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Review

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Ecosystem responses to elevated CO_2 governed by plant–soil interactions and the cost of nitrogen acquisition

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Summary

Land ecosystems sequester on average about a quarter of anthropogenic CO_2 emissions. It has been proposed that nitrogen (N) availability will exert an increasingly limiting effect on plants' ability to store additional carbon (C) under rising CO_2, but these mechanisms are not well understood. Here, we review findings from elevated CO_2 experiments using a plant economics framework, highlighting how ecosystem responses to elevated CO_2 may depend on the costs and benefits of plant interactions with mycorrhizal fungi and symbiotic N-fixing microbes. We found that N-acquisition efficiency is positively correlated with leaf-level photosynthetic capacity and plant growth, and negatively with soil C storage. Plants that associate with ectomycorrhizal fungi and N-fixers may acquire N at a lower cost than plants associated with arbuscular mycorrhizal fungi. However, the additional growth in ectomycorrhizal plants is partly offset by decreases in soil C pools via priming. Collectively, our results indicate that predictive models aimed at quantifying C cycle feedbacks to global change may be improved by treating N as a resource that can be acquired by plants in exchange for energy, with different costs depending on plant interactions with microbial symbionts.

I. Introduction

The atmospheric CO_2 concentration has risen to > 40% above its pre-industrial level, and it is expected to continue rising for decades (Ciais *et al.*, 2013) even under the most ambitious climate-change mitigation scenarios (Smith *et al.*, 2016). Although it is well established that elevated CO_2 (eCO_2) stimulates photosynthesis at the leaf level (Ainsworth & Long, 2005), there is considerable uncertainty about the extent to which plants will sustain elevated levels of productivity and continued carbon (C) storage as CO_2

concentrations rise. This uncertainty reflects incomplete understanding of how eCO_2 alters plant C allocation, decomposition of soil organic matter (SOM), and plant mortality and biomass turnover (Malhi *et al.*, 2015) – all processes modulated by the availability of soil resources.

One of the largest areas of uncertainty about the magnitude of the eCO_2 fertilization effect concerns the role of nutrient availability (Hungate *et al.*, 2003). Relatively tight stoichiometric constraints imply that if the nutrient requirements to increase plant growth are not met (Fay *et al.*, 2015), nutrient availability will inevitably limit the terrestrial C sink (Huang *et al.*, 2015). Nitrogen (N) availability, in particular, appears to limit plant productivity in many terrestrial ecosystems at present (Vitousek & Howarth, 1991; LeBauer & Treseder, 2008; Menge *et al.*, 2012), and is widely considered to be among the most important factors limiting the productivity response of ecosystems to eCO_2 (Körner, 2006; Reich *et al.*, 2006a; Huang *et al.*, 2015; Terrer *et al.*, 2016).

Although numerous experiments have been conducted over the past two decades to investigate the role of N in constraining CO_2-induced stimulation of photosynthesis and primary production, there is still no general explanation for the disparity of responses observed among different ecosystems (Bazzaz, 1990; Saxe *et al.*, 1998; Nowak *et al.*, 2004; Körner, 2006; Reich *et al.*, 2006b; Norby & Zak, 2011). In some studies, low N availability was found to be the primary constraint responsible for the transient, small or nonexistent CO_2 fertilization effect (Schneider *et al.*, 2004; Norby *et al.*, 2010; Reich & Hobbie, 2013; Sigurdsson *et al.*, 2013). In other studies, plant production was stimulated by eCO_2 despite apparent N-limitation (McCarthy *et al.*, 2010; Hungate *et al.*, 2013; Talhelm *et al.*, 2014). As such, most reviews have concluded that the magnitude of the CO_2 effect varies on a site-by-site basis, leaving the observed inter-site variation unexplained.

One hypothesis predicts that the N-limitation on plant responses to eCO_2 is modulated by the type of N-acquisition strategy, which, in turn, is largely determined by symbiotic plant–microbial interactions (Alberton *et al.*, 2005; Drake *et al.*, 2011; Phillips *et al.*, 2013; Terrer *et al.*, 2016). For example, ecosystems where the dominant plants can acquire 'additional N' by stimulating biological N_2-fixation (BNF) or accelerating SOM decomposition (e.g. via priming effects) are predicted to sustain high rates of net primary productivity (NPP) under elevated CO_2. In a recent meta-analysis, Terrer *et al.* (2016) found that N availability and the type of microbial symbiont associated with the plant roots were important factors explaining the observed changes in standing biomass across eCO_2 experiments, with a strong and significant interaction between these two factors. Plants associated with ectomycorrhizal (ECM) fungi showed an eCO_2-driven *c.* 28% enhancement in biomass even under low N. By contrast, plants associated with arbuscular mycorrhizal (AM) fungi were unresponsive to eCO_2 (*c.* 0%) under low N, unless associated with N_2-fixers (*c.* 8%). These conclusions proved consistent for aboveground productivity as well as biomass (Terrer *et al.*, 2017).

Although greater numbers of long-term eCO_2 experiments with both AM and ECM trees are needed to further test this hypothesis (Norby *et al.*, 2017), differences in the nutrient economies of *symbiotic types* may offer a consistent framework to better understand and model the interactions between the C and N cycles (Phillips *et al.*, 2013; Lin *et al.*, 2017). By symbiotic types we refer to the capacity of plant species to employ symbionts in their N-acquisition strategy, such as N-uptake mediated through AM and ECM fungi or symbiotic BNF. Nevertheless, the conclusions of Terrer *et al.* (2016) raise additional hypotheses: (1) do ECM plants and N_2-fixers take up more N than AM plants in response to eCO_2? (2) Is the role of N availability in constraining the eCO_2 effect on plant biomass caused by limitations on leaf-level photosynthesis? And (3) how do changes in N availability under eCO_2 affect soil C stocks and the ecosystem C balance?

Here, we explore these questions by reviewing observations from eCO_2 experiments with a focus on the C cost of N-acquisition. We acknowledge that other factors such as water availability (Morgan *et al.*, 2004) or phosphorus (P) availability (Ellsworth *et al.*, 2017) may be equally important in mediating terrestrial ecosystem responses to eCO_2. These are, however, beyond the scope of the current review, which focuses on the effects of N availability, the most commonly limiting nutrient globally (LeBauer & Treseder, 2008). Importantly, we do not treat N-limitation as an 'on–off' property but rather refer to the cost of N-acquisition – or, its inverse, the return on investment – as a continuum. As such, our plant economics approach can be applied to other soil resources, provided that the necessary data are sufficiently available. In Section II we define and apply the return on investment approach, which is used in Section III as a link driving ecosystem-level effects triggered by eCO_2. In Section IV we discuss the conclusions and propose a conceptual framework, with indications of productive directions for model and experimental improvements.

II. The return on investment approach

1. Methods

We define the *return on investment* as a ratio of the marginal relative increase in N-acquisition (N_{acq}) and the marginal relative increase in belowground C allocation (C_{bg}). We quantify the return on investment with data from eCO_2 experiments using differences in measured N_{acq} and C_{bg} under elevated ('ele') and ambient ('amb') CO_2 treatments:

$$\text{Return on investment} = \frac{\frac{\partial N_{acq}}{N_{acq}}}{\frac{\partial C_{bg}}{C_{bg}}} \approx \frac{\frac{N_{acq}(\text{ele}) - N_{acq}(\text{amb})}{N_{acq}(\text{amb})}}{\frac{C_{bg}(\text{ele}) - C_{bg}(\text{amb})}{C_{bg}(\text{amb})}} = \psi_N^{-1}, \qquad \text{Eqn 1}$$

ψ_N can be interpreted as the C cost of acquiring N, and corresponds to the inverse of the return on investment. It quantifies how plants' N_{acq} rates relate to increasing belowground C allocation, and thereby estimates the degree to which aboveground growth is limited by N.

Although N_{acq} is often measured in eCO_2 experiments (e.g. Feng *et al.*, 2015), estimating C_{bg} (C investment in N_{acq}) remains a conceptual and methodological challenge. C_{bg} is not confined to root production (C_{root}), but also includes C transferred to root exudates, mycorrhizal fungi and symbiotic N-fixing bacteria ($C_{transfer}$; see Vicca *et al.*, 2012), and is therefore indicative of 'investments' for N uptake (or nutrient uptake in general):

$$C_{bg} = C_{root} + C_{transfer}. \qquad \text{Eqn 2}$$

$C_{transfer}$ implies a cost for the plant by reducing the C available for biomass productivity (BP):

$$BP = NPP - C_{transfer}. \qquad \text{Eqn 3}$$

We therefore refer to $C_{transfer}$ as the component of the C budget that may be used by plants to acquire N. Several lines of evidence suggest that is not allocated to plant biomass and, indeed, plants increase allocation to $C_{transfer}$ as soil resources decrease in availability (Treseder, 2004; Hobbie, 2006; Högberg *et al.*, 2010; Drake *et al.*, 2011; Phillips *et al.*, 2011; Aoki *et al.*, 2012; Nouri *et al.*, 2014), and that such increases in allocation to $C_{transfer}$ come at the expense of plant biomass production (Vicca *et al.*, 2012) and can reduce net ecosystem productivity (Fernández-Martínez *et al.*, 2014). This may explain why root colonization by mycorrhizal fungi is often increased by eCO_2 (increased N demand) but decreased by N-fertilization (decreased N demand), indicating that plants increase the investment in $C_{transfer}$ as a means to meet N requirements (Treseder, 2004). Moreover, differences in the C cost of nutrient acquisition may also explain why the proportion of C allocated to C_{bg} (and by extension $C_{transfer}$) is inversely related to N availability at global scales (Gill & Finzi, 2016), with greater belowground investment in boreal relative to tropical regions.

Here, we estimated ψ_N^{-1} (Fig. 1; Eqn 1) for as many eCO_2 studies as possible, that is, those with data on both N_{acq} and C_{bg}. Even though $C_{transfer}$ represents a fraction of 10–40% of NPP (Chapin *et al.*, 2011; Pritchard, 2011), there have been few measurements of C allocation to fungi and exudates in eCO_2 experiments (Phillips *et al.*, 2011). We used fine-root production, fine-root biomass or root biomass as a proxy for C_{bg}, thus assuming a constant ratio of $C_{transfer}$ to C_{root} and therefore:

$$\frac{\partial C_{bg}}{C_{bg}} = \frac{\partial C_{root}}{C_{root}}. \qquad \text{Eqn 4}$$

Eqn 4 is supported for several ECM species (Hobbie, 2006; Hobbie & Hobbie, 2008), but uncertainties regarding its validity remain for AM and N-fixing species. We included data from previous syntheses on eCO_2-driven N_{acq} (Finzi *et al.*, 2007; Feng *et al.*, 2015), and searched from the Web of Science for C_{bg} data, recent additional years and additional field studies Free-Air CO_2 enrichment (FACE) and open top chamber (OTC) with available data on both N_{acq} and C_{bg}. In total, we used observations from 20 grassland and forest ecosystem experiments corresponding to 12 different sites (Table 1). For species in the Aspen-FACE experiment (Table 1) we excluded all years before canopy development was complete, as recommended elsewhere (Norby *et al.*, 2005).

2. Results

In the absence of N-fertilization, N_{acq} increased significantly (+24%, $P < 0.001$) under eCO_2 in ECM plants, whereas the effect was not significant (−5.6%, $P = 0.1056$) in AM plants. In Fig. 1(a),

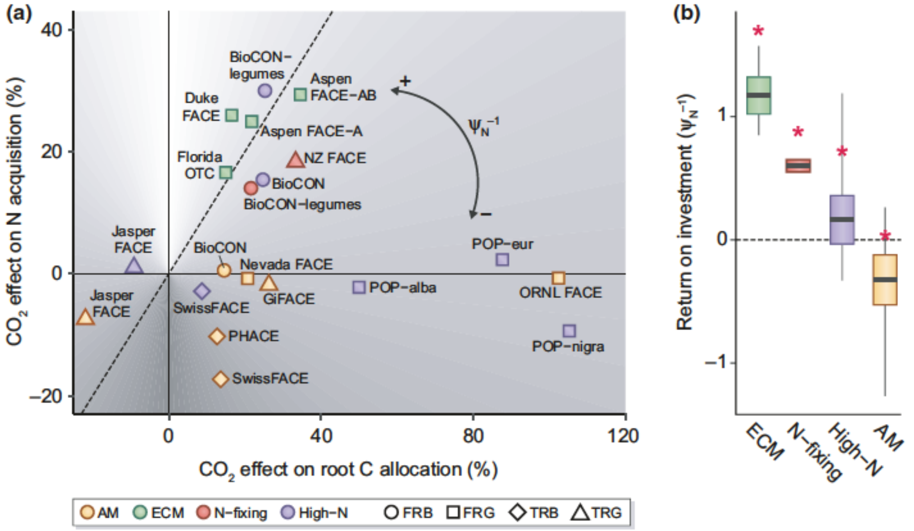


Fig. 1 Plant economics spectrum of the efficiency of plants in acquiring additional nitrogen (N) under elevated CO_2. (a) Relationship between the elevated CO_2 (eCO_2)-induced relative change (%) in root carbon (C_{root}) ('investments') and aboveground N-acquisition ('returns'). (b) Mean, SE, max and min return on investment (ψ_N^{-1}, Eqn 1). Colours represent four different N-acquisition strategies characterized by the type of symbiotic association, that is: arbuscular mycorrhizas (AM), ectomycorrhizas (ECM) and N-fixing species (N-fixing), or high N availability (High-N). Shapes in (a) represent the type of root data used to estimate belowground C allocation (C_{bg}), that is: fine-root biomass (FRB), fine-root growth (FRG), total-root biomass (TRB) or total-root growth (TRG). Black dashed line in (a) represents the 1 : 1 line. The slope of the grey lines in the background in (a) represents ψ_N^{-1}, with lower returns (higher costs) as dark grey. Asterisks in (b) are ψ_N^{-1} estimates at Duke FACE (ECM) and BioCON (AM, N-fixing, and High-N) using C_{bg} instead of C_{root} data (Eqn 2). N_{acq}, product of total or aboveground biomass production and N concentration. When sites include data at the species-level, the site name is followed by a species code (Table 1). Sources of site-level data are given in Table 1.

