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

César Terrer[1], Sara Vicca[2], Benjamin D. Stocker[1,3], Bruce A. Hungate[4,5], Richard P. Phillips[6], Peter B. Reich[7,8], Adrien C. Finzi[9], I. Colin Prentice[1].

[1]AXA Chair Programme in Biosphere and Climate Impacts, Department of Life Sciences, Imperial College London, Silwood Park Campus, Buckhurst Road, Ascot SL5 7PY, UK; [2]Centre of Excellence PLECO (Plants and Ecosystems), Department of Biology, University of Antwerp, 2610 Wilrijk, Belgium; [3]CREAF, Cerdanyola del Vallès, 08193 Catalonia, Spain; [4]Center for Ecosystem Science and Society, Northern Arizona University, Flagstaff, AZ 86011, USA; [5]Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011, USA; [6]Department of Biology, Indiana University, Bloomington, IN 47405, USA; [7]Department of Forest Resources, University of Minnesota, St. Paul, MN 55108, USA; [8]Hawkesbury Institute for the Environment, Western Sydney University, Penrith NSW 2751, Australia; [9]Department of Biology, Boston University, Boston MA 02215, USA.

Author for correspondence:
César Terrer
Tel: +44 7476820487
Email: c.terrer@imperial.ac.uk

Heading: Revisiting the effects of nitrogen availability on ecosystem responses to elevated CO_2.
Twitter: @c_terrer
Key words: CO_2, Nitrogen, mycorrhizae, FACE, photosynthesis, soil carbon, N_2-fixation, SOM

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 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 more than 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). While 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 (Reich *et al.*, 2006a; Körner, 2006; Huang *et al.*, 2015; Terrer *et al.*, 2016).

While 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 non-existent 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 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 ~28% enhancement in biomass even under low N. By contrast, plants associated with arbuscular mycorrhizal (AM) fungi were unresponsive to eCO_2 (~0%) under low N, unless associated with N_2-fixers (~8%). These conclusions proved consistent for aboveground productivity as well as biomass (Terrer *et al.*, 2017).

Although more 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: i) do ECM plants and N_2-fixers take up more N than AM plants in response to eCO_2? ii) is the role of N availability in constraining the eCO_2 effect on plant biomass caused by limitations on leaf-level photosynthesis? And iii) 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 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

A. 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:

$$\text{Return on investment} = \frac{\frac{\partial N_{acq}}{N_{acq}}}{\frac{\partial C_{bg}}{C_{bg}}} \approx \frac{\frac{N_{acq}(ele) - N_{acq}(amb)}{N_{acq}(amb)}}{\frac{C_{bg}(ele) - C_{bg}(amb)}{C_{bg}(amb)}} = \Psi_N^{-1}, \quad (Eq.\ 1)$$

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.

While 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):

$$C_{bg} = C_{root} + C_{transfer}, \qquad (Eq.\ 2)$$

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

$$BP = NPP - C_{transfer} \qquad (Eq.\ 3)$$

We therefore refer to $C_{transfer}$ as the non-plant biomass component of the C budget that may be used by plants to acquire N. Several lines of evidence suggest that, indeed, plants increase allocation to $C_{transfer}$ as soil resources decrease in availability (Treseder, 2004; Hobbie, 2006; Högberg *et al.*, 2010; Phillips *et al.*, 2011; Drake *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, Eq. 1) for as many eCO_2 studies as possible, i.e. those with data on both N_{acq} and C_{bg}. Even though $C_{transfer}$ represents a fraction of 10-40% of NPP (Pritchard, 2011; Chapin *et al.*, 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:

$$\frac{\partial C_{bg}}{C_{bg}} = \frac{\partial C_{root}}{C_{root}} \qquad (Eq.\ 4)$$

Eq. 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).

B. 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. 1A, 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). On the other hand, for a given increase in the amount of C invested belowground, AM plants achieved a much lower enhancement in N_{acq} than ECM plants. In some cases, AM plants acquired less N than 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 non-fertilised 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 is limited by the lack of $C_{transfer}$ data (Eq. 2). In order to test the validity of Eq. 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) (asterisks in Fig. 1B). These data can be used to estimate the cost of N_{acq} in 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} per g 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 (Adair *et al.*, 2009) due 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, 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 $C_{\mathrm{root}}/C_{\mathrm{transfer}}$ ratio (Eq. 4) is a key need for this field, its uncertainty does not stand in the way of the exercise presented here, but does indicate uncertainty about the exact slope in Fig. 1A. 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 ectomycorrhizal (ECM) fungi generally associated with more beneficial effects on their plant host's fitness than arbuscular mycorrhizal (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). eCO_2 did not commonly enhance aboveground N_{acq} in AM plants 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 (A), plant biomass production (B) and SOM decomposition (C) – all factors that influence ecosystem responses to eCO_2 and ecosystem feedbacks to climate change. This approach allows us to characterise systems within a response spectrum spanned by the return on investment.

