al.*, 2005; Larsen *et al.*, 2011).

Multifactor manipulation experiments are more expensive than single factor experiments, because the number of study plots double for each additional factor involved. Therefore, these experiments are still scarce (14 sites in this analysis, see also Rustad, 2008), and most ecosystem models are therefore currently parameterized and tested with results obtained in single factor experiments. This article addresses this restriction by synthesizing the published effects of warming and elevated atmospheric CO₂ concentration, applied separately and in combination, on terrestrial ecosystem biomass production [main terrestrial carbon (C) pool

taking up atmospheric CO₂], soil respiration (main flux releasing CO₂ to the atmosphere), and nitrogen (N) mineralization (main limiting factor of plant CO₂ uptake in most natural northern ecosystems).

Methods

Most of the data included in this analysis were extracted from figures and tables in published papers. Other data, not published in the peer-reviewed literature, were obtained via personal communication (Table S1–2). We collected data from 150 manipulation experiment sites across a range of different ecosystems and climates, reporting data on total biomass, above-ground biomass, root biomass, fine root biomass, soil C, heterotrophic respiration, soil respiration, and soil N availability and foliar N content, resulting in 821 entries for the meta-analysis (Table S3). When several years of data were reported in the same study, we calculated a weighted mean, using the reciprocal of the measurement variance as a weight factor. Several studies used different species in the same experiment or included other manipulations, e.g. fertilization, ozone, different soil types, different management types. Results from different treatments, plant species, soils, or measurement protocols within the same experiment were considered independent experiments and were used as different inputs in the meta-analysis. General site information, source references, and sampling methods are described in Tables S1–6. The database is freely available after contacting the corresponding author.

Data were analyzed with meta-analytical techniques using MetaWin 2.1 software (Rosenberg *et al.*, 2000). As in conventional meta-analysis, each individual observation was weighted by the reciprocal of the mixed-model variance (Hedges *et al.*, 1999). We used standard deviation (SD) values reported in the individual studies, or calculated the SD from the reported standard error and the number of replicates. Studies that did not report standard error or deviation were not included in the database. The natural log of the response ratio ($r = \text{response in treatment plots} / \text{response in untreated plots}$) was the metric used in the analyses, and is reported as the percentage change to elevated [CO₂] and elevated temperature. 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 treatment effects for the different parameters. If the number of studies used to calculate a mean and confidence interval is lower than 20, the confidence interval can be too narrow (Hedges *et al.*, 1999).

Therefore, we used the confidence interval based on resampling methods for the assessment of statistical differences (2500 iterations). As compared to standard confidence intervals, confidence intervals based on such bootstrapping tests are larger and the resulting estimates are more conservative (Adams *et al.*, 1997). If the calculated 95% confidence interval did not encompass zero, the response to the manipulation was considered significant. Significant between-group differences (i.e., categorical analyses for treatment comparisons) were identified on the basis of the within- and between-group heterogeneity. Between-group differences are reported statistically significant at $P < 0.05$. From the distribution of individual responses, we also derived median effect sizes as a comparison to the weighted effect sizes calculated in the meta-analysis.

In addition to the typical meta-analysis in which all available data were used, we also performed a more conservative comparison of the single factor and combined factor manipulation experiments by taking into account only those experiments where all three treatments were tested simultaneously: elevated [CO₂] only, warming only, and combined [CO₂]-enrichment and warming. Experiments included in this analysis are listed in Table 1.

What single factor experiments revealed

Because the effects of single factor elevated [CO₂] and elevated temperature are relatively well understood and have previously been synthesized (Ceulemans & Mousseau, 1994; Curtis & Wang, 1998; Rustad *et al.*, 2001; Nowak *et al.*, 2004; Pendall *et al.*, 2004; Ainsworth & Long, 2005; Norby *et al.*, 2005; De Graaff *et al.*, 2006; Hyvönen *et al.*, 2007; Dieleman *et al.*, 2010; Way & Oren, 2010; Wu *et al.*, 2011; Elmendorf *et al.*, 2012), we will only briefly touch upon these single factor manipulation effects.

Elevated [CO₂] increases plant photosynthesis and growth, although responses can be limited in mature tree stands and nutrient-limited systems (Körner, 2006), and tend to decline with the duration of the experiment (Leuzinger *et al.*, 2011). In many ecosystems, the indirect [CO₂] effect via reduced stomatal conductance and subsequent water savings (Volk *et al.*, 2000; Morgan *et al.*, 2004) could be much more sustainable than the direct [CO₂] effect on photosynthesis (Holtum & Winter, 2010). As a consequence of the enhanced plant production, the increased demand for nutrients stimulates belowground C allocation and fine root and mycorrhizal growth (Fig. 1a) (e.g. Rogers *et al.*, 1994; Curtis & Wang,

Table 1 Sites that applied both single factor [CO₂] and warming treatments, and a simultaneous [CO₂] and warming treatment

Site name	Country	System	Treatments	Source references
Natural/Outdoor systems				
Brandjberg	Denmark	Temperate heathland	CO ₂ x Warming x Drought	(Andresen <i>et al.</i> , 2009; Larsen <i>et al.</i> , 2011; Selsted <i>et al.</i> , 2012)
Flakaliden	Sweden	Picea abies forest	CO ₂ x Warming	(Comstedt <i>et al.</i> , 2006; Kostianinen <i>et al.</i> , 2009)
Ginninderra	Australia	Phalaris aquatic swards	CO ₂ x Warming	(Volder <i>et al.</i> , 2007)
Jasper ridge	USA	California annual grassland	CO ₂ x Warming x Fertilization x Water	(Dukes <i>et al.</i> , 2005)
Mekrijärvi	Finland	Pinus sylvestris forest	CO ₂ x Warming	(Pajari, 1995; Niinistö <i>et al.</i> , 2004)
Oak ridge grasslands	USA	Model grassland	CO ₂ x Warming x Water	(Wan <i>et al.</i> , 2007; Kardol <i>et al.</i> , 2010)
Oak ridge maples	USA	Acer rubrum, Acer saccharum	CO ₂ x Warming	(Edwards & Norby, 1999; Norby <i>et al.</i> , 2000; Wan <i>et al.</i> , 2004)
PHACE	USA	Northern mixed-grass prairie	CO ₂ x Warming	(Dijkstra <i>et al.</i> , 2010; Morgan <i>et al.</i> , 2011)
Stillberg	Switzerland	Treeline larch and pine system	CO ₂ x Warming	(Hagedorn <i>et al.</i> , 2010; Dawes <i>et al.</i> , 2011)
TasFACE	Australia	Species-rich temperate grassland	CO ₂ x Warming	(Hovenden <i>et al.</i> , 2008; Pendall <i>et al.</i> , 2011)
Controlled mesocosms/ phytotrons				
Duke phytotron	USA	Pinus and Robinia seedlings	CO ₂ x Warming x Fertilization	(Larigauderie <i>et al.</i> , 1994; King <i>et al.</i> , 1996; Uselman <i>et al.</i> , 2000)
NERC Ecotron	UK	Grassland model ecosystem	CO ₂ x Warming	(Kandeler <i>et al.</i> , 1998)
Risø	Denmark	Pisum sativum	CO ₂ x Warming	(Gavito <i>et al.</i> , 2003)
USEPA	USA	Pseudotsuga seedlings	CO ₂ x Warming	(Lin <i>et al.</i> , 2001; Olszyk <i>et al.</i> , 2003; Tingey <i>et al.</i> , 2006)