the slope represents N_{acq}-efficiency (ψ_N^{-1}), with lighter shading representing higher 'returns'. Most ECM experiments plotted close to the 1 : 1 line, suggesting proportionality between the relative changes in investment and acquisition (e.g. a 1% increase in C investment belowground translates into a 1% increase in N_{acq}). Systems where N_2-fixers were present exhibited a similar relationship between N_{acq} and C_{bg} as ECM systems. This finding is based on two experiments: plots from the BioCON experiment with legume species only (Reich & Hobbie, 2013), and all plots from the New Zealand (NZ) FACE experiment, with a mix of N_2-fixers *Trifolium repens* L. and *Trifolium subterraneum* L. and other grassland AM-species (Newton *et al.*, 2014). AM plants achieved a much lower enhancement in N_{acq} than ECM plants and N-fixers for a given amount of C invested belowground. In some cases, AM plants acquired less N under elevated than ambient CO_2 despite increasing belowground C investments (Fig. 1a). This relates to results by Feng *et al.* (2015), who found reduced N_{acq} under eCO_2. The simultaneous increase in C_{bg} indicates a strong reduction in N_{acq} efficiency. N-fertilization generally increased ψ_N^{-1} compared to nonfertilized AM systems (e.g. BioCON, SwissFACE), but it did not consistently help plants achieve the high ψ_N^{-1}-levels of ECM and N_2-fixers in this dataset (Fig. 1b).

C_{bg} data in Fig. 1 are limited by the lack of $C_{transfer}$ data (Eqn 2). In order to test the validity of Eqn 4 and the patterns in Fig. 1, we estimated ψ_N^{-1} using data from four experiments where C_{bg} ($C_{root} + C_{transfer}$) was inferred from plant C balance (Litton *et al.*, 2007. These data can be used to estimate the cost of N_{acq} without assuming Eqn 4, in relative (asterisks in Fig. 1b) and absolute terms. For example, in the Duke FACE experiment (ECM), Drake *et al.* (2011) estimated that plants under eCO_2 invested 88 g of $C_{bg}\,g^{-1}$ of N_{acq}, including 12 g of $C_{transfer}$. At BioCON (AM), the estimated cost of N_{acq} under eCO_2 and low N was 2033 g $C_{bg}\,g^{-1}\,N_{acq}$ (Adair *et al.*, 2009) resulting from the low capacity of plants to acquire additional N. In N_2-fixing legumes, however, eCO_2 stimulated N_{acq} at a rate of 97 g $C_{bg}\,g^{-1}\,N_{acq}$, similar to ECM-trees at Duke. These patterns (asterisks in Fig. 1b) using both C_{root} and $C_{transfer}$ data, indicate that the cost of N_{acq} varies across N_{acq}-strategies, supporting the conclusions in Fig. 1. Although assessing the assumption of a constant $C_{root} : C_{transfer}$ ratio (Eqn 4) is a key need for this field, its uncertainty does not stand in the way of the exercise presented here, yet it does indicate uncertainty about the exact slope in Fig. 1(a). Regardless of the slope, marginal N-gains in ECM are larger than in AM plants (Sulman *et al.*, 2017). In order to estimate the true costs, however, more data about the investment in symbiotic associations ($C_{transfer}$) under eCO_2 are necessary (see list of data limitations of the approach in Table 2).

In view of these results, the ability of plants to acquire additional N under eCO_2 appears to vary among symbiotic types and levels of N availability. The important role of mycorrhizal fungi as factors determining ecosystem processes (under current climate) is becoming increasingly apparent (Wurzburger *et al.*, 2017), with ECM fungi generally associated with more beneficial effects on their plant host's fitness than AM fungi (Bennett *et al.*, 2017; Teste *et al.*, 2017). Current evidence suggests that the role of AM fungi in N_{acq} depends on soil N availability, as the fungi may have limited capacity to take up (or transfer) N when in low supply (Reynolds *et al.*, 2005; Johnson *et al.*, 2015). On the one hand, eCO_2 did not commonly enhance aboveground N_{acq} in AM plants under low N in this dataset (Fig. 1a), whereas root investment was increased, leading to a negative mean ψ_N^{-1} (Fig. 1b). This is consistent with the hypothesis that AM fungi associate with plants along a continuum of interactions ranging from beneficial to parasitic (Johnson *et al.*, 1997), with negative effects for the plant under low N availability (Reynolds *et al.*, 2005; Johnson *et al.*, 2015). On the other hand, AM fungi are commonly associated with enhanced plant N_{acq} when N availability is moderate or high (Johnson *et al.*, 2015; Thirkell *et al.*, 2016). The negative ψ_N^{-1} in AM under low N may also reflect increased tissue C : N ratios and N-use efficiency under eCO_2. Whether this is a plant strategy controlled by acclimation of photosynthesis or merely a consequence of insufficient N_{acq} is unclear. ECM species in this dataset could acquire additional N 'on demand' via increased C investments, which may be explained by the capacity of many ECM fungal species to produce extracellular enzymes that break down SOM and transfer organic and inorganic forms of N to the host plant (Lindahl & Tunlid, 2015; Shah *et al.*, 2015).

III. CO_2 response spectrum

Here we focus on the return on investment approach to summarize findings regarding the role of N_{acq} in shaping leaf-level photosynthesis (1), plant biomass production (2) and SOM decomposition (3) – all factors that influence ecosystem responses to eCO_2 and ecosystem feedbacks to climate change. This approach allows us to characterize systems within a response spectrum spanned by the return on investment.

1. eCO_2 effects on photosynthetic capacity

Background Theoretical considerations based on optimal use of resources predict a decrease in the maximum rate of carboxylation (V_{cmax}) under eCO_2 (Wang *et al.*, 2017). This prediction arises because the actual rate of assimilation under average field conditions is necessarily limited by available light, and because the response of light-limited assimilation to the leaf-internal partial pressure of CO_2 (c_i) is less steep than the response of V_{cmax}-limited assimilation. Therefore, if light availability and the ratio of c_i to ambient CO_2 partial pressure (c_a) are unchanged, an increase in c_a means that a lower V_{cmax} is required for the V_{cmax}-limited rate to match the light-limited rate. However, existing theories do not explicitly consider the costs of achieving and maintaining a given value of V_{cmax}, related to the cost of N_{acq} because Rubisco constitutes a substantial proportion of total foliar N (Spreitzer & Salvucci, 2002).

Question Is the role of N availability in constraining the eCO_2 effect on biomass caused by limitations on leaf-level photosynthesis?

Observations The downregulation of V_{cmax} by eCO_2 in nonfertilized soils is inversely related to ψ_N^{-1} (Fig. 2a, $P < 0.01$), suggesting

Table 1 List of Free-Air CO_2 enrichment (FACE) and open top chamber (OTC) sites analysed in this review, along with some site characteristics and sources for data used in Figs 1–4

Site	Location	Ecosystem, species	*n*	Symbiont	Root data	N_{acq}	V_{cmax}, A_{sat}	ANPP	Soil C
Aspen FACE	Rhinelander, WI, USA	Forest (deciduous): *Populus tremuloides* (A) & *Betula papyrifera* (B)	Low–medium	ECM	Talhelm *et al.* (2014)*	Talhelm *et al.* (2014)	Ellsworth *et al.* (2004); Darbah *et al.* (2010)	Talhelm *et al.* (2014)	Talhelm *et al.* (2014)
Duke FACE	Durham, NC, USA	Forest (conifer): *Pinus taeda*	Low	ECM	McCarthy *et al.* (2010); Drake *et al.* (2011); A. C. Finzi, pers. comm.*	Finzi *et al.* (2007); A. C. Finzi, pers. comm.	Ellsworth *et al.* (2012)	A. C. Finzi, pers. comm.	Lichter *et al.* (2008)
Florida OTC	Cape Canaveral, FL, USA	Forest (deciduous): *Quercus myrtifolia*, *Q. geminata* and *Q. chapmanii*	Low	ECM	Hungate *et al.* (2013); B. A. Hungate, pers. comm.*	Hungate *et al.* (2013); B. A. Hungate, pers. comm.	Li *et al.* (1999)	Hungate *et al.* (2013); B. A. Hungate, pers. comm.	van Groenigen *et al.* (2014)
Nevada FACE	Las Vegas, NV, USA	Desert scrub dominated by *Larrea tridentata* and *Ambrosia dumosa*	Low	AM	Ferguson & Nowak (2011)*	Housman *et al.* (2012); Smith *et al.* (2014)	Ainsworth & Long (2005)	Smith *et al.* (2014)	Evans *et al.* (2014)
ORNL FACE	Oak Ridge, TN, USA	Forest (deciduous): *Liquidambar styraciflua*	Low	AM	Norby *et al.* (2010); R. J. Norby, pers. comm.*	Norby *et al.* (2010); R. J. Norby, pers. comm.	Warren *et al.* (2015)	Norby *et al.* (2010); R. J. Norby, pers. comm.	Iversen *et al.* (2012)
PHACE	Cheyenne, WY, USA	Mixed-grass prairie	Low	AM	Mueller *et al.* (2016)****;	D. M. Blumenthal, pers. comm.	Blumenthal *et al.* (2013)	D. M. Bluementhal, pers. comm.	–
BioCON	Cedar Creek, MN, USA	Grassland dominated by C_3, C_4 grasses, legumes and forbs	Low (ambient) & medium (4)	AM, N-fixing	P. B. Reich, pers. comm.**	P. B. Reich, pers. comm.	Crous *et al.* (2010); Lee *et al.* (2011)	Reich & Hobbie (2013); P. B. Reich, pers. comm.	http://www.cedarcreek.umn.edu/research/data/dataset?ache141
NZ FACE	Bulls, Manawatu, New Zealand	Grassland dominated by legumes, C_3 and C_4 grasses	Low	N-fixing, AM	Allard *et al.* (2005)***	P. C. D. Newton, pers. comm.	von Caemmerer *et al.* (2001)	P. C. D. Newton, pers. comm.	Ross *et al.* (2013)
Swiss FACE	Eschikon, Switzerland	Ryegrass dominated by *Lolium perenne*	Medium (14) and high (56)	AM	Bazot *et al.* (2006)****	Schneider *et al.* (2004); M. K. Schneider, pers. comm.	Rogers *et al.* (1998)	Schneider *et al.* (2004)	van Kessel *et al.* (2006)
POP	Tuscany, Italy	Forest (deciduous): *Populus alba*, *P. nigra* & *P. euramericana*	High	ECM + AM	Finzi *et al.* (2007)*	Finzi *et al.* (2007)	Hovenden (2003)	Finzi *et al.* (2007)	Hoosbeek & Scarascia-Mugnozza (2009)
Jasper FACE	San Mateo, CA, USA	California grassland dominated by annual nonnative grasses	Low (ambient) and high (7)	AM	Zhu *et al.* (2016)***	B. A. Hungate, pers. comm.	-	Zhu *et al.* (2016)	B. A. Hungate, pers. comm.
GiFACE	Giessen, Germany	Grassland, including legumes (<1% initially)	Medium (4)	AM, N-fixing	Janze (2006)***	C. I. Kammann, pers. comm.	-	Andresen *et al.* (2017)	Lenhart *et al.* (2016)

The amount of N-fertilization applied is indicated in parentheses (units in g m^{-2} yr^{-1}).
ECM, ectomycorrhiza; AM, arbuscular mycorrhiza.
*Fine-root growth; **fine-root biomass; ***root growth; ****root biomass.

Table 2 List of major gaps in the framework outlined here concerning the interactions between the carbon (C) and nutrient cycles under elevated CO_2, and recommendations for experiments and methods to fill some of these gaps

Gap	Recommendations
Quantification of the C cost of N-acquisition under eCO_2	Improve the quantification of the plant C investment (in response to eCO_2) in N-acquisition (C_{bg}) by systematically measuring fine-root production and estimating fine-root transfers to exudation and microbial symbionts. Mycorrhizal growth can be used as a proxy for $C_{transfer}$ to mycorrhizas Extend the quantification and report of measurements of plant total N-acquisition Quantification of N derived from N_2-fixation eCO_2 experiments with ericoid mycorrhizal plants eCO_2 experiments with AM and ECM trees in the same site Quantification of the bottom range of N availability for ECM-mutualistic N-acquisition
Quantification of the C cost of P acquisition under eCO_2	eCO_2 experiments in tropical forests are highly needed Study the role of AM and ECM fungi as above but under P-limitations
Quantification of soil C storage under eCO_2	Quantification of changes in soil C pools Quantification of autotrophic and heterotrophic soil respiration Analysis of C stabilization pathways for litters with different C : N ratio
Methodological bias in eCO_2 experiments	Mesocosm experiments are excellent tools to quantify allocation to exudates and symbionts Field experiments should make use of natural and undisturbed soils Quantification of soil parameters pH, %N, %C, P% and other nutrients to assess nutrient availability Minimize the effect of expanding canopies, prioritising mature plants in steady-state Minimum of 5–10 yr of eCO_2 fumigation to allow soil dynamics start developing

C, carbon; N, nitrogen; C_{bg}, belowground carbon; eCO_2, elevated CO_2; P, phosphorus; AM, arbuscular mycorrhiza; ECM, ectomycorrhiza.

that the decline of V_{cmax} under eCO_2 is generally less pronounced in plants that can acquire N more efficiently. This is consistent with meta-analyses that suggest that downregulation is related to low N supply, with a stronger V_{cmax} decline under low N (−22%, Ainsworth & Long, 2005) than under high N (−12%, Ainsworth & Long, 2005), and a stronger reduction in grasses (AM, −17%, Ainsworth & Long, 2005) than in trees (most of which were ECM, −6%, Ainsworth & Long, 2005) and legumes (N_2-fixers, −12%, Ainsworth & Long, 2005) (Nowak *et al.*, 2004; Ainsworth & Long, 2005; Ainsworth & Rogers, 2007).

Despite downregulation of V_{cmax}, a stimulating effect of eCO_2 on leaf-level photosynthesis (A_{sat}) in C_3 plants is observed (Fig. 2b), with an overall stimulation of 35%, similar to the 31% effect from the meta-analysis by Ainsworth & Long (2005). Following the same pattern as for V_{cmax}, the eCO_2 effect on A_{sat} is generally larger in ECM than in AM plants (Fig. 2b). For example, in the Duke FACE experiment, downregulation of V_{cmax} was not significant, and eCO_2 increased A_{sat} in pine (ECM) by an average of 67% despite moderately low soil fertility (Ellsworth *et al.*, 2012). At the AM-forest FACE experiment in Oak Ridge (ORNL), eCO_2 reduced foliar N (due to low N availability), and resulted in a 21% stimulation of A_{sat} (Warren *et al.*, 2015) (although with small sample sizes and only occasional measurements rendered this effect nonsignificant).

The effect of eCO_2 on A_{sat} in legumes (Ainsworth & Long, 2005; Wang *et al.*, 2012) and N-fertilized plants, however, was not higher than in AM nonfertilized plants (Fig. 2b), contrary to our expectation. For example, at the Swiss and BioCON FACE experiments, AM-associated grassland species growing under eCO_2 had eCO_2 effects on A_{sat} of similar magnitude for both low and high N treatments (Rogers *et al.*, 1998; Lee *et al.*, 2011). We speculate that A_{sat} did not increase with N-fertilization at BioCON because the downward shift in leaf %N with eCO_2 was larger in the N-fertilized than in the ambient treatments (−14% vs −9%) (Lee *et al.*, 2011), perhaps because N-fertilization was modest and plants under eCO_2 and high N increased growth (and thus demand) and remained both C and N limited (Reich & Hobbie, 2013). The lower effect on A_{sat} in legumes than in grasses (Fig. 2b, Ainsworth & Long, 2005) could have resulted from light limitation for legumes in dense canopy conditions or limitations from other soil resources beyond N; further research will be required to elucidate the mechanisms.

The ecosystem-level effect on photosynthesis (gross primary productivity, GPP) requires scaling the leaf-level response taking into account leaf area index (LAI). If eCO_2 decreases LAI, GPP might not increase despite a positive leaf-level effect. Negative effects of eCO_2 on LAI are not common. Rather, a meta-analysis showed that eCO_2 enhanced LAI by 21% in trees, with no significant effect in grasslands (Ainsworth & Long, 2005). Norby & Zak (2011) suggested that only trees with low LAI ($< 3.5\ m^2$ leaf m^{-2} ground) could increase LAI further in response to eCO_2, although this effect might disappear when nutrient availability is low (Duursma *et al.*, 2016).

Another important factor to consider is the temporal acclimation of the photosynthetic response to eCO_2. Stomatal density has been shown to decrease with historical CO_2 concentrations (Peñuelas & Matamala, 1990; Franks *et al.*, 2013), but a meta-analysis of eCO_2 experiments did not find a significantly negative effect for an average $[CO_2]$ of 571 ppm (Ainsworth & Rogers, 2007). Furthermore, a meta-analysis found that eCO_2 increased the number of leaves (Ainsworth & Long, 2005), an effect that might compensate for any potential reduction on stomatal density at the ecosystem level. The experiments shown in Fig. 2(b) did not generally find a decreasing A_{sat} response over time, but the long-term acclimation to eCO_2 requires further investigation (Franks *et al.*, 2013).

Conclusions Although the influence of N on the eCO_2 effect on V_{cmax} has been long known, it has commonly been linked to plant functional groups rather than to actual N_{acq}-strategies (e.g. Ainsworth & Long, 2005). We have shown that the strength of the V_{cmax} decline under eCO_2 changes with the efficiency of plants in acquiring extra N (ψ_N^{-1}), with the strongest decline under low N in AM systems where N-acquisition costs might increase most strongly. This affects leaf-level photosynthesis, with a smaller effect of eCO_2 in AM than in ECM plants. However, the role of N-fertilization and N_2-fixation on the eCO_2 effect on A_{sat} needs

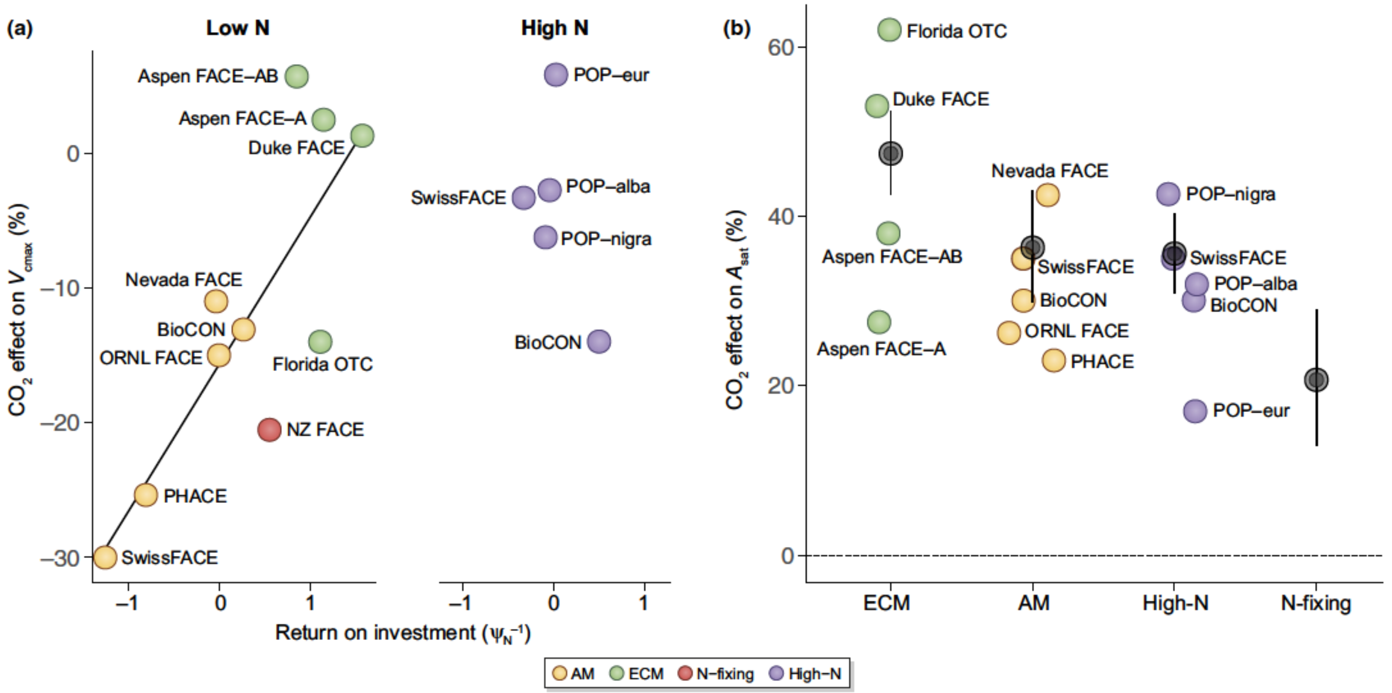


Fig. 2 Effects of elevated CO_2 on leaf-level photosynthesis and its modulation by nitrogen (N)-acquisition efficiency. (a) Relationship between the effect of elevated CO_2 on maximum rate of carboxylation (V_{cmax}) and the N return on investment (ψ_N^{-1}, Eqn 1) under low (left panel) and high (right panel) N availability. (b) Summary of the effect of elevated CO_2 on light saturated photosynthesis (A_{sat}). The black dots in (b) are mean effects ± CI from a meta-analysis by Ainsworth & Long (2005) for trees, grasses, N-fertilized plants and legumes. Sources of site-level data are given in Table 1.

further investigation. In any case, despite partial downregulation of V_{cmax}, N availability does not usually preclude an effect of eCO_2 on A_{sat}. Hence, the lack of a significant eCO_2 effect on plant biomass in AM communities under low N (Terrer *et al.*, 2016) cannot be fully explained by downregulation of leaf-level photosynthesis; changes in C allocation are hence crucial for understanding these responses.

2. CO_2 effects on biomass production

Background When N availability is low, a positive growth enhancement effect of eCO_2 depends on a plant's ability to (1) increase its rate of N_{acq} from the soil (Oren *et al.*, 2001; Finzi *et al.*, 2007), and/or (2) use the assimilated N more efficiently. The N-use efficiency (NUE) of growth can be defined as biomass produced per unit of N_{acq}, and is reflected in the overall plant C : N stoichiometry and retranslocation efficiency of N upon leaf shedding. Zaehle *et al.* (2014) found that models' predicted enhancement of productivity under eCO_2 is commonly associated with an increase in NUE, in conflict with the conclusions from observational studies that found the effect driven by increased N_{acq} (Finzi *et al.*, 2007; Feng *et al.*, 2015).

Question What are the mechanisms that drive the differences among sites in the magnitude of the CO_2 fertilization effect on biomass production?

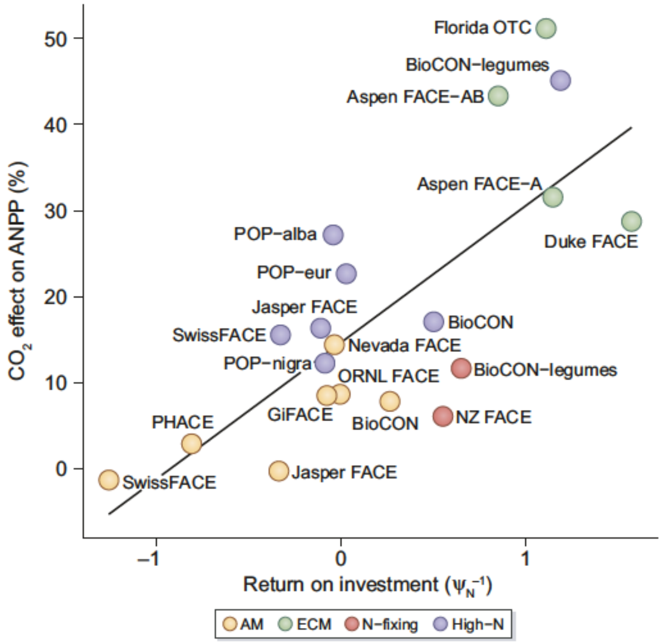


Fig. 3 Relationship between the effect of elevated CO_2 on aboveground biomass production (ANPP) and the nitrogen (N) return on investment (ψ_N^{-1}, Eqn 1). Sources of site-level data are given in Table 1.

Observations We found a significantly positive relationship between ψ_N^{-1} and the eCO_2 effect on aboveground biomass productivity (ANPP) (Fig. 3, $P < 0.001$), resulting in the largest eCO_2-driven ANPP enhancement in ECM > N-fertilized > N_2-fixing > AM strategies. This suggests that N_{acq}-efficiency is a primary driver of the eCO_2 effect on productivity. Note that although the change in biomass is part of the ψ_N^{-1} calculation, increased C investment belowground reduces ψ_N^{-1}; thus, the positive relationship in Fig. 3 is not necessarily an artefact of using C_{root} in both (see also Feng *et al.*, 2015).