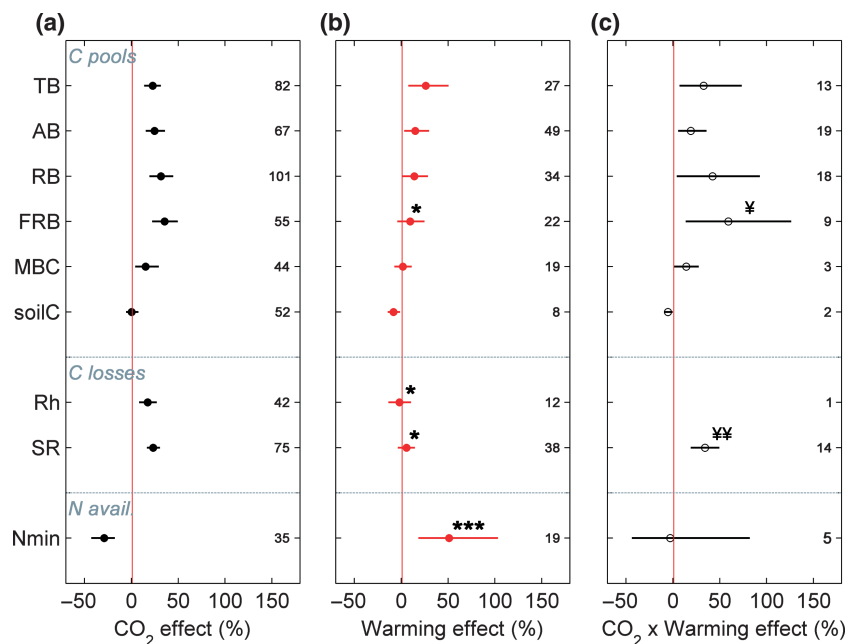


Fig. 1 Overall meta-analysis effect sizes for elevated [CO₂] (a), warming (b) and the combined elevated [CO₂] and warming treatment (c) reported as the percentage change relative to the control. Data listed are total biomass (TB), aboveground biomass (AB), root biomass (RB), fine root biomass (FRB), soil C content (soilC), heterotrophic respiration (Rh), soil respiration (SR), and mineral N availability (Nmin). Positive values indicate a positive treatment effect, negative values indicate a decrease. Error bars represent the 95% confidence interval. Data are the weighted means for n data points. The number of studies is given along the Y-axis. Significant differences in the response to [CO₂] enrichment vs. the warming response are indicated (* indicates differences with the [CO₂] responses, ¥ indicates differences with the warming responses. * or ¥ indicates a significant difference at $P < 0.05$; ** or ¥¥ indicates a significant difference at $P < 0.01$, *** or ¥¥¥ indicates a significant difference at $P < 0.001$). References to all individual experiments included in this meta-analysis are listed in Tables S5 and S6.

1998; Ceulemans *et al.*, 1999; De Graaff *et al.*, 2006; Litton *et al.*, 2007; Dieleman *et al.*, 2010). The associated increase in fine root turnover and rhizodeposition generally enhances substrate availability to soil organisms (Dieleman *et al.*, 2010), whose greater biomass and/or activity may stimulate the decomposition of organic material (Zak *et al.*, 2000; Heath *et al.*, 2005; Dijkstra & Cheng, 2007; Fontaine *et al.*, 2007; Hagedorn *et al.*, 2008; Kuzyakov, 2011). Results of the current meta-analysis support this pattern: heterotrophic- and total soil respiration generally increased under [CO₂] enrichment (Fig. 1a). The simultaneous increase in soil C inputs and soil C losses under elevated [CO₂] leaves soil C stocks largely unchanged (Schlesinger & Lichter, 2001; Dieleman *et al.*, 2010). Although other meta-analyses indicated increased soil C (Jastrow *et al.*, 2005; Luo *et al.*, 2006), especially in N-fertilized ecosystems (Hungate *et al.*, 2009), elevated [CO₂] thus mainly accelerates soil C cycling in terrestrial ecosystems (Lukac *et al.*, 2009).

As a consequence of increasing plant and microbial biomass stocks in elevated [CO₂], more nutrients are immobilized, which may result in progressive N limitation (PNL, for concept see Luo *et al.*, 2004) in unfertilized and infertile ecosystems. Our meta-analysis supports this hypothesis, showing a substantial reduction in mineral N availability (Nmin; NO₃⁻ and NH₄⁺ concentration) in response to increased [CO₂]

(Fig. 1a), which suggests either a gradual depletion of soil N levels, or a more conservative use of N in an increasingly more closed N cycle. In general, plant biomass and soil C fluxes thus respond positively to elevated [CO₂] in manipulation experiments, but nutrient availability is expected to constrain this stimulation in the long run unless total ecosystem nitrogen content increases due to CO₂-induced increases in nitrogen uptake or CO₂-induced decreases in nitrogen losses (Luo *et al.*, 2004, 2006).

In contrast to elevated [CO₂], which affects different terrestrial ecosystems quite uniformly at the leaf level, resulting in increased photosynthesis, biomass production, and resource demands, warming is a more complex driver that affects multiple processes (e.g. photosynthesis, respiration, evapotranspiration, N mineralization) in various direct as well as indirect ways (Luo, 2007). Plant productivity, for example, can be influenced by warming directly through accelerated reaction rates, cell division and elongation, but is also affected indirectly through altered water (De Boeck *et al.*, 2008) and nutrient availabilities or a lengthening of the growing season (Jarvis & Linder, 2000; Saxe *et al.*, 2001). In addition, optimal growth temperatures differ between biomes at different latitudes, which will largely determine the responsiveness of different ecosystems to different degrees of warming (Way & Oren, 2010).

This greater complexity of the warming response results in average response patterns that differ strongly from those to [CO₂]-enrichment, not only in size and statistical significance, but especially in the direction of the responses. First, N availability declines substantially under elevated [CO₂], while warming typically stimulates nutrient availability via enhanced net N mineralization rates (Rustad *et al.*, 2001; Pendall *et al.*, 2004; Hyvönen *et al.*, 2007; but Niu *et al.*, 2010) (Fig. 1a and b). Second, in contrast to elevated [CO₂] experiments, higher temperatures enhance aboveground biomass, but do not stimulate (fine) root biomass (Fig. 1b) (Way & Oren, 2010). This apparent difference in the impact on biomass distribution patterns may be partly due to the increased N availability in warming experiments (Fig. 1b), as higher nutrient availability reduces the need to develop an elaborate belowground nutrient acquisition system (Melillo *et al.*, 2011). A third striking difference is the lack of stimulation of soil respiration in the warming experiments (Fig. 1b). Whereas elevated [CO₂] typically increases soil respiration (Zak *et al.*, 2000; Dieleman & Janssens, 2011; Selsted *et al.*, 2012) (Fig. 1a), soil respiration does not show a consistent long-term response to warming (Fig. 1b). Although soil respiration generally increases when temperature rises on a short time scale (± 5 years, Rustad *et al.*, 2001; Melillo *et al.*, 2002), several mechanisms can prevent a persistent positive warming effect on microbial and soil respiration (Davidson & Janssens, 2006). Particularly important in this regard are depletion of labile soil organic matter pools following extended stimulation of microbial decomposition during earlier phases in the warming

experiments (Kirschbaum, 2004; Eliasson *et al.*, 2005; Knorr *et al.*, 2005; Hartley *et al.*, 2007; Bradford *et al.*, 2008), warming-induced water limitation of microbial activity (Suseela *et al.*, 2012), and thermal acclimatization of root and/or microbial respiration (Atkin & Tjoelker, 2003; Vicca *et al.*, 2010).

Elevated [CO₂] and warming thus elicit very different ecosystem responses, but *in situ* these two global change factors are changing concurrently. Therefore, combined [CO₂] enrichment and warming experiments are crucial to test whether ecosystem processes in a warmer, [CO₂]-enriched world will mirror those observed in warming, those in elevated [CO₂], or whether their responses will be additive, antagonistic or even synergistic.

Average responses to combined [CO₂] and warming

To date, combined warming and [CO₂] enrichment studies have been rare and only few C-cycle related variables were reported for at least five manipulation experiments (Fig. 1c). Because of this we performed a more extensive analysis on response variables: a first analysis based on conventional meta-analysis with weighted effect estimates, and a second analysis based on histograms and median effect estimates to study the distribution of effect sizes in our dataset (see Method section).

For total, aboveground and belowground biomass, the weighted meta-analysis estimates of the responses to elevated [CO₂] and warming were considerably higher compared to the median effect estimates (Fig. 2). As meta-analysis gives

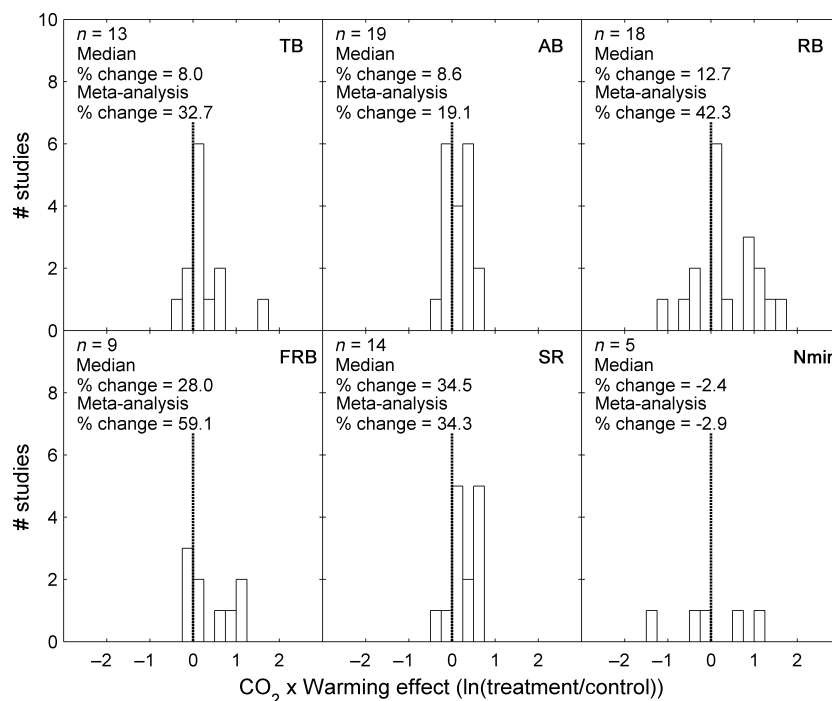


Fig. 2 Histograms for the combined elevated [CO₂] and warming treatment effect on total biomass (TB), aboveground biomass (AB), root biomass (RB), fine root biomass (FRB), soil respiration (SR), and mineral N availability (Nmin). The number of studies, the median effect estimates and the meta-analysis effect estimates are given for each dataset.

more weight to larger studies (i.e. studies with more treatment replicates), this indicates that larger studies tended to report larger responses. However, while the median effect estimates were smaller, the number of studies reporting positive responses was consistently larger than the number of studies reporting negative responses (Fig. 2). Hence, a positive effect of a combined warming and elevated [CO₂] treatment on biomass production was supported by both analyses. In addition, the trend for a larger belowground biomass response compared to the aboveground biomass response was also apparent in both analyses. The increased allocation to belowground biomass translated into a strong positive fine root biomass response in both analyses (Fig. 2).

Both the median effect estimate and the meta-analysis estimate (Figs 1c and 2) indicated a stimulated soil respiration in response to combined warming and elevated [CO₂] treatment. Indeed, of 14 studies, only two reported a negative response to the combined treatment, adding confidence to the observation of a consistently higher soil respiration rate in a warmer climate and higher [CO₂]. Effects on mineral N availability, on the other hand, were very variable (Fig. 2), from very negative to very positive, suggesting a large dependence on site-specific circumstances.

To summarize, effects of combined elevated [CO₂] and warming on plant biomass compartments tended to be variable, but nevertheless positive, with a tendency for greater increase in C allocation to belowground biomass. Feedbacks related to availability of nutrients might be part of the explanation, but we could not conclusively test this mechanism.

Most convincing, however, is the consistent increase in soil respiration in the vast majority of experiments. While our results thus suggest a consistent increase of C release to the atmosphere, we reported relative changes here, precluding inferences on the actual C balance. Our results are consistent with a study comparing four ecosystem models that found that combined [CO₂] and warming treatment stimulated net primary production (NPP) and decomposition (Rh) (Luo *et al.*, 2008). However, these authors found that combined [CO₂] and warming generally resulted in a net increase of C storage in a range of different terrestrial ecosystems, suggesting that effects on plant net primary production and soil C inputs are proportionally larger than effects on decomposition of soil organic matter. The currently available data from combined [CO₂] and warming experiments are, however, too small to test this model outcome.

Which factor dominates the response to combined [CO₂] and warming?

Direct comparison of the average meta-analytical effect estimates for combined warming and CO₂-fumigation (as shown in Fig. 1c) with those for single factor manipulation (Fig. 1a and b) was hampered by the low number of experiments combining elevated [CO₂] and warming treatments as opposed to a multitude of single factor experiments (14 vs. 130 experimental sites). Therefore, we performed the meta-analysis taking into account only those ecosystem manipulation experiments that tested all three treatments (i.e. [CO₂] only,

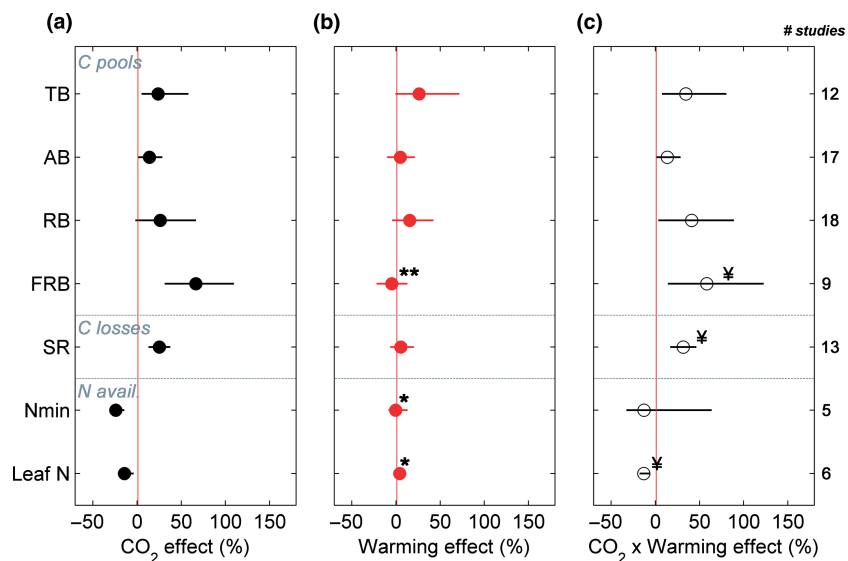


Fig. 3 Meta-analysis effect sizes for experiments where all three treatments were tested simultaneously: elevated [CO₂] (a), warming (b) and the combined elevated [CO₂] and warming treatment (c), reported as the percentage change relative to the control. Data listed are total biomass (TB), aboveground biomass (AB), root biomass (RB), fine root biomass (FRB), soil respiration (SR), mineral N availability (Nmin) and foliar N concentration (Leaf N). Positive values indicate a positive treatment effect, negative values indicate a decrease. Error bars represent the 95% confidence interval. Data are the weighted means for *n* data points. The number of studies is given along the Y-axis. Significant differences in the response to [CO₂] enrichment vs. the warming response are indicated (*indicates differences with the [CO₂] responses, † indicates differences with the warming responses. * or † indicates a significant difference at $P < 0.05$; ** or †† indicates a significant difference at $P < 0.01$). References to all individual experiments included in this meta-analysis are listed in Tables S5 and 6.

warming only and the combined treatment, hereafter 'paired meta-analysis' (Fig. 3, Table 1). Responses to warming-only and [CO₂]-only treatments (Fig. 3a and b) were very similar to those in the much larger, comprehensive dataset (Fig. 1a and b), indicating that the experiments used in this more robust, but restricted paired meta-analysis provided a representative sample.

A first observation in the paired meta-analysis is that the uncertainties in the combined treatment, tended to be larger than those in the single factor experiments (error bars in Fig. 3c vs. those in 3a and b). In other words: responses to the combined treatment were much less consistent across experiments. Second, across all tested variables, the response pattern in the combined treatment appeared to be more similar to that of the [CO₂]-only treatment than to the warming-only treatment: root biomass (+41% in the combined treatment) and especially fine root biomass (+58%) were stimulated more than aboveground biomass (only +15%), and soil respiration increased substantially (+32%) (Fig. 3). This suggests that, averaged over all multifactor manipulation experiments, [CO₂] manipulation affected ecosystem carbon cycling more strongly than warming. The impact of CO₂ enrichment may have been greater because the degree of CO₂ alteration in the experiments was typically larger in relative terms than the imposed temperature change (i.e. [CO₂] is often (nearly) doubled (Fig. S1), while temperature treatments generally remain within the temperature range plants experience in the current climate (Fig. S2)). This is, however, not a drawback or artifact, because the much larger relative increase in [CO₂] than in temperature is consistent with the projected future scenarios. As mentioned before, the effect of a warming treatment will depend on optimal growth temperature and the magnitude of the treatment relative to the prevailing temperatures at a particular site (Way & Oren, 2010). For this reason, future warming in high latitude/altitude ecosystems could still be proportionally more important than [CO₂] increases, although our meta-analysis indicates otherwise.