ECM plants consistently showed the largest increases in ANPP, and this was associated with the highest ψ_N^{-1} (Fig. 3). For example, FACE experiments with ECM-associated loblolly pine (Duke FACE) and aspen (Aspen FACE) trees showed a large (22–39%) and sustained effect on total biomass productivity despite moderate–low N availability (McCarthy *et al.*, 2010; Talhelm *et al.*, 2014). Furthermore, N-fertilization in the Duke FACE experiment did not increase productivity further (McCarthy *et al.*, 2010), consistent with the observation of increased aboveground growth in most AM trees in response to N-deposition, but not in ECM trees (Thomas *et al.*, 2010). Efficient N_{acq} stimulated trees at the Duke and Aspen FACE experiments to increasingly allocate more C to wood (with low [N]), enhancing NUE (Zaehle *et al.*, 2014) as a consequence of this biomass allocation shift.

Under high N availability, *Populus alba*, *P. euramericana* and *P. nigra* in the POP-FACE experiment in Italy, dominated by both ECM and AM fungi, showed a lower ψ_N^{-1} than other ECM species (Figs 1, 3) due to the lack of an eCO_2-driven N_{acq} enhancement; N_{acq} was already high in both CO_2 treatment plots due to previous agricultural use and irrigation (Liberloo *et al.*, 2006). Instead, trees at POP-FACE sustained the eCO_2 fertilization effect by increasing NUE (Finzi *et al.*, 2007), which was likely influenced by increased allocation to wood (low [N]).

AM systems showed a wider range of responses, presumably driven by their variable capacity to acquire N, either through N-fertilization or association with N_2-fixers. For example, AM-grassland *Lolium perenne* at SwissFACE showed a positive CO_2-induced aboveground biomass enhancement under high N, but not in low N plots (Schneider *et al.*, 2004), consistent with the lower cost of N_{acq} associated with N-fertilization (Fig. 3). *Medicago sativa* in this same experiment, however, showed a positive effect on ANPP and N_{acq} even under low N, consistent with its N_2-fixing capacity (Lüscher *et al.*, 2000) (data not included in Fig. 3 because no indication of C_{bg} was found). Likewise at BioCON, the eCO_2-enhancement in productivity was larger in N_2-fixing legumes than in nonlegume AM species (Fig. 3; see Mueller *et al.*, 2013).

AM trees at ORNL FACE apparently showed the opposite pattern to that of Aspen and Duke FACE ECM trees. As AM fungi may have little effect on plant N_{acq}, we speculate that these trees relied primarily on increased allocation to fine roots (with high [N]) to explore a larger proportion of the soil (Norby *et al.*, 2010; Iversen *et al.*, 2012), thus allocating less C to wood and decreasing NUE. Because this strategy caused only a slight, initial stimulation of total N_{acq}, and because NUE was already high from the start (Finzi *et al.*, 2007), the trees at the ORNL site could not meet the higher N demand imposed by higher CO_2 supply – thus limiting the stand's capacity to increase ANPP (Fig. 3). Interestingly, the authors reported an increasing abundance of the N_2-fixer *Elaeagnus umbellata* by the end of the experiment, with evidence for N_2-fixation (Norby & Zak, 2011).

Although N return on investment is a primary factor determining the ANPP response to eCO_2, nutrients other than N, as well as water, are required for plant growth and may increase variability in Fig. 3. For example, the ANPP response of AM species in the Nevada Desert FACE from 1998 to 2007 (Fig. 3) showed pronounced interannual variation because growth was limited by water availability, with stronger increases in ANPP under eCO_2 in wet than dry years (Housman *et al.*, 2006; Smith *et al.*, 2014; see also Fatichi *et al.*, 2016). However, these periodic increases in productivity did not result in increased above- or belowground biomass at the end of the experiment (Newingham *et al.*, 2013). Opposite responses have been found for other grassland experiments in dry regions, with greater biomass responses to eCO_2 in dry than wet years (Morgan *et al.*, 2004, 2011). Results from the TasFACE experiment, however, suggest that these contrasting results might have been driven by the effects of seasonal precipitation on the N cycle (Hovenden *et al.*, 2014), with spring rainfall causing negative effects on N availability, thus limiting the eCO_2-response.

The eCO_2 effect on plant growth and its relationship with symbiotic type may also be prone to environmental factors other than N, including P availability, climatic conditions and disturbance. The role of symbiotic types in acquiring P under eCO_2 is uncertain, as only few experiments have been conducted in low-P conditions. For example, ECM-dominated *Eucalyptus* trees in a water- and P-limited soil showed a positive leaf-level photosynthesis response to eCO_2, but no increase in aboveground growth (Ellsworth *et al.*, 2017) despite enhanced P and N availability (Hasegawa *et al.*, 2016; Ochoa-Hueso *et al.*, 2017). More research is needed to investigate whether AM plants may acquire P more efficiently and show a stronger eCO_2 response than ECM plants under low-P. An indication of the influence of weather and disturbance may be provided by the scrub-oak OTC experiment in Florida, which showed the largest increase in ANPP (Fig. 2). There, N_{acq} in the ECM species may have been additionally stimulated by disturbance, initially by fire and later by a hurricane, both associated by a pulse of belowground resource availability (Hungate *et al.*, 2013).

Although ECM ecosystems typically showed a strong eCO_2 response of ANPP and a high ψ_N^{-1}, this pattern may not persist under extremely N-scarce conditions. For example, a Norway spruce in Sweden on moraine soil and with a very thin soil organic layer did not show a significant eCO_2-effect on aboveground growth except when N-fertilized (Sigurdsson *et al.*, 2013). Following the mutualism–parasitism continuum hypothesis (Johnson *et al.*, 1997), and as suggested by some models for boreal N-poor forests (Franklin *et al.*, 2014; Baskaran *et al.*, 2017), there may be a point at the lower range of N availability below which ECM fungi do not transfer enough N to the plant to elicit and sustain higher rates of eCO_2-growth.

Conclusion Although several factors likely modulate growth responses to eCO_2, N return on investment is a primary control explaining the variety of responses observed in eCO_2 experiments. Under low N availability, a sustained CO_2 effect requires a mechanism by which plants can increase N_{acq}, via association with ECM fungi or N_2-fixers. AM plants generally do not increase N_{acq} under eCO_2 (Fig. 1), so increases in productivity (Fig. 3), if any, are sustained through increased NUE. In soils with high N availability where N_{acq} is already high, plants may sustain enhanced growth rates through increased NUE too. But changes in NUE also respond to shifts in competition strategies, with greater allocation to leaves (high [N]) during stand development, and greater allocation to wood (low [N]) after canopy closure, leading to increased NUE as trees age (Gholz *et al.*, 1985). Therefore, there is generally limited scope for enhanced NUE as a strategy to sustain increased demand under eCO_2 in the long-term, which rather seems to be a consequence of changes in allocation to the different plant biomass pools. If enhanced root exploration or symbiotic uptake do not result in efficient N_{acq}, the CO_2 effect disappears when available N in the rhizosphere does not meet plant N demand.

3. eCO_2 effects on priming and soil C content

Background In previous sections, we discussed the capacity of ECM and N_2-fixing plants to acquire additional N under eCO_2, which feeds back on plant productivity. Both N-acquisition through SOM decomposition (outputs) and productivity (inputs) affect soil C storage. Meta-analyses show that, indeed, eCO_2 increases belowground C inputs through enhanced fine-root production by 44% (Dieleman *et al.*, 2010) and rhizodeposition by 37.9% (Nie *et al.*, 2013). Although greater inputs of root-derived C may increase soil C storage, much of the C that is released to the soil can also stimulate microbes to accelerate SOM decay and N release via 'priming effects' (Cheng *et al.*, 2014; Finzi *et al.*, 2015). Indeed, meta-analyses have shown that increases in soil C inputs under eCO_2 are offset by loses (Hungate *et al.*, 2009; van Groenigen *et al.*, 2014). These studies, however, did not account for potential differential effects among symbiotic types. The quantification of priming effects has important implications on the magnitude of the terrestrial CO_2 sink, but these effects are difficult to measure and model (Georgiou *et al.*, 2015).

Question How do changes in N availability under eCO_2 affect soil C storage?

Observations We found a pattern of changes in soil C storage across N-acquisition strategies, with eCO_2 generally stimulating soil C losses in ECM, and soil C storage in AM systems under low N availability. The marginally significant relationship between soil C storage and ψ_N^{-1} (Fig. 4; $P = 0.0503$), however, highlights that other factors beyond ψ_N^{-1} are at play.

Enhanced N-mining activity in ECM under eCO_2 involves CO_2 release through heterotrophic respiration, minimizing net accumulation of soil C with eCO_2 (Fig. 4). For example, the large CO_2 fertilization effect on ANPP in Duke FACE (ECM) (McCarthy *et al.*, 2010) was likely driven by increased allocation to ECM fungi (Drake *et al.*, 2011) and root exudation (Phillips *et al.*, 2011), which stimulated microbial activity and SOM decomposition (priming), increasing N availability to plants (see also Cheng *et al.*, 2014). This, however, was accompanied by increased soil respiration (Oishi *et al.*, 2014), reducing soil C content (Fig. 4). In the *Populus tremuloides* (ECM) community from the Aspen FACE experiment, eCO_2 increased litter inputs, but also decreased soil C content (Fig. 4), suggesting strong stimulation in SOM decomposition (Talhelm *et al.*, 2014). Likewise in the Florida OTC experiment, eCO_2 increased plant productivity of scrub oaks (ECM) under low N availability (Fig. 3) through enhanced N-mineralization (Langley *et al.*, 2009), but the stimulation of SOM decomposition yielded no effect on C storage at the ecosystem level (Hungate *et al.*, 2013).

By contrast, several AM-ecosystems under low N have shown limited eCO_2-effects on N-mineralization and plant productivity, together with significant increases in soil C content. For example, the lack of a significant eCO_2 effect on biomass after 10 yr in the Nevada Desert FACE (AM) (Newingham *et al.*, 2013) was accompanied by a significantly positive effect on soil C content (Evans *et al.*, 2014), with increased fungal activity (Jin & Evans, 2010), but not fine-root inputs (Ferguson & Nowak, 2011) – suggesting $C_{transfer}$ as the main driver of this effect (Jin & Evans, 2010). The same pattern of smaller than average biomass responses but soil C accumulation was observed, for example, in an AM-forest ecosystem at ORNL (Iversen *et al.*, 2012), an AM-grassland ecosystem in Australia (Pendall *et al.*, 2011) and a shortgrass steppe in the US (Pendall & King, 2007), accompanied by a doubling in rhizodeposition (Pendall *et al.*, 2004).

Other AM ecosystems, however, do not follow this pattern. In the SwissFACE experiment, neither the AM grass *Lolium perenne* nor the N_2-fixer *Trifolium repens* showed an increase in soil C storage after 10 yr of eCO_2 (van Kessel *et al.*, 2006), despite a positive effect on photosynthesis (Ainsworth *et al.*, 2003) and a lack of N-mineralization and ANPP response under low N availability (Schneider *et al.*, 2004). eCO_2 did not increase soil C content at GiFACE either (Lenhart *et al.*, 2016), but the presence of legumes may have contributed to an increase in the allocation of $C_{transfer}$ to N_2-fixation, rather than soil C stabilization, which would explain the strong increase in abundance of legume species from *c.* 1% at the beginning of the experiment to 10% in later years, together with an increasingly positive overall effect on plant biomass (Andresen *et al.*, 2017). A certain degree of CO_2-driven enhancement of

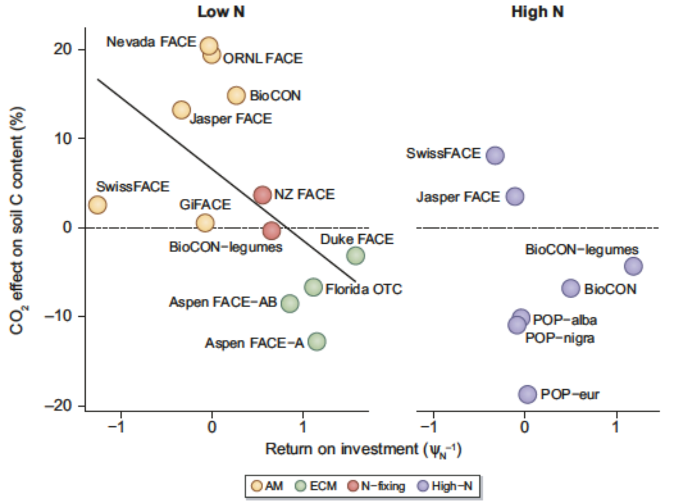


Fig. 4 Relationship between the effect of elevated CO_2 on soil carbon (C) content (%) and the nitrogen (N) return on investment (ψ_N^{-1}, Eqn 1). Sources of site-level data are given in Table 1.

N-mineralization in grasslands might also follow from increased soil water (e.g. Pendall *et al.*, 2003).

Although there have been reports of AM plants accelerating litter decomposition under eCO_2 (Cheng *et al.*, 2012), there is little evidence that AM plants can increase the decay of SOM under eCO_2, particularly in low N soils. Thus, CO_2-induced priming effects in AM systems are likely to be more short-lived relative to those occurring in ECM-dominated ecosystems (Sulman *et al.*, 2017).

An intermediate situation might be found for N_2-fixers (Fig. 4), which can obtain (additional) N from the atmosphere. eCO_2 generally increases growth in legumes (Fig. 3; Ainsworth & Long, 2005), and thus likely also enhances soil C inputs, but whether SOM decomposition offsets additional inputs is uncertain. For example, eCO_2 increased C inputs through biomass and productivity (Fig. 3) in a grassland FACE experiment with N_2-fixers in New Zealand. But eCO_2 also increased N-mineralization (Rütting *et al.*, 2010) and N availability (Newton *et al.*, 2010), yielding a modest increase in soil C storage (Ross *et al.*, 2013; Fig. 4). Various factors are probably at play to determine the balance between inputs and outputs, including species composition, litter quality, climate and nutrient and water availability.

The eCO_2 effects on soil C under high N availability do not appear to follow a clear pattern in this dataset (Fig. 4). Meta-analyses show that N-fertilization may increase the positive effects of eCO_2 on soil respiration further (Zhou *et al.*, 2016), but the effect of N has been shown to be negative in trees (Janssens *et al.*, 2010), and positive in grasslands and croplands (Zhou *et al.*, 2014). Whether this variability indicate different effects of N-fertilization among N-acquisition strategies or plant functional types remains to be disentangled.

These differences in the sign and magnitude of the effects of eCO_2 on N-mineralization, priming and soil C storage across symbiotic types might explain the large variability and non-significance of these effects found in several meta-analyses (de Graaff *et al.*, 2006; Hungate *et al.*, 2009; van Groenigen *et al.*, 2014). The reasons for these different patterns among symbiotic types, however, remain elusive. Recent empirical observations and model analyses suggest that labile litter (low C : N) is quickly assimilated by microbes, and this microbial necromass contributes to the formation of stable SOM in greater proportion than recalcitrant litter (high C : N), which decomposes slowly (Knicker, 2011; Castellano *et al.*, 2015; Cotrufo *et al.*, 2015). On the other hand, the stabilization of labile litter in SOM should protect plant material, constraining the eCO_2-driven priming effect (Sulman *et al.*, 2014, 2017). Thus, recalcitrant litter should be more easily primed provided that it is 'unprotected'. A recent meta-analysis showed that, overall, AM trees produce litter that is significantly more labile than ECM trees (Lin *et al.*, 2017). Therefore, AM litter may be more easily stabilized by microbes, protecting new C from priming, whereas recalcitrant ECM litter may be more susceptible to priming, stimulating N-mineralization and N availability. This would explain the limited CO_2-driven priming observed in some AM experiments, together with increased soil C content in AM-low N systems.

Conclusions Evidence from eCO_2 experiments suggests that mycorrhizal status plays a key role in determining the sign of the eCO_2 effect on soil C storage. Under low N availability, some AM- and ECM-dominated ecosystems show opposite patterns. In some AM-dominated ecosystems, eCO_2-driven priming is more limited than in ECM-dominated ecosystems, which results in lower C losses in the former. By contrast, many ECM systems show strong priming effect and N-acquisition in response to eCO_2. This mechanism, however, enhances SOM decomposition and may thus partially offset the increase in biomass storage and limit CO_2 sequestration at the ecosystem level. The result is a C-allocation shift in AM vs ECM ecosystems, which may result in enhanced soil-C gains in AM and enhanced biomass-C gains in ECM. It is, however, the final balance between the (changes in) C inputs and outputs that eventually determines whether soil C storage increases, decreases or remains unaltered.

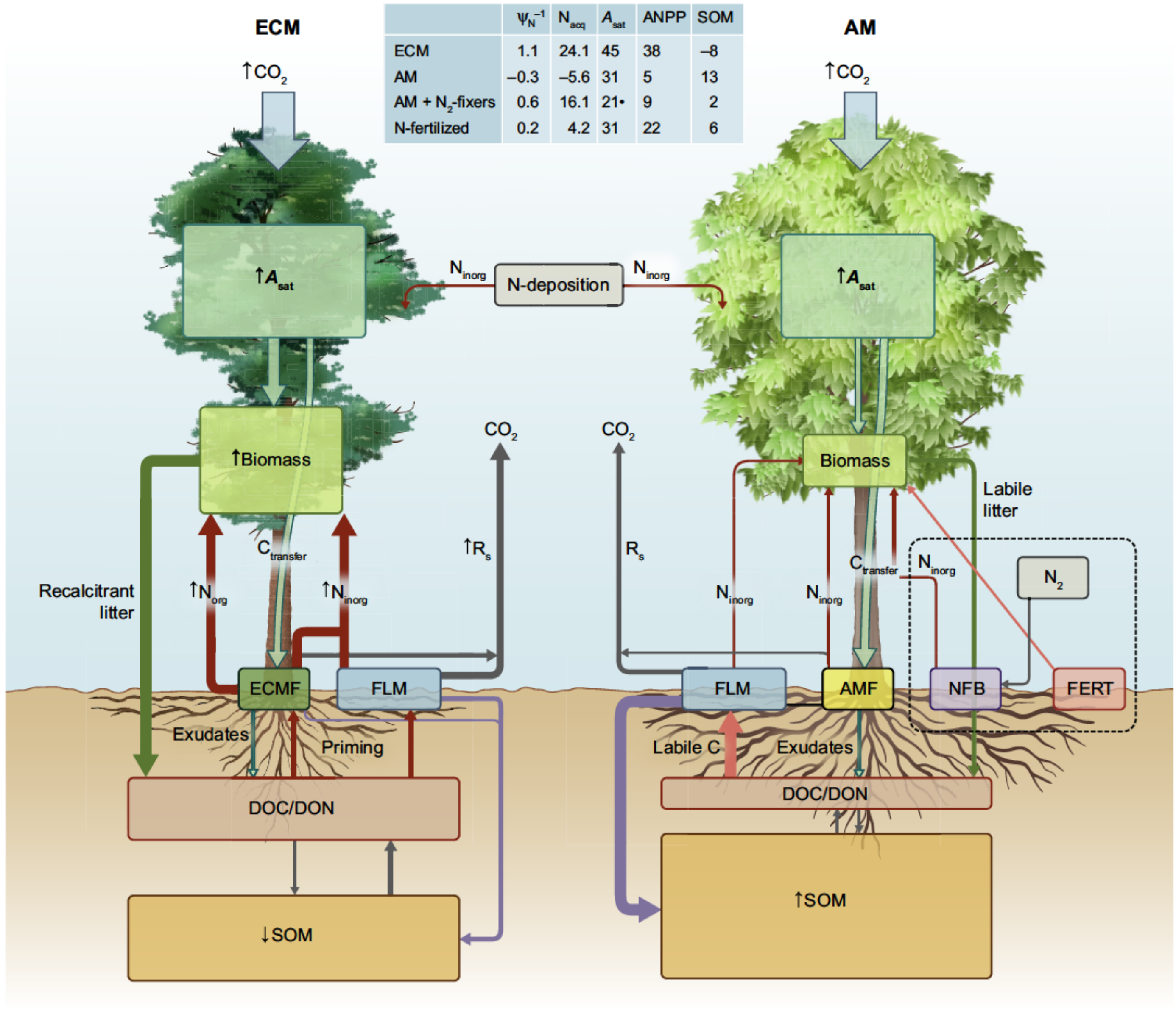
IV. Discussion

We used a plant economics approach to quantify the C cost of N-acquisition and explore how this relates to the eCO_2-response in different measured variables. Under eCO_2, plants in nutrient-limited ecosystems may allocate part of the additional assimilation permitted by eCO_2 in ways that increase N_{acq}: (1) allocation to fine roots (Iversen, 2010), (2) allocation to mycorrhizal fungi (Drake *et al.*, 2011) and (3) allocation to root exudates to increase soil priming (Phillips *et al.*, 2012). Therefore, N_{acq} is a process that requires C resources that could otherwise be allocated to growth. Given the diversity of N_{acq} strategies of investigated plants, soil conditions, and N-fertilization treatments, we expected different costs associated with N_{acq} in plants exposed to eCO_2. These costs might help explain discrepant responses in processes that require or are affected by N, such as leaf-level photosynthetic capacity, plant-level growth and soil C storage, and place different systems within a continuous spectrum of ecosystem responses to eCO_2.

We show that the type of plant mycorrhizal association and N-fixing capability determines their position within this spectrum. ECM plants can acquire N more efficiently than AM plants under eCO_2, although N_{acq} by AM plants can be enhanced when grown with N_2-fixing plants or when N-fertilized. This efficiency in N_{acq} partly explains the magnitude of the eCO_2 effects on leaf-level photosynthesis, aboveground productivity and soil C storage. eCO_2 generally increases the amount of assimilates that plants produce per unit leaf area, even in plants with high costs associated with N_{acq}. However, the eCO_2 stimulation of aboveground growth tends to be smaller when the cost of N_{acq} is high, and vice versa. Contrarily to aboveground growth responses, the eCO_2 effect on soil C storage tends to decrease with decreasing costs.

Elevated CO_2 generally increases leaf-level photosynthesis regardless of N_{acq}-costs, but the cost of N_{acq} strongly affects the C allocation patterns. When costs are low (ECM in Fig. 5), plants can efficiently acquire N and sustain a growth response, which, on the other hand, can reduce SOM. We hypothesized that plants that associate with ECM fungi acquire N more efficiently than those with AM for two reasons: (1) many ECM fungi have the enzymes necessary to mine organic N (Shah *et al.*, 2015), and (2) litter produced by ECM plants has a high C : N ratio (Lin *et al.*, 2017) that promotes slow decomposition (Cotrufo *et al.*, 2015) and facilitates priming (Sulman *et al.*, 2014, 2017). A similar effect can be achieved by AM plants when N availability is high or in the presence of N_2-fixers (Fig. 5). The effects of eCO_2 on litter production, root exudation and allocation to ECM, as well as potentially increasing litter C : N ratios, may amplify these effects.

When costs are high (AM in Fig. 5), a positive growth response to eCO_2 cannot be sustained as a consequence of insufficient N uptake. This is because (1) AM fungi do not produce the enzymes required to increase priming in response to eCO_2 (Hodge & Storer, 2015), and (2) litter produced by AM plants has a lower C : N ratio (Lin *et al.*, 2017), promoting



	ψ_N^{-1}	N_{acq}	A_{sat}	ANPP	SOM
ECM	1.1	24.1	45	38	–8
AM	–0.3	–5.6	31	5	13
AM + N_2-fixers	0.6	16.1	21•	9	2
N-fertilized	0.2	4.2	31	22	6

Fig. 5 Conceptual framework, representing the effects of elevated CO_2 under low nitrogen (N)-acquisition costs in ectomycorrhizal (ECM) systems (left) and high costs in arbuscular mycorrhizal (AM) systems (right). The area within dashed lines represents plant N-acquisition through N_2-fixation and external N-fertilization. N inputs through N_2-fixation and N-fertilization are relevant in ECM systems as well, but not drawn here. Tabulated values represent the inverse of the carbon (C) cost of N-acquisition (ψ_N^{-1}, Eqn 1) and mean CO_2-effects (%) on N-acquisition (N_{acq}), leaf-level photosynthesis (A_{sat}), aboveground biomass production (ANPP) and soil organic matter (SOM) for ECM, AM, AM with N_2-fixing capacity and N-fertilized systems derived from Figs 2–4. The CO_2 effect on A_{sat} for AM + N_2-fixers corresponds to the value reported in the meta-analysis by Ainsworth & Long (2005) for legumes. $C_{transfer}$, C exported to mycorrhizas, root exudation and symbiotic N_2-fixation; ECMF, ectomycorrhizal fungi; AMF, arbuscular mycorrhizal fungi; FLM, free-living microbes; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; R_s, soil respiration; N_2, atmospheric N; NFB, N_2-fixing bacteria; FERT, N-fertilization. Differences in box-size between AM and ECM systems represent differentiated changes in pool or flux size by elevated CO_2, and arrows inside boxes represent the sign of the CO_2 effect.

greater stabilization of SOM (Sulman *et al.*, 2014, 2017). Thereby, AM plants have limited ability to prime the labile SOM that they live on. If soil C inputs into the soil are higher than C losses, however, eCO_2 may result in an increase in soil C storage. These allocation patterns of eCO_2-driven extra C in AM and ECM plants result is a spectrum of ecosystem responses to eCO_2, driven primarily by the cost of N_{acq}.

It has been observed in several studies that an eCO_2-driven increase in photosynthesis did not translate into an increase in plant biomass production (Bader *et al.*, 2013; Newingham *et al.*, 2013; Sigurdsson *et al.*, 2013; Ellsworth *et al.*, 2017). This has raised the question: 'Where does the carbon go?' Potential candidates are autotrophic respiration (R_a) and $C_{transfer}$. The majority of experiments do not show a positive effect of eCO_2 on R_a (Smith, 2017), and there is no evidence that the R_a : GPP ratio consistently increases under eCO_2 (van Oijen *et al.*, 2010; Smith & Dukes, 2013). This implies that any increase in GPP without an increase in biomass production most likely increases the proportion of GPP allocated to $C_{transfer}$ ($GPP = BP + C_{transfer} + R_a$). Indeed, root exudation and mycorrhizal abundance have been observed to increase under eCO_2 (Treseder, 2004; Alberton *et al.*, 2005; Phillips *et al.*, 2011; Nie *et al.*, 2013), pointing at $C_{transfer}$ as an important flux of the 'missing' C.