The similarity between the response to the combined treatment and that to the [CO₂]-only treatment, as suggested by the general mean response pattern in Fig. 3, is not that clear when looking across individual experiments (Fig. 4). While for aboveground and fine root biomass, the response to the combined treatment was clearly more similar to the [CO₂]-only treatment rather than to the warming-only treatment, this is not the case for total below-ground biomass or N mineralization (Fig. 4). Assessing which factor dominates the response to combined [CO₂] and warming requires acknowledging the processes that underlie the response of interest and understanding which factors control their responses. For example, the dominance of the [CO₂] impact in the response of soil respiration to the combined treatment is very likely due to the dominant effect of elevated [CO₂] on fine root biomass, both across experiments (Fig. 4) and when averaged over all experiments (Fig. 3). Very often, however, understanding which factor dominates the response to combined warming and [CO₂] enrichment is not straightforward. In one of the few [CO₂] × warming studies on mature trees, for example, warming enhanced shoot-scale net photosynthesis early in the

season only, while elevated [CO₂] increased maximum net photosynthesis only late in the season (Slaney *et al.*, 2007; Hall *et al.*, 2009). Both climate change factors thus contributed to the increase in the combined treatment, but this would not have become apparent without detailed process measurements. Observations limited to only part of the season would have attributed increased carbon assimilation by new shoots in the combined treatment to either warming or elevated [CO₂], when in fact it should be attributed to their combined effect. Thus, for process responses that are monitored only infrequently, (seasonal) variation in the relative contribution of individual treatment effects to the response in the combined treatment may have contributed to the large variation in process responses within and between individual experiments.

Is there evidence for progressive nitrogen limitation in combined elevated [CO₂] and warming?

Although the stimulation of plant productivity in elevated [CO₂] is known to gradually decline due to progressive nitrogen limitation (Oren *et al.*, 2001; Hungate *et al.*, 2003; Luo *et al.*, 2004), warming is known to accelerate organic matter mineralization and thus to enhance nutrient availability. The combined warming and elevated [CO₂] experiments provide the opportunity to test whether or not PNL occurs when these contrasting determinants of soil N availability are combined.

As expected, our meta-analysis reveals that elevated [CO₂] decreased soil N availability (Figs 1a and 3a), but that it increased (or tended to increase) under warming (Figs 1b and 3b). On average, these opposite responses counterbalanced each other in the combined elevated [CO₂] and warming experiments, resulting in little change in N availability relative to the control treatments (Figs 1c and 3c). Furthermore, across individual experiments, responses of soil N availability in the combined [CO₂] and warming experiments were positively correlated with responses in the warming-only experiments, but were not significantly correlated with the responses in the [CO₂]-only experiments (Fig. 4). These two observations are highly relevant, because they imply that results of [CO₂]-only experiments overstate the likelihood that PNL will occur in future.

However, we also assembled a dataset for foliar N concentrations as an indirect indication of PNL [Figs 2c and 4 (inset)]. Plant nutrient concentrations are not only influenced by the soil nutrient availability, but also by the dilution effects of the enlarged biomass and by the competitive immobilization by soil microbes. Interestingly, our meta-analysis indicated that foliar N concentration did decline significantly in combined elevated [CO₂] and warming (Fig. 3c). Moreover, across the individual experiments, foliar N concentration responses showed a contrasting pattern compared to the response of soil nutrient availability: a positive correlation between the combined treatment effects and the single factor [CO₂] effects (thus also in line with the responses of soil respiration and biomass production), compared to a marginally significant negative correlation for single factor warming effects [Figs 3 and 4 (inset)]. While it is important to express the foliar N concentration

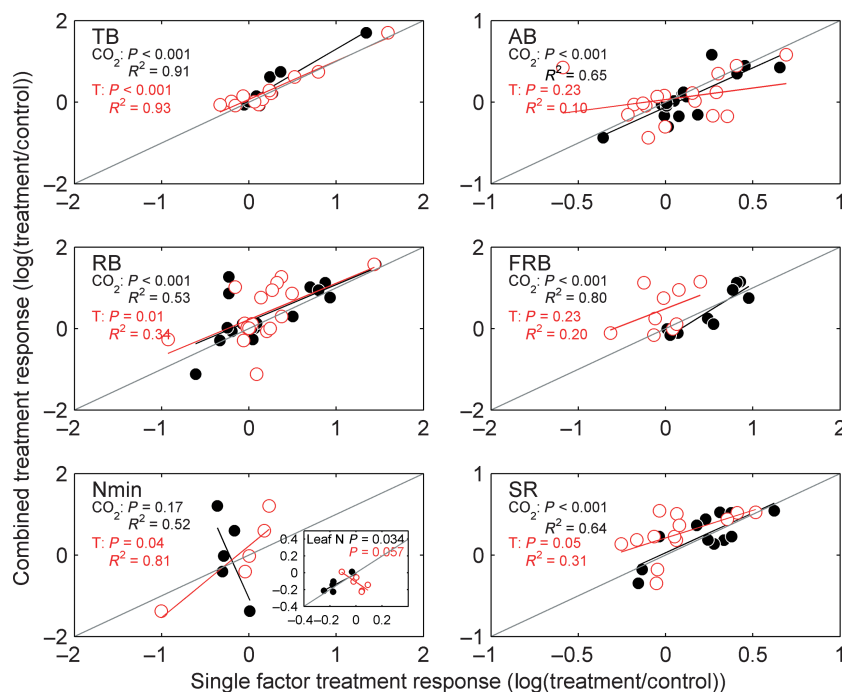


Fig. 4 Meta-analysis effect sizes for single factor [CO₂] effects (black filled circles) and single factor warming effects (red open circles) plotted against the combined [CO₂] × warming effect sizes. Data are reported as log (Treatment/Control). Data listed are total biomass (TB), aboveground biomass (AB), root biomass (RB), fine root biomass (FRB), mineral N availability (Nmin), foliar N concentration (Leaf N, inset), and soil respiration (SR). *P*-values and *R*² values for linear regressions between single factor and combined treatment effects are given. Correlations were considered statistically significant at *P* < 0.05.

on structural dry mass since there are major seasonal variations in foliar starch concentration that are also affected by the treatments (Jarvis & Linder, 2000), these analyses of the response of foliar N concentration to the combined treatments both suggest that PNL can be expected to occur also when [CO₂] and temperature increase together. In addition, many soil warming studies revealed only short-lived stimulation of organic matter decomposition (Luo *et al.*, 2001; Melillo *et al.*, 2002; but see Vicca *et al.*, 2009), implying that also the stimulatory effect of warming on net N mineralization might not be sustainable in the long term and therefore that warming can only postpone the occurrence of PNL. Our dataset for mineral N availability is, however, dominated by short-term experiments (2–5 years) and at this stage there clearly are insufficient long-term data available to support a robust conclusion on the occurrence of PNL in a future warmer and [CO₂]-enriched world.

The fact that the current set of manipulation experiments does not provide a conclusive indication about the occurrence of PNL in a future warmer and [CO₂]-enriched world is not only related to the limited size and duration of the data set, but probably more to the fact that local conditions determine the likelihood that PNL will occur. In theory, PNL is most likely to occur in northern and temperate-zone ecosystems in which biomass production is typically N-limited than in tropical ecosystems where N is typically not the limiting nutrient (Aerts & Chapin, 2000). Due to the growing intensity and global distribution of atmospheric deposition of reactive N com-

pounds (Galloway *et al.*, 2004), however, more and more extra-tropical ecosystems are shifting from a state of N (co-) limitation to a state of N-saturation (Aber *et al.*, 1998). Both PNL and N-saturation are plant-centered concepts that are often used to explain plant responses to elevated [CO₂] and to N deposition, but these concepts are not independent from one another. Ecosystems where N inputs exceed N demands will simultaneously evolve in the direction of N-saturation and be less prone to exhibit PNL with rising atmospheric [CO₂]. In other words, PNL is highly unlikely to occur in ecosystems with a very open and leaky N cycle (high N input rates combined with high rates of N leaching and N volatilization), characterized by excess plant available N and high temporal variability in ecosystem N content at annual or decadal timescale (Luo *et al.*, 2006; Liao *et al.*, 2008; Lu *et al.*, 2011). In the long term, PNL can thus be expected under elevated [CO₂], but only where the N cycle is closed (Rastetter *et al.*, 1997) and where warming-induced increases in net N mineralization are unlikely to cope with the increasing N immobilization in plant biomass, litter, and soil organic matter.

Are responses synergistic, antagonistic, or additive?

When applied in combination, interactive effects of elevated [CO₂] and warming may not simply result in additive responses, but might elicit synergistic or antagonistic responses. For example, accelerated nutrient mineralization caused by warming could counterbalance [CO₂]-induced

nutrient limitations and allow the full [CO₂] fertilization effect to be expressed. In this case, the [CO₂] effect could be larger in the combined treatment than in the [CO₂]-only treatment. Similarly, increased water use efficiency (WUE), caused by elevated [CO₂], could overcome warming-induced water limitation and thereby allow the full warming effect on biomass production (Morgan *et al.*, 2011). Hence, synergistic responses of biomass production to warming and elevated [CO₂] are not unrealistic (Norby & Luo, 2004).

The responses of plant biomass in combined treatment experiments relative to the sum of the single factor treatment responses are shown in Fig. 5. In the case of synergistic responses, individual experiments should be situated above the 1 : 1 line, which is clearly the exception rather than the rule. However, this is not that surprising *per se*, since warming will only tend to enhance the [CO₂] response if the response is actually suppressed by nutrient limitations. Similarly, elevated [CO₂] will only tend to increase the warming response if it helps to overcome a warming-induced water limitation. Therefore, synergistic responses should only be expected where nutrient limitation is currently suppressing the [CO₂] response and where water constraints are currently limiting the growth stimulation by warming. The general absence of a synergistic response therefore suggests that either current experiments rarely involve nutrient- or water-limited ecosystems, or that warming does not sufficiently mitigate nutrient limitation and/or elevated [CO₂] does not sufficiently mitigate drought.

In combination, warming and elevated [CO₂] clearly increase total and belowground biomass (Fig. 3c), but this effect is less than synergistic (Fig. 5), especially for aboveground biomass. This suggests the possible occurrence of antagonistic mechanisms. One such potential antagonistic mechanism is that both elevated [CO₂] and warming typically increase leaf area (Wullschlegel *et al.*, 2002; McCarthy *et al.*, 2007; Way & Oren, 2010). Consequently, the evapotranspiration in the combined treatment might be increased in response to the higher vapor pressure deficit and higher leaf area, despite the improved WUE as a consequence of elevated [CO₂] alone. In this case, the combined [CO₂] and warming treatment would deplete soil water reserves more rapidly than warming alone (but see Morgan *et al.*, 2011). Similarly, a reduction of root biomass (relative to the increased leaf area) as a consequence of higher nutrient availability due to the warming treatment might make plants more susceptible to periodic droughts (Way & Oren, 2010), possibly limiting the balancing effect of the increasing WUE in elevated [CO₂].

Differences in plant growth strategies might also affect the capacity of ecosystems to respond to a combined treatment. For example, Medlyn *et al.* (2001) have shown that stomatal conductance in evergreen trees is less responsive to elevated [CO₂], and therefore water savings might be lower, increasing their susceptibility to warming-induced droughts. If other biogeochemical processes (e.g. root exudation) differ in similar ways between species and ecosystems types, this might affect responses in a combined treatment as well. Furthermore,

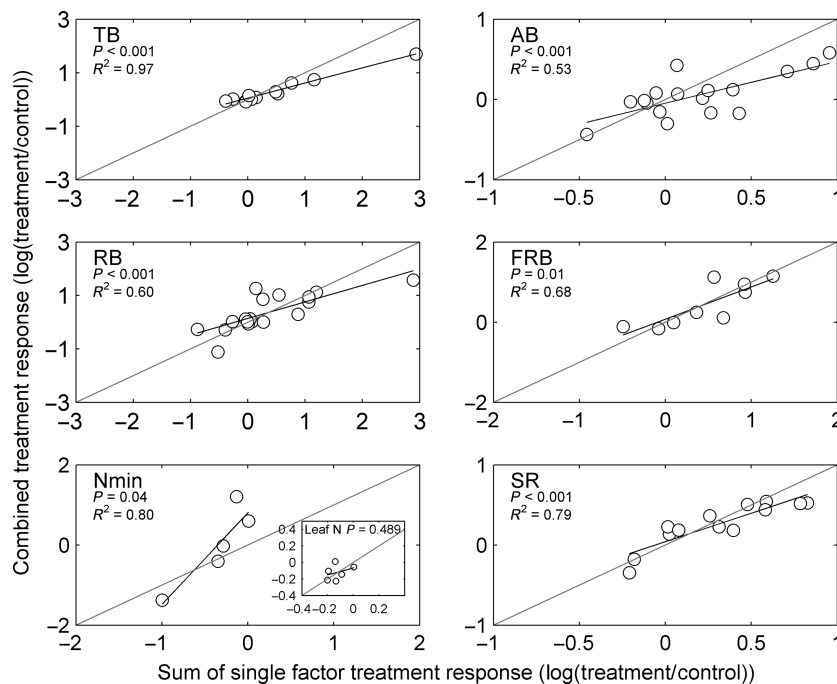


Fig. 5 Meta-analysis effect sizes for the calculated sum of single factor [CO₂] effects and single factor warming effects, plotted against the combined [CO₂] × warming effect sizes. Data are reported as log (Treatment/Control). Data listed are total biomass (TB), aboveground biomass (AB), root biomass (RB), fine root biomass (FRB), mineral N availability (Nmin), foliar N concentration (Leaf N), and soil respiration (SR). *P*-values and *R*² values for linear regressions between single factor and combined treatment effects are given. Correlations were considered statistically significant at *P* < 0.05.

increased competition in a mixed C₃ (favored by elevated [CO₂]) and C₄ (favored by warming) plant community might increase competition for resources and limit the full effect of both drivers (see Morgan *et al.*, 2011; for shift toward more C₄). Last, co-limitation of primary productivity by N and phosphorus (P) (Harpole *et al.*, 2011) may constrain combined effects of [CO₂] and warming when reduced N availability with [CO₂] is counteracted by reduced P availability with warming (Dijkstra *et al.*, in prep.). Such antagonistic responses appear to be the rule rather than the exception in the response of aboveground biomass, where the response to combined [CO₂] and warming is smaller than the response to [CO₂] alone in the vast majority of experiments (Fig. 3). Fine root biomass responded much less to warming than to [CO₂] enrichment, whether analyses encompassed all studies (Figs 1b and 2b), or when only studies that included both the single and combined treatments were evaluated (Fig. 3). Small responses of root biomass to warming are consistent with increased nutrient availability, which typically affects fine root biomass very little, but induces a substantial allocation shift in favor of aboveground biomass (Litton *et al.*, 2007; Brassard *et al.*, 2009).

Limitations and recommendations

Although the current set of experiments indicated a significant increase in biomass and soil respiration in response to elevated [CO₂] and warming, this may not be robust because the current set of experiments may not be large enough to equally represent the range of different ecosystem types. The amount of available data did not allow us to test for treatment duration effects, for differences among manipulation types or intensities, for differences among vegetation types, etc. This further highlights the urgent need for more multifactorial experiments. Nevertheless, with the currently available data, we have shown that the combined treatments elicited responses that were more similar to [CO₂]-only than to warming-only experiments. Therefore, model testing with data from [CO₂] enrichment-only experiments is to be preferred over testing with data from warming-only experiments.

Secondary effects of warming and [CO₂] enrichment (i.e. changes in water and nutrient availability) determine the ultimate response of terrestrial ecosystems. We were not able to test their influence due to a lack of data, or differences in reported parameters. Therefore, more attention should be given to standardized protocols for experimental design and measurements, not only for biomass production and ecosystem C fluxes, but especially for water availability/stress, as well as for nutrient availability.