A large part of the framework outlined here (see Fig. 5) is not represented in the current generation of Dynamic Global Vegetation Models (Sitch *et al.*, 2015). Although these models may produce eCO_2-induced increases in growth that are consistent in magnitude with observations (but see De Kauwe *et al.*, 2017), the importance of underlying mechanisms governing N constraints are inappropriately represented (Zaehle *et al.*, 2014). Common to most modelling approaches is to account for the limiting effects of N by reducing the ratio of NPP to GPP, hence increasing R_a, and to increase the C : N ratio of new tissue production to match the plant C and N budgets under *a priori* defined stoichiometric constraints (Zaehle *et al.*, 2014; Thomas *et al.*, 2015). Models do not generally consider $C_{transfer}$ as a separate component of the plant C budget (Medlyn *et al.*, 2015), and 'spill-over' R_a has no effects on modelled N_{acq}. Furthermore, little or no adjustment of above- vs belowground C allocation is simulated in response to shifts in the availability of above- and belowground resources (De Kauwe *et al.*, 2014; Zaehle *et al.*, 2014). Indeed, Zaehle *et al.* (2014) found that the eCO_2-induced increase in simulated N_{acq} was strongly underestimated in the Duke FACE experiment.

In order to better represent the effects of eCO_2 discussed here, a next generation of models for the coupled C and nutrient cycles in land ecosystems should be centred around nutrient cost considerations to simulate flexible C allocation in response to changing above- and belowground resource availabilities. Key mechanisms that determine these relationships are the capacity for BNF, mycorrhizal type-specific plant–soil interactions, rhizosphere $C_{transfer}$ and its effects on SOM decomposition rates. In Table 2 we suggest some examples of types of observational data required to further explore some of the gaps detected here.

Our results suggest that the N-limitation on ecosystem responses to eCO_2 are most likely displayed in a continuum, in which the ability of the plants to acquire additional N in exchange for energy (carbon) plays a key role. Many ecosystems with ECM-associated plants and N_2-fixers have the capacity to enhance N_{acq} under increasing demand, highlighting the importance of plant-mediated control on N availability, as opposed to the traditional view of a rigid N-limitation. Due to the limited temporal coverage of available experiments, the persistence of enhanced plant growth rates under eCO_2 remains uncertain. Our findings underline the importance of the cost of N-acquisition, an avenue that if explored by experimentalists and modellers working together may provide a way forward to better understand the interactions between the C and N cycles under rising CO_2.

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References

Adair EC, Reich PB, Hobbie SE, Knops JMH. 2009. Interactive effects of time, CO_2, N, and diversity on total belowground carbon allocation and ecosystem carbon storage in a grassland community. *Ecosystems* **12**: 1037–1052.

Ainsworth EA, Davey PA, Hymus GJ, Osborne CP, Rogers A, Blum H, Nösberger J, Long SP. 2003. Is stimulation of leaf photosynthesis by elevated carbon dioxide concentration maintained in the long term? A test with *Lolium perenne* grown for 10 years at two nitrogen fertilization levels under Free Air CO_2 Enrichment (FACE). *Plant, Cell & Environment* **26**: 705–714.

Ainsworth EA, Long SP. 2005. What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. *New Phytologist* **165**: 351–372.

Ainsworth EA, Rogers A. 2007. The response of photosynthesis and stomatal conductance to rising $[CO_2]$: mechanisms and environmental interactions. *Plant, Cell & Environment* **30**: 258–270.

Alberton O, Kuyper TW, Gorissen A. 2005. Taking mycocentrism seriously: mycorrhizal fungal and plant responses to elevated CO_2. *New Phytologist* **167**: 859–868.

Allard V, Newton PCD, Lieffering M, Soussana JF, Carran RA, Matthew C. 2005. Increased quantity and quality of coarse soil organic matter fraction at elevated

CO_2 in a grazed grassland are a consequence of enhanced root growth rate and turnover. *Plant and Soil* **276**: 49–60.

Andresen LC, Yuan N, Seibert R, Moser G, Kammann CI, Luterbacher J, Erbs M, Müller C. 2017. Biomass responses in a temperate European grassland through 17 years of elevated CO_2. *Global Change Biology*. doi: 10.1111/gcb.13705.

Aoki M, Fujii K, Kitayama K. 2012. Environmental control of root exudation of low-molecular weight organic acids in tropical rainforests. *Ecosystems* **15**: 1194–1203.

Bader MKF, Leuzinger S, Keel SG, Siegwolf RTW, Hagedorn F, Schleppi P, Körner C. 2013. Central European hardwood trees in a high-CO_2 future: synthesis of an 8-year forest canopy CO_2 enrichment project. *Journal of Ecology* **101**: 1509–1519.

Baskaran P, Hyvönen R, Berglund SL, Clemmensen KE, Ågren GI, Lindahl BD, Manzoni S. 2017. Modelling the influence of ectomycorrhizal decomposition on plant nutrition and soil carbon sequestration in boreal forest ecosystems. *New Phytologist* **213**: 1452–1465.

Bazot S, Ulff L, Blum H, Nguyen C, Robin C. 2006. Effects of elevated CO_2 concentration on rhizodeposition from *Lolium perenne* grown on soil exposed to 9 years of CO_2 enrichment. *Soil Biology and Biochemistry* **38**: 729–736.

Bazzaz F. 1990. The response of natural ecosystems to the rising global CO_2 levels. *Annual Review of Ecology and Systematics* **21**: 167–196.

Bennett JA, Maherali H, Reinhart KO, Lekberg Y, Hart MM, Klironomos J. 2017. Plant–soil feedbacks and mycorrhizal type influence temperate forest population dynamics. *Science* **355**: 181–184.

Blumenthal DM, Resco V, Morgan JA, Williams DG, LeCain DR, Hardy EM, Pendall E, Bladyka E. 2013. Invasive forb benefits from water savings by native plants and carbon fertilization under elevated CO_2 and warming. *New Phytologist* **200**: 1156–1165.

von Caemmerer S, Ghannoum O, Conroy JP, Clark H, Newton PC. 2001. Photosynthetic responses of temperate species to free air CO_2 enrichment (FACE) in a grazed New Zealand pasture. *Functional Plant Biology* **28**: 439–450.

Castellano MJ, Mueller KE, Olk DC, Sawyer JE, Six J. 2015. Integrating plant litter quality, soil organic matter stabilization, and the carbon saturation concept. *Global Change Biology* **21**: 3200–3209.

Chapin FS III, Matson PA, Vitousek P. 2011. *Principles of terrestrial ecosystem ecology*. New York, NY, USA: Springer Science & Business Media.

Cheng L, Booker FL, Tu C, Burkey KO, Zhou L, Shew HD, Rufty TW, Hu S. 2012. Arbuscular mycorrhizal fungi increase organic carbon decomposition under elevated CO_2. *Science* **337**: 1084–1087.

Cheng W, Parton WJ, Gonzalez Meler MA, Phillips R, Asao S, McNickle GG, Brzostek E, Jastrow JD. 2014. Synthesis and modeling perspectives of rhizosphere priming. *New Phytologist* **201**: 31–44.

Ciais P, Sabine C, Bala G, Bopp L, Brovkin V, Canadell J, Chhabra A, DeFries R, Galloway J, Heimann M *et al.* 2013. Carbon and other biogeochemical cycles. In: Stocker TF, Qin D, Plattner GK, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM, eds. *Climate Change 2013: the physical science basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge, UK & New York, NY, USA: Cambridge University Press, 465–570.

Cotrufo MF, Soong JL, Horton AJ, Campbell EE, Haddix ML, Wall DH, Parton WJ. 2015. Formation of soil organic matter via biochemical and physical pathways of litter mass loss. *Nature Geoscience* **8**: 776–779.

Crous KY, Reich PB, Hunter MD, Ellsworth DS. 2010. Maintenance of leaf N controls the photosynthetic CO_2 response of grassland species exposed to 9 years of free-air CO_2 enrichment. *Global Change Biology* **16**: 2076–2088.

Darbah JNT, Sharkey TD, Calfapietra C, Karnosky DF. 2010. Differential response of aspen and birch trees to heat stress under elevated carbon dioxide. *Environmental Pollution* **158**: 1008–1014.

De Kauwe MG, Medlyn BE, Walker AP, Zaehle S, Asao S, Guenet B, Harper AB, Hickler T, Jain AK, Luo Y *et al.* 2017. Challenging terrestrial biosphere models with data from the long-term multifactor Prairie Heating and CO_2 Enrichment experiment. *Global Change Biology* **348**: 895.

De Kauwe MG, Medlyn BE, Zaehle S, Walker AP, Dietze MC, Wang Y-P, Luo Y, Jain AK, El Masri B, Hickler T *et al.* 2014. Where does the carbon go? A model-data intercomparison of vegetation carbon allocation and turnover processes at two temperate forest free-air CO_2 enrichment sites. *New Phytologist* **203**: 883–899.

Dieleman WIJ, Luyssaert S, Rey A, De Angelis P, Barton CVM, Broadmeadow MSJ, Broadmeadow SB, Chigwerewe KS, Crookshanks M, Dufrêne E *et al.* 2010. Soil [N] modulates soil C cycling in CO_2-fumigated tree stands: a meta-analysis. *Plant, Cell & Environment* **33**: 2001–2011.

Drake JE, Gallet Budynek A, Hofmockel KS, Bernhardt ES, Billings SA, Jackson RB, Johnsen KS, Lichter J, McCarthy HR, McCormack ML *et al.* 2011. Increases in the flux of carbon belowground stimulate nitrogen uptake and sustain the long-term enhancement of forest productivity under elevated CO_2. *Ecology Letters* **14**: 349–357.

Duursma RA, Gimeno TE, Boer MM, Crous KY, Tjoelker MG, Ellsworth DS. 2016. Canopy leaf area of a mature evergreen Eucalyptus woodland does not respond to elevated atmospheric [CO_2] but tracks water availability. *Global Change Biology* **22**: 1666–1676.

Ellsworth DS, Anderson IC, Crous KY, Cooke J, Drake JE, Gherlenda AN, Gimeno TE, Macdonald CA, Medlyn BE, Powell JR *et al.* 2017. Elevated CO_2 does not increase eucalypt forest productivity on a low-phosphorus soil. *Nature Climate Change* **320**: 279–282.

Ellsworth DS, Reich PB, Naumburg ES, Koch GW, Kubiske ME, Smith SD. 2004. Photosynthesis, carboxylation and leaf nitrogen responses of 16 species to elevated pCO_2 across four free-air CO_2 enrichment experiments in forest, grassland and desert. *Global Change Biology* **10**: 2121–2138.

Ellsworth DS, Thomas R, Crous KY, Palmroth S, Ward E, Maier C, DeLucia E, Oren R. 2012. Elevated CO_2 affects photosynthetic responses in canopy pine and subcanopy deciduous trees over 10 years: a synthesis from Duke FACE. *Global Change Biology* **18**: 223–242.

Evans RD, Koyama A, Sonderegger DL, Charlet TN, Newingham BA, Fenstermaker LF, Harlow B, Jin VL, Ogle K, Smith SD *et al.* 2014. Greater ecosystem carbon in the Mojave Desert after ten years exposure to elevated CO_2. *Nature Climate Change* **4**: 394–397.

Fatichi S, Leuzinger S, Paschalis A, Langley JA, Donnellan Barraclough A, Hovenden MJ. 2016. Partitioning direct and indirect effects reveals the response of water-limited ecosystems to elevated CO_2. *Proceedings of the National Academy of Sciences, USA* **113**: 12757–12762.

Fay PA, Prober SM, Harpole WS, Knops JMH, Bakker JD, Borer ET, Lind EM, MacDougall AS, Seabloom EW, Wragg PD *et al.* 2015. Grassland productivity limited by multiple nutrients. *Nature Plants* **1**: 15080.

Feng Z, Rütting T, Pleijel H, Wallin G, Reich PB, Kammann CI, Newton PCD, Kobayashi K, Luo Y, Uddling J. 2015. Constraints to nitrogen acquisition of terrestrial plants under elevated CO_2. *Global Change Biology* **21**: 3152–3168.

Ferguson SD, Nowak RS. 2011. Transitory effects of elevated atmospheric CO_2 on fine root dynamics in an arid ecosystem do not increase long-term soil carbon input from fine root litter. *New Phytologist* **190**: 953–967.

Fernández-Martínez M, Vicca S, Janssens IA, Sardans J, Luyssaert S, Campioli M, Chapin FS III, Ciais P, Malhi Y, Obersteiner M *et al.* 2014. Nutrient availability as the key regulator of global forest carbon balance. *Nature Climate Change* **4**: 471–476.

Finzi AC, Abramoff RZ, Spiller KS, Brzostek ER, Darby BA, Kramer MA, Phillips RP. 2015. Rhizosphere processes are quantitatively important components of terrestrial carbon and nutrient cycles. *Global Change Biology* **21**: 2082–2094.

Finzi AC, Norby RJ, Calfapietra C, Gallet Budynek A, Gielen B, Holmes WE, Hoosbeek MR, Iversen CM, Jackson RB, Kubiske ME *et al.* 2007. Increases in nitrogen uptake rather than nitrogen-use efficiency support higher rates of temperate forest productivity under elevated CO_2. *Proceedings of the National Academy of Sciences, USA* **104**: 14014–14019.