We did not include precipitation changes in this analysis although they form an essential part of the changing climate and have been shown to affect responses in combined elevated [CO₂] and warming (e.g. N mineralization response at Brandbjerg, DK, Larsen *et al.*, 2011). Similarly, increasing amounts of reactive N deposition could strongly affect the responses observed here. The replication of combined [CO₂] and warming experiments on dry vs. wet, and nutrient-poor vs. -rich sites could be an alternative approach where both

water and nutrient effects can be incorporated in analyses like ours.

Conclusions

Elevated [CO₂] and warming exert fundamentally different effects on C storage and C and nutrient cycling in terrestrial ecosystems. In a combined treatment, effects of elevated [CO₂] often dominated the response, suggesting a larger sensitivity of terrestrial ecosystems to rising [CO₂] compared to rising temperatures. This dominance of [CO₂] in the combined treatments is probably attributable to the larger imposed relative changes in [CO₂] than in temperature, as is consistent with projected changes. Responses to single factor treatments were rarely additive, and interactions may lead to overestimation of effects based on the single factor results. Our results suggest that ecosystem models should ideally be tested against results from multifactor experiments to optimize their model structures.

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References

- Aber J, McDowell W, Nadelhoffer K *et al.* (1998) Nitrogen saturation in temperate forest ecosystems - hypotheses revisited. *BioScience*, **48**, 921–934.
- Adams DC, Gurevitch J, Rosenberg MS (1997) Resampling tests for meta-analysis of ecological data. *Ecology*, **78**, 1277–1283.
- Aerts R, Chapin FS (2000) The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Advances in Ecological Research*, **30**, 1–67.
- Ainsworth EA, Long SP (2005) What have we learned from 15 years of free-air CO₂ enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy. *New Phytologist*, **165**, 351–371.
- Andresen LC, Michelsen A, Jonasson S, Beier C, Ambus P (2009) Glycine uptake in heath plants and soil microbes responds to elevated temperature, CO₂ and drought. *Acta Oecologica-International Journal of Ecology*, **35**, 786–796.
- Atkin OK, Tjoelker MG (2003) Thermal acclimation and the dynamic response of plant respiration to temperature. *Trends in Plant Science*, **8**, 343–351.

- Beier C, Emmett BA, Gundersen P *et al.* (2004) Novel approaches to study climate change effects on terrestrial ecosystems in the field: drought and passive nighttime warming. *Ecosystems*, **7**, 583–597.
- Bradford MA, Fierer N, Reynolds JF (2008) Soil carbon stocks in experimental mesocosms are dependent on the rate of labile carbon, nitrogen and phosphorus inputs to soils. *Functional Ecology*, **22**, 964–974.
- Brassard BW, Chen HYH, Bergeron Y (2009) Influence of environmental variability on root dynamics in northern forests. *Critical Reviews in Plant Sciences*, **28**, 179–197.
- Ceulemans R, Mousseau M (1994) Tansley review No-71 - effects of elevated atmospheric CO₂ on woody plants. *New Phytologist*, **127**, 425–446.
- Ceulemans R, Janssens IA, Jach ME (1999) Effects of CO₂ enrichment on trees and forests: lessons to be learned in view of future ecosystem studies. *Annals of Botany*, **84**, 577–590.
- Comstedt D, Boström B, Marshall JD, Holm A, Slaney M, Linder S, Ekblad A (2006) Effects of elevated atmospheric carbon dioxide and temperature on soil respiration in a boreal forest using delta C-13 as a labeling tool. *Ecosystems*, **9**, 1266–1277.
- Curtis PS, Wang XZ (1998) A meta-analysis of elevated CO₂ effects on woody plant mass, form, and physiology. *Oecologia*, **113**, 299–313.
- Davidson EA, Janssens IA (2006) Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature*, **440**, 165–173.
- Dawes MA, Hagedorn F, Zumbunn T, Handa IT, Hättenschwiler S, Wipf S, Rixen C (2011) Growth and community responses of alpine dwarf shrubs to *in situ* CO₂ enrichment and soil warming. *New Phytologist*, **191**, 806–818.
- De Boeck H, Lemmens C, Zavalloni C *et al.* (2008) Biomass production in experimental grasslands of different species richness during three years of climate warming. *Biogeosciences*, **5**, 585–594.
- De Graaff MA, Van Groenigen KJ, Six J, Hungate B, Van Kessel C (2006) Interactions between plant growth and soil nutrient cycling under elevated CO₂: a meta-analysis. *Global Change Biology*, **12**, 2077–2091.
- Dieleman WIJ, Janssens IA (2011) Can publication bias affect ecological research? A case study on soil respiration under elevated CO₂? *New Phytologist*, **190**, 517–521.
- Dieleman WIJ, Luyssaert S, Rey A *et al.* (2010) Soil [N] modulates soil C cycling in CO₂-fumigated tree stands: a meta-analysis. *Plant Cell and Environment*, **33**, 2001–2011.
- Dijkstra FA, Cheng W (2007) Interactions between soil and tree roots accelerate long-term soil carbon decomposition. *Ecology Letters*, **10**, 1046–1053.
- Dijkstra FA, Blumenthal DM, Morgan JA, Pendall E, Carrillo Y, Follett RF (2010) Contrasting effects of elevated CO₂ and warming on nitrogen cycling in a semiarid grassland. *New Phytologist*, **187**, 426–437.
- Dukes JS, Chiariello NR, Cleland EE *et al.* (2005) Responses of grassland production to single and multiple global environmental changes. *PLoS Biology*, **3**, 1829–1837.
- Edwards NT, Norby RJ (1999) Below-ground respiratory responses of sugar maple and red maple saplings to atmospheric CO₂ enrichment and elevated air temperature. *Plant and Soil*, **206**, 85–97.
- Eliasson PE, Mcmurtrie RE, Pepper DA, Strömberg M, Linder S, Ågren GI (2005) The response of heterotrophic CO₂ flux to soil warming. *Global Change Biology*, **11**, 167–181.
- Elmendorf SC, Henry GHR, Hollister RD *et al.* (2012) Global assessment of experimental climate warming on tundra vegetation: heterogeneity over space and time. *Ecology Letters*, **15**, 164–175.
- Fontaine S, Barot S, Barre P, Bdioui N, Mary B, Rumpel C (2007) Stability of organic carbon in deep soil layers controlled by fresh carbon supply. *Nature*, **450**, 277–U210.
- Friedlingstein P, Cox P, Betts R *et al.* (2006) Climate-carbon cycle feedback analysis: results from the C⁴MIP model intercomparison. *Journal of Climate*, **19**, 3337–3353.
- Galloway JN, Dentener FJ, Capone DG *et al.* (2004) Nitrogen cycles: past, present, and future. *Biogeochemistry*, **70**, 153–226.
- Gavito ME, Schweiger P, Jakobsen I (2003) P uptake by arbuscular mycorrhizal hyphae: effect of soil temperature and atmospheric CO₂ enrichment. *Global Change Biology*, **9**, 106–116.
- Hagedorn F, Van Hees PAW, Handa IT, Hättenschwiler S (2008) Elevated atmospheric CO₂ fuels leaching of old dissolved organic matter at the alpine treeline. *Global Biogeochemical Cycles*, **22**.
- Hagedorn F, Martin M, Rixen C *et al.* (2010) Short-term responses of ecosystem carbon fluxes to experimental soil warming at the Swiss alpine treeline. *Biogeochemistry*, **97**, 7–19.
- Hall M, Rantfors M, Slaney M, Linder S, Wallin G (2009) Carbon dioxide exchange of buds and developing shoots of boreal Norway spruce exposed to elevated or ambient CO₂ concentration and temperature in whole-tree chambers. *Tree Physiology*, **29**, 467–481.
- Harpole WS, Ngai JT, Cleland EE *et al.* (2011) Nutrient co-limitation of primary producer communities. *Ecology Letters*, **14**, 852–862.
- Hartley IP, Heinemeyer A, Ineson P (2007) Effects of three years of soil warming and shading on the rate of soil respiration: substrate availability and not thermal acclimation mediates observed response. *Global Change Biology*, **13**, 1761–1770.
- Heath J, Ayres E, Possell M *et al.* (2005) Rising atmospheric CO₂ reduces sequestration of root-derived soil carbon. *Science*, **309**, 1711–1713.
- Hedges LV, Gurevitch J, Curtis PS (1999) The meta-analysis of response ratios in experimental ecology. *Ecology*, **80**, 1150–1156.
- Holtum JAM, Winter K (2010) Elevated [CO₂] and forest vegetation: more a water issue than a carbon issue? *Functional Plant Biology*, **37**, 694–702.
- Hovenden MJ, Newton PCD, Carran RA *et al.* (2008) Warming prevents the elevated CO₂-induced reduction in available soil nitrogen in a temperate, perennial grassland. *Global Change Biology*, **14**, 1018–1024.
- Hungate BA, Dukes JS, Shaw MR, Luo Y, Field CB (2003) Nitrogen and climate change. *Science*, **302**, 1512–1513.
- Hungate BA, Van Groenigen KJ, Six J *et al.* (2009) Assessing the effect of elevated carbon dioxide on soil carbon: a comparison of four meta-analyses. *Global Change Biology*, **15**, 2020–2034.
- Hyvönen R, Ågren GI, Linder S *et al.* (2007) Tansley Review. The likely impact of elevated [CO₂], nitrogen deposition, increased temperature and management on carbon sequestration in temperate and boreal forest ecosystems: a literature review. *New Phytologist*, **173**, 463–480.
- Ipcc (2007) Climate Change 2007: *Synthesis Report Contribution of Working Groups I, II and III to the Fourth Assessment Report of the International Panel on Climate Change*, (eds Pachauri RK, Reisinger A). IPCC, Geneva, Switzerland.
- Jarvis P, Linder S (2000) Botany - Constraints to growth of boreal forests. *Nature*, **405**, 904–905.
- Jastrow JD, Miller RM, Matamala R, Norby RJ, Boutton TW, Rice CW, Owensby CE (2005) Elevated atmospheric carbon dioxide increases soil carbon. *Global Change Biology*, **11**, 2057–2064.
- Kandeler E, Tschirko D, Bardgett RD, Hobbs PJ, Kampichler C, Jones TH (1998) The response of soil microorganisms and roots to elevated CO₂ and temperature in a terrestrial model ecosystem. *Plant and Soil*, **202**, 251–262.
- Kardol P, Campy CE, Souza L, Norby RJ, Weltzin JF, Classen AT (2010) Climate change effects on plant biomass alter dominance patterns and community evenness in an experimental old-field ecosystem. *Global Change Biology*, **16**, 2676–2687.
- King JS, Thomas RB, Strain BR (1996) Growth and carbon accumulation in root systems of *Pinus taeda* and *Pinus ponderosa* seedlings as affected by varying CO₂, temperature and nitrogen. *Tree Physiology*, **16**, 635–642.
- Kirschbaum MUF (2004) Soil respiration under prolonged soil warming: are rate reductions caused by acclimation or substrate loss? *Global Change Biology*, **10**, 1870–1877.
- Knorr W, Prentice IC, House JI, Holland EA (2005) Long-term sensitivity of soil carbon turnover to warming. *Nature*, **433**, 298–301.
- Körner C (2006) Plant CO₂ responses: an issue of definition, time and resource supply. *New Phytologist*, **172**, 393–411.
- Kostiainen K, Kaakinen S, Saranpää P, Sigurdsson BD, Lundqvist S, Linder S, Vapaavuori E (2009) Stem wood properties of mature Norway spruce after 3 years of continuous exposure to elevated [CO₂] and temperature. *Global Change Biology*, **15**, 368–379.
- Kuzyakov Y (2011) Prime time for microbes. *Nature Climate Change*, **1**, 295–297.
- Larigauderie A, Reynolds JF, Strain BR (1994) Root response to CO₂ enrichment and nitrogen supply in loblolly-pine. *Plant and Soil*, **165**, 21–32.
- Larsen KS, Andresen LC, Beier C *et al.* (2011) Reduced N cycling in response to elevated CO₂, warming, and drought in a Danish heathland: synthesizing results of the CLIMATE project after two years of treatments. *Global Change Biology*, **17**, 1884–1899.
- Leuzinger S, Luo Y, Beier C, Dieleman WIJ, Vicca S, Körner C (2011) Do global change experiments overestimate impacts on terrestrial ecosystems? *Trends in Ecology & Evolution*, **26**, 236–241.
- Liao C, Peng R, Luo Y *et al.* (2008) Altered ecosystem carbon and nitrogen cycles by plant invasion: a meta-analysis. *New Phytologist*, **177**, 706–714.
- Lin GH, Rygielwicz PT, Ehleringer JR, Johnson MG, Tingey DT (2001) Time-dependent responses of soil CO₂ efflux components to elevated atmospheric [CO₂] and temperature in experimental forest mesocosms. *Plant and Soil*, **229**, 259–270.
- Litton CM, Raich JW, Ryan MG (2007) Carbon allocation in forest ecosystems. *Global Change Biology*, **13**, 2089–2109.
- Lu M, Yang Y, Luo Y *et al.* (2011) Responses of ecosystem nitrogen cycle to nitrogen addition: a meta-analysis. *New Phytologist*, **189**, 1040–1050.

- Lukac M, Lagomarsino A, Moscatelli MC, De Angelis P, Cotrufo MF, Godbold DL (2009) Forest soil carbon cycle under elevated CO₂ - a case of increased through-pot? *Forestry*, **82**, 75–86.
- Luo YQ (2007) Terrestrial carbon-cycle feedback to climate warming. *Annual Review of Ecology Evolution and Systematics*, **38**, 683–712.
- Luo YQ, Wan SQ, Hui DF, Wallace LL (2001) Acclimatization of soil respiration to warming in a tall grass prairie. *Nature*, **413**, 622–625.
- Luo Y, Su B, Currie WS *et al.* (2004) Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide. *BioScience*, **54**, 731–739.
- Luo YQ, Hui DF, Zhang DQ (2006) Elevated CO₂ stimulates net accumulations of carbon and nitrogen in land ecosystems: a meta-analysis. *Ecology*, **87**, 53–63.
- Luo Y, Gerten D, Le Maire G *et al.* (2008) Modeled interactive effects of precipitation, temperature, and [CO₂] on ecosystem carbon and water dynamics in different climatic zones. *Global Change Biology*, **14**, 1–14.
- McCarthy HR, Oren R, Finzi AC, Ellsworth DS, Kim H, Johnsen KH, Millar B (2007) Temporal dynamics and spatial variability in the enhancement of canopy leaf area under elevated atmospheric CO₂. *Global Change Biology*, **13**, 2479–2497.
- Medlyn BE, Barton CVM, Broadmeadow MSJ *et al.* (2001) Stomatal conductance of forest species after long-term exposure to elevated CO₂ concentration: a synthesis. *New Phytologist*, **149**, 247–264.
- Melillo JM, Steudler PA, Aber JD *et al.* (2002) Soil warming and carbon-cycle feedbacks to the climate system. *Science*, **298**, 2173–2176.
- Melillo JM, Butler S, Johnson J *et al.* (2011) Soil warming, carbon-nitrogen interactions, and forest carbon budgets. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 9508–9512.
- Morgan J, Pataki D, Körner C *et al.* (2004) Water relations in grassland and desert ecosystems exposed to elevated atmospheric CO₂. *Oecologia*, **140**, 11–25.
- Morgan JA, Lecain DR, Pendall E *et al.* (2011) C₄ grasses prosper as carbon dioxide eliminates desiccation in warmed semi-arid grassland. *Nature*, **476**, 202–U101.
- Niinistö SM, Silvola J, Kellomäki S (2004) Soil CO₂ efflux in a boreal pine forest under atmospheric CO₂ enrichment and air warming. *Global Change Biology*, **10**, 1363–1376.
- Niu S, Sherry RA, Zhou X, Wan S, Luo Y (2010) Nitrogen regulation of the climate-carbon feedback: evidence from a long-term global change experiment. *Ecology*, **91**, 3261–3273.
- Norby JN, Luo Y (2004) Evaluating ecosystem responses to rising atmospheric CO₂ and global warming in a multi-factor world. *New Phytologist*, **162**, 281–293.
- Norby RJ, Lang TM, Hartz-Rubin JS, O'Neill EG (2000) Nitrogen resorption in senescing tree leaves in a warmer, CO₂-enriched atmosphere. *Plant and Soil*, **224**, 15–29.
- Norby RJ, Delucia EH, Gielen B *et al.* (2005) Forest response to elevated CO₂ is conserved across a broad range of productivity. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 18052–18056.
- Nowak RS, Ellsworth DS, Smith SD (2004) Functional responses of plants to elevated atmospheric CO₂ - do photosynthetic and productivity data from FACE experiments support early predictions? *New Phytologist*, **162**, 253–280.
- Olszyk DM, Johnson MC, Tingey DT *et al.* (2003) Whole-seedling biomass allocation, leaf area, and tissue chemistry for Douglas-fir exposed to elevated CO₂ and temperature for 4 years. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere*, **33**, 269–278.
- Oren R, Ellsworth DS, Johnsen KH *et al.* (2001) Soil fertility limits carbon sequestration by forest ecosystems in a CO₂-enriched atmosphere. *Nature*, **411**, 469–472.
- Pajari B (1995) Soil respiration in a poor upland site of Scots pine stand subjected to elevated temperatures and atmospheric carbon concentration. *Plant and Soil*, **168**, 563–570.
- Pendall E, Bridgman S, Hanson PJ *et al.* (2004) Below-ground process responses to elevated CO₂ and temperature: a discussion of observations, measurement methods, and models. *New Phytologist*, **162**, 311–322.
- Pendall E, Osanai Y, Williams AL, Hovenden MJ (2011) Soil carbon storage under simulated climate change is mediated by plant functional type. *Global Change Biology*, **17**, 505–514.
- Rastetter EB, Agren GI, Shaver GR (1997) Responses of N-limited ecosystems to increased CO₂: a balanced-nutrition, coupled-element-cycles model. *Ecological Applications*, **7**, 444–460.
- Rogers HH, Runion GB, Krupa SV (1994) Plant responses to atmospheric CO₂ enrichment with emphasis on roots and the rhizosphere. *Environmental Pollution*, **83**, 155–189.
- Rosenberg MS, Adams DC, Gurevitch J. (2000) *MetaWin: Statistical Software for Meta-Analysis*, Sinauer Associates, Inc., Sunderland, Massachusetts.
- Rustad LE (2008) The response of terrestrial ecosystems to global climate change: towards an integrated approach. *Science of the Total Environment*, **404**, 222–235.
- Rustad L, Campbell J, Marion G *et al.* (2001) A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia*, **126**, 543–562.
- Saxe H, Cannell MGR, Johnsen B, Ryan MG, Vourlitis G (2001) Tree and forest functioning in response to global warming. *New Phytologist*, **149**, 369–399.
- Schlesinger WH, Lichter J (2001) Limited carbon storage in soil and litter of experimental forest plots under increased atmospheric CO₂. *Nature*, **411**, 466–469.
- Selsted MB, Van Der Linden L, Ibrom A *et al.* (2012) Soil respiration is stimulated by elevated CO₂ and reduced by summer drought: three years of measurements in a multifactor ecosystem manipulation experiment in a temperate heathland (CLIMAITE). *Global Change Biology*, **18**, 1216–1230.
- Shaw MR, Zavaleta ES, Chiariello NR, Cleland EE, Mooney HA, Field CB (2002) Grassland responses to global environmental changes suppressed by elevated CO₂. *Science*, **298**, 1987–1990.
- Slaney M, Wallin G, Medhurst J, Linder S (2007) Impact of elevated carbon dioxide concentration and temperature on bud burst and shoot growth of boreal Norway spruce. *Tree Physiology*, **27**, 301–312.
- Suseela V, Conant RT, Wallenstein MD, Dukes JS (2012) Effects of soil moisture on the temperature sensitivity of heterotrophic respiration vary seasonally in an old-field climate change experiment. *Global Change Biology*, **18**, 336–348.
- Tingey DT, Lee EH, Waschmann R, Johnson MG, Rygielwicz PT (2006) Does soil CO₂ efflux acclimatize to elevated temperature and CO₂ during long-term treatment of Douglas-fir seedlings? *New Phytologist*, **170**, 107–118.
- Usselman SM, Qualls RG, Thomas RB (2000) Effects of increased atmospheric CO₂, temperature, and soil N availability on root exudation of dissolved organic carbon by a N-fixing tree (*Robinia pseudoacacia* L.). *Plant and Soil*, **222**, 191–202.
- Van Groenigen KJ, Osenberg CW, Hungate BA (2011) Increased soil emissions of potent greenhouse gases under increased atmospheric CO₂. *Nature*, **475**, 214–U121.
- Vicca S, Fizez L, Kockelbergh F *et al.* (2009) No signs of thermal acclimation of heterotrophic respiration from peat soils exposed to different water levels. *Soil Biology & Biochemistry*, **41**, 2014–2016.
- Vicca S, Janssens IA, Wong S, Cernusak LA, Farquhar GD (2010) Zea mays rhizosphere respiration, but not soil organic matter decomposition was stable across a temperature gradient. *Soil Biology & Biochemistry*, **42**, 2030–2033.
- Volder A, Gifford RM, Evans JR (2007) Effects of elevated atmospheric CO₂, cutting frequency, and differential day/night atmospheric warming on root growth and turnover of Phalaris swards. *Global Change Biology*, **13**, 1040–1052.
- Volk M, Niklaus PA, Körner C (2000) Soil moisture effects determine CO₂ responses of grassland species. *Oecologia*, **125**, 380–388.
- Wan SQ, Norby RJ, Pregitzer KS, Ledford J, O'Neill EG (2004) CO₂ enrichment and warming of the atmosphere enhance both productivity and mortality of maple tree fine roots. *New Phytologist*, **162**, 437–446.
- Wan S, Norby RJ, Ledford J, Weltzin JF (2007) Responses of soil respiration to elevated CO₂, air warming, and changing soil water availability in a model old-field grassland. *Global Change Biology*, **13**, 2411–2424.
- Way DA, Oren R (2010) Differential responses to changes in growth temperature between trees from different functional groups and biomes: a review and synthesis of data. *Tree Physiology*, **30**, 669–688.
- Wu Z, Dijkstra P, Koch GW, Penuelas J, Hungate B (2011) Responses of terrestrial ecosystems to temperature and precipitation change: a meta-analysis of experimental manipulation. *Global Change Biology*, **17**, 927–942.
- Wullschlegel SD, Tschaplinski TJ, Norby RJ (2002) Plant water relations at elevated CO₂ - implications for water-limited environments. *Plant Cell and Environment*, **25**, 319–331.
- Zak DR, Pregitzer KS, King JS, Holmes WE (2000) Elevated atmospheric CO₂, fine roots and the response of soil microorganisms: a review and hypothesis. *New Phytologist*, **147**, 201–222.