Franklin O, Näsholm T, Högberg P, Högberg MN. 2014. Forests trapped in nitrogen limitation – an ecological market perspective on ectomycorrhizal symbiosis. *New Phytologist* **203**: 657–666.

Franks PJ, Adams MA, Amthor JS, Barbour MM, Berry JA, Ellsworth DS, Farquhar GD, Ghannoum O, Lloyd J, McDowell N *et al.* 2013. Sensitivity of plants to changing atmospheric CO_2 concentration: from the geological past to the next century. *New Phytologist* **197**: 1077–1094.

Georgiou K, Koven CD, Riley WJ, Torn MS. 2015. Toward improved model structures for analyzing priming: potential pitfalls of using bulk turnover time. *Global Change Biology* **21**: 4298–4302.

Gholz HL, Fisher RF, Prichett WL. 1985. Nutrient dynamics in slash pine plantation ecosystems. *Ecology* **66**: 647–659.

Gill AL, Finzi AC. 2016. Belowground carbon flux links biogeochemical cycles and resource-use efficiency at the global scale. *Ecology Letters* **19**: 1419–1428.

de Graaff M-A, van Groenigen KJ, Six J, Hungate B, van Kessel C. 2006. Interactions between plant growth and soil nutrient cycling under elevated CO_2: a meta-analysis. *Global Change Biology* **12**: 2077–2091.

van Groenigen KJ, Qi X, Osenberg CW, Luo Y, Hungate BA. 2014. Faster decomposition under increased atmospheric CO_2 limits soil carbon storage. *Science* **344**: 508–509.

Hasegawa S, Macdonald CA, Power SA. 2016. Elevated carbon dioxide increases soil nitrogen and phosphorus availability in a phosphorus-limited Eucalyptuswoodland. *Global Change Biology* **22**: 1628–1643.

Hobbie EA. 2006. Carbon allocation to ectomycorrhizal fungi correlates with belowground allocation in culture studies. *Ecology* **87**: 563–569.

Hobbie EA, Hobbie JE. 2008. Natural abundance of ^{15}N in nitrogen-limited forests and tundra can estimate nitrogen cycling through mycorrhizal fungi: a review. *Ecosystems* **11**: 815–830.

Hodge A, Storer K. 2015. Arbuscular mycorrhiza and nitrogen: implications for individual plants through to ecosystems. *Plant and Soil* **386**: 1–19.

Högberg MN, Briones MJI, Keel SG, Metcalfe DB, Campbell C, Midwood AJ, Thornton B, Hurry V, Linder S, Näsholm T *et al.* 2010. Quantification of effects of season and nitrogen supply on tree below-ground carbon transfer to ectomycorrhizal fungi and other soil organisms in a boreal pine forest. *New Phytologist* **187**: 485–493.

Hoosbeek MR, Scarascia-Mugnozza GE. 2009. Increased litter build up and soil organic matter stabilization in a poplar plantation after 6 years of atmospheric CO_2 enrichment (FACE): final results of POP-EuroFACE compared to other forest FACE experiments. *Ecosystems* **12**: 220–239.

Housman DC, Killingbeck KT, Dave Evans R, Charlet TN, Smith SD. 2012. Foliar nutrient resorption in two Mojave Desert shrubs exposed to Free-Air CO_2 Enrichment (FACE). *Journal of Arid Environments* **78**: 26–32.

Housman DC, Naumburg E, Huxman TE, Charlet TN, Nowak RS, Smith SD. 2006. Increases in desert shrub productivity under elevated carbon dioxide vary with water availability. *Ecosystems* **9**: 374–385.

Hovenden MJ. 2003. Photosynthesis of coppicing poplar clones in a free-air CO_2 enrichment (FACE) experiment in a short-rotation forest. *Functional Plant Biology* **30**: 391–400.

Hovenden MJ, Newton PCD, Wills KE. 2014. Seasonal not annual rainfall determines grassland biomass response to carbon dioxide. *Nature* **511**: 583–586.

Huang W, Houlton BZ, Marklein AR, Liu J, Zhou G. 2015. Plant stoichiometric responses to elevated CO_2 vary with nitrogen and phosphorus inputs: evidence from a global-scale meta-analysis. *Scientific Reports* **5**: 18 225.

Hungate BA, Dijkstra P, Wu Z, Duval BD, Day FP, Johnson DW, Megonigal JP, Brown ALP, Garland JL. 2013. Cumulative response of ecosystem carbon and nitrogen stocks to chronic CO_2 exposure in a subtropical oak woodland. *New Phytologist* **200**: 753–766.

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, Jastrow JD, Luo Y, de Graaff M-A, van Kessel C, Osenberg CW. 2009. Assessing the effect of elevated carbon dioxide on soil carbon: a comparison of four meta-analyses. *Global Change Biology* **15**: 2020–2034.

Iversen CM. 2010. Digging deeper: fine-root responses to rising atmospheric CO_2 concentration in forested ecosystems. *New Phytologist* **186**: 346–357.

Iversen CM, Keller JK, Garten CT Jr, Norby RJ. 2012. Soil carbon and nitrogen cycling and storage throughout the soil profile in a sweetgum plantation after 11 years of CO_2-enrichment. *Global Change Biology* **18**: 1684–1697.

Janssens IA, Dieleman W, Luyssaert S, Subke JA, Reichstein M, Ceulemans R, Ciais P, Dolman AJ, Grace J, Matteucci G *et al.* 2010. Reduction of forest soil respiration in response to nitrogen deposition. *Nature Geoscience* **3**: 315–322.

Janze S. 2006. *Auswirkungen von erhöhtem CO_2 auf die Vegetation eines Grünlandes (Effects of increased CO_2 concentrations on the vegetation of a temperate grassland).* Giessen, Germany: Giessener Elektronische Bibiliothek, Universität Giessen.

Jin VL, Evans RD. 2010. Microbial ^{13}C utilization patterns via stable isotope probing of phospholipid biomarkers in Mojave Desert soils exposed to ambient and elevated atmospheric CO_2. *Global Change Biology* **16**: 2334–2344.

Johnson NC, Graham JH, Smith FA. 1997. Functioning of mycorrhizal associations along the mutualism–parasitism continuum. *New Phytologist* **135**: 575–585.

Johnson NC, Wilson GWT, Wilson JA, Miller RM, Bowker MA. 2015. Mycorrhizal phenotypes and the Law of the Minimum. *New Phytologist* **205**: 1473–1484.

van Kessel C, Boots B, De Graaff M-A, Harris D, Blum H, Six J. 2006. Total soil C and N sequestration in a grassland following 10 years of free air CO_2 enrichment. *Global Change Biology* **12**: 2187–2199.

Knicker H. 2011. Soil organic N – an under-rated player for C sequestration in soils? *Soil Biology and Biochemistry* **43**: 1118–1129.

Körner C. 2006. Plant CO_2 responses: an issue of definition, time and resource supply. *New Phytologist* **172**: 393–411.

Langley JA, McKinley DC, Wolf AA, Hungate BA, Drake BG, Megonigal JP. 2009. Priming depletes soil carbon and releases nitrogen in a scrub-oak ecosystem exposed to elevated CO_2. *Soil Biology and Biochemistry* **41**: 54–60.

LeBauer DS, Treseder KK. 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology* **89**: 371–379.

Lee TD, Barrott SH, Reich PB. 2011. Photosynthetic responses of 13 grassland species across 11 years of free-air CO_2 enrichment is modest, consistent and independent of N supply. *Global Change Biology* **17**: 2893–2904.

Lenhart K, Kammann C, Boeckx P, Six J, Müller C. 2016. Quantification of ecosystem C dynamics in a long-term FACE study on permanent grassland. *Rapid Communications in Mass Spectrometry* **30**: 963–972.

Li JH, Dijkstra P, Hinkle CR, Wheeler RM, Drake BG. 1999. Photosynthetic acclimation to elevated atmospheric CO_2 concentration in the Florida scrub-oak species *Quercus geminata* and *Quercus myrtifolia* growing in their native environment. *Tree Physiology* **19**: 229–234.

Liberloo M, Calfapietra C, Lukac M, Godbold D, Luo ZB, Polle A, Hoosbeek MR, Kull O, Marek M, Raines C *et al.* 2006. Woody biomass production during the second rotation of a bio-energy *Populus* plantation increases in a future high CO_2 world. *Global Change Biology* **12**: 1094–1106.

Lichter J, Billings SA, Ziegler SE, Gaindh D, Ryals R, Finzi AC, Jackson RB, Stemmler EA, Schlesinger WH. 2008. Soil carbon sequestration in a pine forest after 9 years of atmospheric CO_2 enrichment. *Global Change Biology* **14**: 2910–2922.

Lin G, McCormack ML, Ma C, Guo D. 2017. Similar below-ground carbon cycling dynamics but contrasting modes of nitrogen cycling between arbuscular mycorrhizal and ectomycorrhizal forests. *New Phytologist* **213**: 1440–1451.

Lindahl BD, Tunlid A. 2015. Ectomycorrhizal fungi – potential organic matter decomposers, yet not saprotrophs. *New Phytologist* **205**: 1443–1447.

Litton CM, Raich JW, Ryan MG. 2007. Carbon allocation in forest ecosystems. *Global Change Biology* **13**: 2089–2109.

Lüscher A, Hartwig UA, Suter D, Nösberger J. 2000. Direct evidence that symbiotic N_2 fixation in fertile grassland is an important trait for a strong response of plants to elevated atmospheric CO_2. *Global Change Biology* **6**: 655–662.

Malhi Y, Doughty CE, Goldsmith GR, Metcalfe DB, Girardin CAJ, Marthews TR, del Aguila-Pasquel J, Aragão LEOC, Araujo-Murakami A, Brando P *et al.* 2015. The linkages between photosynthesis, productivity, growth and biomass in lowland Amazonian forests. *Global Change Biology* **21**: 2283–2295.

McCarthy HR, Oren R, Johnsen KH, Gallet Budynek A, Pritchard SG, Cook CW, LaDeau SL, Jackson RB, Finzi AC. 2010. Re-assessment of plant carbon dynamics at the Duke free-air CO_2 enrichment site: interactions of atmospheric [CO_2] with nitrogen and water availability over stand development. *New Phytologist* **185**: 514–528.

Medlyn BE, Zaehle S, De Kauwe MG, Walker AP, Dietze MC, Hanson PJ, Hickler T, Jain AK, Luo Y, Parton W *et al.* 2015. Using ecosystem experiments to improve vegetation models. *Nature Climate Change* **5**: 528–534.

Menge DNL, Hedin LO, Pacala SW. 2012. Nitrogen and phosphorus limitation over long-term ecosystem development in terrestrial ecosystems. *PLoS ONE* **7**: e42045.

Morgan JA, LeCain DR, Pendall E, Blumenthal DM, Kimball BA, Carrillo Y, Williams DG, Heisler-White J, Dijkstra FA, West M. 2011. C_4 grasses prosper as carbon dioxide eliminates desiccation in warmed semi-arid grassland. *Nature* **476**: 202–205.

Morgan JA, Pataki DE, Körner C, Clark H, Del Grosso SJ, Grünzweig JM, Knapp AK, Mosier AR, Newton PCD, Niklaus PA *et al.* 2004. Water relations in grassland and desert ecosystems exposed to elevated atmospheric CO_2. *Oecologia* **140**: 11–25.

Mueller KE, Blumenthal DM, Pendall E, Carrillo Y, Dijkstra FA, Williams DG, Follett RF, Morgan JA. 2016. Impacts of warming and elevated CO_2 on a semi-arid grassland are non-additive, shift with precipitation, and reverse over time. *Ecology Letters* **19**: 956–966.

Mueller KE, Hobbie SE, Tilman D, Reich PB. 2013. Effects of plant diversity, N fertilization, and elevated carbon dioxide on grassland soil N cycling in a long-term experiment. *Global Change Biology* **19**: 1249–1261.

Newingham BA, Vanier CH, Charlet TN, Ogle K, Smith SD, Nowak RS. 2013. No cumulative effect of 10 years of elevated [CO_2] on perennial plant biomass components in the Mojave Desert. *Global Change Biology* **19**: 2168–2181.

Newton PCD, Lieffering M, Bowatte WMSD, Brock SC, Hunt CL, Theobald PW, Ross DJ. 2010. The rate of progression and stability of progressive nitrogen limitation at elevated atmospheric CO_2 in a grazed grassland over 11 years of Free Air CO_2 enrichment. *Plant and Soil* **336**: 433–441.

Newton PCD, Lieffering M, Parsons AJ, Brock SC, Theobald PW, Hunt CL, Luo D, Hovenden MJ. 2014. Selective grazing modifies previously anticipated responses of plant community composition to elevated CO_2 in a temperate grassland. *Global Change Biology* **20**: 158–169.

Nie M, Lu M, Bell J, Raut S, Pendall E. 2013. Altered root traits due to elevated CO_2: a meta-analysis. *Global Ecology and Biogeography* **22**: 1095–1105.