Supporting Information

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

Figure S1. Histograms presenting the frequency distribution of [CO₂] treatments in combined warming and elevated [CO₂] studies for total biomass (TB), aboveground biomass (AB), root biomass (RB), fine root biomass (FRB), soil respiration (SR), and mineral N availability (Nmin). Mean treatment intensity and standard deviations (SD) are given.

Figure S2. Histograms presenting the frequency distribution of temperature treatments in combined warming and elevated [CO₂] studies for total biomass (TB), aboveground biomass (AB), root biomass (RB), fine root biomass (FRB), soil respiration (SR), and mineral N availability (Nmin). Mean treatment intensity and standard deviations (SD) are given.

Table S1. General information about the sites included in the database. The experiments performed at these sites can include one of the treatments or species listed in the columns below. Site location, annual precipitation and temperature are given where available.

Table S2. All experiments included in the database are listed, fumigation and warming techniques used in the experiments, and the amount of [CO₂] or T increment are indicated.

Table S3. All experiments included in the database are listed, and stars indicate which experiment provided what kind of data.

Table S4. All experiments included in the database are listed, with the methodology used to obtain the respective datapoints. For more detailed methodologies, we refer to the source references (to be found in the supplementary Table S5).

Table S5. All experiments included in the database are listed. Source references used for the respective sites are given (Full references are given in supplementary Table S6).

Table S6. Full source references used in the database.

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