Norby RJ, De Kauwe MG, Walker AP, Werner C, Zaehle S, Zak DR. 2017. Comment on 'Mycorrhizal association as a primary control of the CO_2 fertilization effect'. *Science* **355**: 358–358.

Norby RJ, DeLucia EH, Gielen B, Calfapietra C, Giardina CP, King JS, Ledford J, McCarthy HR, Moore DJP, Ceulemans R *et al.* 2005. Forest response to elevated CO_2 is conserved across a broad range of productivity. *Proceedings of the National Academy of Sciences, USA* **102**: 18 052–18 056.

Norby RJ, Warren JM, Iversen CM, Medlyn BE, McMurtrie RE. 2010. CO_2 enhancement of forest productivity constrained by limited nitrogen availability. *Proceedings of the National Academy Sciences, USA* **107**: 19 368–19 373.

Norby RJ, Zak DR. 2011. Ecological lessons from free-air CO_2 enrichment (FACE) experiments. *Annual Review of Ecology* **42**: 181–203.

Nouri E, Breuillin-Sessoms F, Feller U, Reinhardt D. 2014. Phosphorus and nitrogen regulate arbuscular mycorrhizal symbiosis in *Petunia hybrida*. *PLoS ONE* **9**: e90841.

Nowak RS, Ellsworth DS, Smith SD. 2004. Functional responses of plants to elevated atmospheric CO_2 – do photosynthetic and productivity data from FACE experiments support early predictions? *New Phytologist* **162**: 253–280.

Ochoa-Hueso R, Hughes J, Delgado-Baquerizo M, Drake JE, Tjoelker MG, Piñeiro J, Power SA. 2017. Rhizosphere-driven increase in nitrogen and phosphorus availability under elevated atmospheric CO_2 in a mature Eucalyptus woodland. *Plant and Soil* **1**: 1.

van Oijen M, Schapendonk A, Höglind M. 2010. On the relative magnitudes of photosynthesis, respiration, growth and carbon storage in vegetation. *Annals of Botany* **105**: 793–797.

Oishi AC, Palmroth S, Johnsen KH, McCarthy HR, Oren R. 2014. Sustained effects of atmospheric [CO_2] and nitrogen availability on forest soil CO_2 efflux. *Global Change Biology* **20**: 1146–1160.

Oren R, Ellsworth DS, Johnsen KH, Phillips N, Ewers BE, Maier C, Schäfer KV, McCarthy H, Hendrey G, McNulty SG *et al.* 2001. Soil fertility limits carbon sequestration by forest ecosystems in a CO_2-enriched atmosphere. *Nature* **411**: 469–472.

Pendall E, Del Grosso S, King JY, LeCain DR, Milchunas DG, Morgan JA, Mosier AR, Ojima DS, Parton WA, Tans PP *et al.* 2003. Elevated atmospheric CO_2 effects and soil water feedbacks on soil respiration components in a Colorado grassland. *Global Biogeochemical Cycles* **17**: 1046.

Pendall E, King JY. 2007. Soil organic matter dynamics in grassland soils under elevated CO_2: insights from long-term incubations and stable isotopes. *Soil Biology and Biochemistry* **39**: 2628–2639.

Pendall E, Mosier AR, Morgan JA. 2004. Rhizodeposition stimulated by elevated CO_2 in a semiarid grassland. *New Phytologist* **162**: 447–458.

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.

Peñuelas J, Matamala R. 1990. Changes in N and S leaf content, stomatal density and specific leaf area of 14 plant species during the last three centuries of CO_2 increase. *Journal of Experimental Botany* **41**: 1119–1124.

Phillips RP, Brzostek E, Midgley MG. 2013. The mycorrhizal-associated nutrient economy: a new framework for predicting carbon–nutrient couplings in temperate forests. *New Phytologist* **199**: 41–51.

Phillips RP, Finzi AC, Bernhardt ES. 2011. Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO_2 fumigation. *Ecology Letters* **14**: 187–194.

Phillips RP, Meier IC, Bernhardt ES, Grandy AS, Wickings K, Finzi AC. 2012. Roots and fungi accelerate carbon and nitrogen cycling in forests exposed to elevated CO_2. *Ecology Letters* **15**: 1042–1049.

Pritchard SG. 2011. Soil organisms and global climate change. *Plant Pathology* **60**: 82–99.

Reich PB, Hobbie SE. 2013. Decade-long soil nitrogen constraint on the CO_2 fertilization of plant biomass. *Nature Climate Change* **3**: 278–282.

Reich PB, Hobbie SE, Lee T, Ellsworth DS, West JB, Tilman D, Knops JMH, Naeem S, Trost J. 2006a. Nitrogen limitation constrains sustainability of ecosystem response to CO_2. *Nature* **440**: 922–925.

Reich PB, Hungate BA, Luo Y. 2006b. Carbon-nitrogen interactions in terrestrial ecosystems in response to rising atmospheric carbon dioxide. *Annual Review of Ecology, Evolution, and Systematics* **37**: 611–636.

Reynolds HL, Hartley AE, Vogelsang KM, Bever JD, Schultz PA. 2005. Arbuscular mycorrhizal fungi do not enhance nitrogen acquisition and growth of old-field perennials under low nitrogen supply in glasshouse culture. *New Phytologist* **167**: 869–880.

Rogers A, Fischer B, Bryant J, Frehner M, Blum H, Raines C, Long S. 1998. Acclimation of photosynthesis to elevated CO_2 under low-nitrogen nutrition is affected by the capacity for assimilate utilization. Perennial ryegrass under free-Air CO_2 enrichment. *Plant Physiology* **118**: 683–689.

Ross DJ, Newton PCD, Tate KR, Luo D. 2013. Impact of a low level of CO_2 enrichment on soil carbon and nitrogen pools and mineralization rates over ten years in a seasonally dry, grazed pasture. *Soil Biology and Biochemistry* **58**: 265–274.

Rütting T, Clough TJ, Müller C, Lieffering M, Newton PCD. 2010. Ten years of elevated atmospheric carbon dioxide alters soil nitrogen transformations in a sheep-grazed pasture. *Global Change Biology* **16**: 2530–2542.

Saxe H, Ellsworth DS, Heath J. 1998. Tree and forest functioning in an enriched CO_2 atmosphere. *New Phytologist* **139**: 395–436.

Schneider MK, Lüscher A, Richter M, Aeschlimann U, Hartwig UA, Blum H, Frossard E, Nösberger J. 2004. Ten years of free-air CO_2 enrichment altered the mobilization of N from soil in *Lolium perenne* L. swards. *Global Change Biology* **10**: 1377–1388.

Shah F, Nicolás C, Bentzer J, Ellström M, Smits M, Rineau F, Canbäck B, Floudas D, Carleer R, Lackner G *et al.* 2015. Ectomycorrhizal fungi decompose soil organic matter using oxidative mechanisms adapted from saprotrophic ancestors. *New Phytologist* **209**: 1705–1719.

Sigurdsson BD, Medhurst JL, Wallin G, Eggertsson O, Linder S. 2013. Growth of mature boreal Norway spruce was not affected by elevated [CO_2] and/or air temperature unless nutrient availability was improved. *Tree Physiology* **33**: 1192–1205.

Sitch S, Friedlingstein P, Gruber N, Jones SD, Murray-Tortarolo G, Ahlström A, Doney SC, Graven H, Heinze C, Huntingford C *et al.* 2015. Recent trends and drivers of regional sources and sinks of carbon dioxide. *Biogeosciences* **12**: 653–679.

Smith NG. 2017. Plant respiration responses to elevated CO_2: an overview from cellular processes to global impacts. In: Govindjee, Sharkey TD, Tcherkez G, Ghashghaie J, eds. *Contribution to Advances in Photosynthesis and Respiration special volume on plant respiration.* New York, NY, USA: Springer, in press.

Smith NG, Dukes JS. 2013. Plant respiration and photosynthesis in global-scale models: incorporating acclimation to temperature and CO_2. *Global Change Biology* **19**: 45–63.

Smith P, Davis SJ, Creutzig F, Fuss S, Minx J, Gabrielle B, Kato E, Jackson RB, Cowie A, Kriegler E *et al.* 2016. Biophysical and economic limits to negative CO_2 emissions. *Nature Climate Change* **6**: 42–50.

Smith SD, Charlet TN, Zitzer SF, Abella SR, Vanier CH, Huxman TE. 2014. Long-term response of a Mojave Desert winter annual plant community to a whole-ecosystem atmospheric CO_2 manipulation (FACE). *Global Change Biology* 20: 879–892.

Spreitzer RJ, Salvucci ME. 2002. RUBISCO: structure, regulatory interactions, and possibilities for a better enzyme. *Annual Review of Plant Biology* 53: 449–475.

Sulman BN, Brzostek ER, Medici C, Shevliakova E, Menge DNL, Phillips RP. 2017. Feedbacks between plant N demand and rhizosphere priming depend on type of mycorrhizal association. *Ecology Letters* 102: 1043–1053.

Sulman BN, Phillips RP, Oishi AC, Shevliakova E, Pacala SW. 2014. Microbe-driven turnover offsets mineral-mediated storage of soil carbon under elevated CO_2. *Nature Climate Change* 4: 1099–1102.

Talhelm AF, Pregitzer KS, Pregitzer KS, Kubiske ME, Kubiske ME, Zak DR, Campany CE, Burton AJ, Dickson RE, Hendrey GR *et al.* 2014. Elevated carbon dioxide and ozone alter productivity and ecosystem carbon content in northern temperate forests. *Global Change Biology* 20: 2492–2504.

Terrer C, Vicca S, Hungate BA, Phillips RP, Prentice IC. 2016. Mycorrhizal association as a primary control of the CO_2 fertilization effect. *Science* 353: 72–74.

Terrer C, Vicca S, Hungate BA, Phillips RP, Reich PB, Franklin O, Stocker BD, Fisher JB, Prentice IC. 2017. Response to Comment on 'Mycorrhizal association as a primary control of the CO_2 fertilization effect'. *Science* 355: 358.

Teste FP, Kardol P, Turner BL, Wardle DA, Zemunik G, Renton M, Laliberté E. 2017. Plant–soil feedback and the maintenance of diversity in Mediterranean-climate shrublands. *Science* 355: 173–176.

Thirkell TJ, Cameron DD, Hodge A. 2016. Resolving the 'nitrogen paradox' of arbuscular mycorrhizas: fertilization with organic matter brings considerable benefits for plant nutrition and growth. *Plant, Cell & Environment* 39: 1683–1690.

Thomas RQ, Brookshire ENJ, Gerber S. 2015. Nitrogen limitation on land: how can it occur in Earth system models? *Global Change Biology* 21: 1777–1793.

Thomas RQ, Canham CD, Weathers KC, Goodale CL. 2010. Increased tree carbon storage in response to nitrogen deposition in the US. *Nature Geoscience* 3: 13–17.

Treseder KK. 2004. A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO_2 in field studies. *New Phytologist* 164: 347–355.

Vicca S, Luyssaert S, Peñuelas J, Campioli M, Chapin FS III, Ciais P, Heinemeyer A, Högberg P, Kutsch WL, Law BE *et al.* 2012. Fertile forests produce biomass more efficiently. *Ecology Letters* 15: 520–526.

Vitousek P, Howarth R. 1991. Nitrogen limitation on land and in the sea: how can it occur? *Biogeochemistry* 13: 87–115.

Wang D, Heckathorn SA, Wang X, Philpott SM. 2012. A meta-analysis of plant physiological and growth responses to temperature and elevated CO_2. *Oecologia* 169: 1–13.

Wang H, Prentice IC, Davis TW, Keenan T, Wright IJ, Peng C. 2017. Photosynthetic responses to altitude: an explanation based on optimality principles. *New Phytologist* 213: 976–982.

Warren JM, Jensen AM, Medlyn BE, Norby RJ, Tissue DT. 2015. Carbon dioxide stimulation of photosynthesis in *Liquidambar styraciflua* is not sustained during a 12-year field experiment. *AoB Plants* 7: plu074.

Wurzburger N, Brookshire ENJ, McCormack ML, Lankau RA. 2017. Mycorrhizal fungi as drivers and modulators of terrestrial ecosystem processes. *New Phytologist* 213: 996–999.

Zaehle S, Medlyn BE, De Kauwe MG, Walker AP, Dietze MC, Hickler T, Luo Y, Wang Y-P, El Masri B, Thornton P *et al.* 2014. Evaluation of 11 terrestrial carbon–nitrogen cycle models against observations from two temperate Free-Air CO_2 Enrichment studies. *New Phytologist* 202: 803–822.

Zhou L, Zhou X, Shao J, Nie Y, He Y, Jiang L, Wu Z, Bai SH. 2016. Interactive effects of global change factors on soil respiration and its components: a meta-analysis. *Global Change Biology* 22: 3157–3169.

Zhou L, Zhou X, Zhang B, Lu M, Luo Y, Liu L, Li B. 2014. Different responses of soil respiration and its components to nitrogen addition among biomes: a meta-analysis. *Global Change Biology* 20: 2332–2343.

Zhu K, Chiariello NR, Tobeck T, Fukami T, Field CB. 2016. Nonlinear, interacting responses to climate limit grassland production under global change. *Proceedings of the National Academy of Sciences, USA* 113: 10589–10594.