A. 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 down-regulation of V_{cmax} by eCO$_2$ in non-fertilized soils is inversely related to Ψ_N^{-1}(Fig. 2A, $P<0.01$), suggesting 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 down-regulation 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 down-regulation 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, down-regulation 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 non-significant).

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 non-fertilized 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% versus −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 (less than 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. 2B 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 further investigation. In any case, despite partial down-regulation 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.

B. 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 (i) increase its rate of N_{acq} from the soil (Oren *et al.*, 2001; Finzi *et al.*, 2007), and/or (ii) 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?

Observations: we found a significantly positive relationship between $\Psi_N^{-1}$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 and 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-fertilisation 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-fertilisation (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). Similarly at BioCON, the eCO_2-enhancement in productivity was larger in N_2-fixing legumes than in non-legume AM species (Fig. 3) (see Mueller *et al.*, 2013).

AM trees at ORNL FACE apparently showed the opposite pattern than 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 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). Similar 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 these results might have been driven by seasonal - instead of annual precipitation (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 above-ground 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 for 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 e.g. 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-fertilised (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 more allocation to leaves (high [N]) during stand development, and more 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 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.

C. 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). While 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). Similarly 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).

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

While 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 suggest that mycorrhizal status play 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. In 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}: (i) allocation to fine roots (Iversen, 2010), (ii) allocation to mycorrhizal fungi (Drake *et al.*, 2011), and (iii) 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 fertilisation 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 AM-plants for two reasons: (i) many ECM fungi have the enzymes necessary to mine organic N (Shah *et al.*, 2015), and (ii) 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 (i) AM fungi do not produce the enzymes required to increase priming in response to eCO_2 (Hodge & Storer, 2015), and (ii) litter produced by AM plants has a lower C:N ratio (Lin *et al.*, 2017), promoting 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, primarily driven 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 (Newingham *et al.*, 2013; Bader *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 versus 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.

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.

Acknowledgments

We thank M. Schneider, R. Norby, K. Zhu, C. Field, N. Chiariello, G. Moser, C. Müller, C. Kammann, S. Smith, D. Evans, L. Fenstermaker, D. Blumenthal, A. Talhelm, M. Hovenden and P. Newton, who provided data and advice. C.T. was supported by the Imperial College initiative Grand Challenges in Ecosystems and the Environment and the AXA Chair Programme in Biosphere and Climate Impacts. C.T. and R.P thank KNAW, DOE, INTERFACE, and the New Phytologist trust for funding the Workshop “Climate models revisited: the biogeochemical consequences of mycorrhizal dynamics”. S.V. is a postdoctoral fellow of the Fund for Scientific Research – Flanders and acknowledges support from the ClimMani COST action (ES1308) and the European Research Council grant ERC-SyG- 610028 IMBALANCE-P. NSF supported R.P.P. (DEB-1153401), P.B.R (DEB-1234162, DEB-1242531, DEB-1120064) and B.A.H. (DEB-1241094). B.D.S. is funded by the Swiss National Science Foundation. U.S. Department of Energy (BER) supported A.C.F. (DE-SC0006916, DE-SC0012288), RPP and B.A.H. (DE-SC0010632). C.T., B.D.S., S.V. and I.C.P. designed the research and wrote the initial manuscript. C.T. and B.D.S. carried out the analysis. All authors contributed to interpretation and writing of the final paper.

Table 1. List of FACE and OTC sites analysed in this review, along with some site characteristics and sources for data used in Figs. 1-4. The amount of N-fertilization applied is indicated in parenthesis (units in $g\ m^{-2}\ yr^{-1}$).

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); pers.comm *	Finzi *et al.* (2007) & pers.comm	Ellsworth *et al.* (2012)	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); pers.comm *	Hungate *et al.* (2013) & pers.comm	Li *et al.* (1999)	Hungate *et al.* (2013); 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); pers.comm *	Norby *et al.* (2010) & pers.comm	Warren *et al.* (2015)	Norby *et al.* (2010); pers.comm	Iversen *et al.* (2012)
PHACE	Cheyenne, WY, USA	Mixed-grass prairie	Low	AM	Mueller *et al.* (2016) ****	pers.comm	Blumenthal *et al.* (2013)	pers.comm	-
BioCON	Cedar Creek, MN, USA	Grassland dominated by C3, C4 grasses, legumes and forbs	Low (ambient) & medium (4)	AM, N-fixing	pers.comm **	pers.comm	Crous *et al.* (2010); Lee *et al.* (2011)	Reich & Hobbie (2013); pers.comm	http://www.cedarcreek.umn.edu/research/data/dataset?ache141
NZ FACE	Bulls, Manawatu, New Zealand	Grassland dominated by legumes, C3 and C4 grasses	Low	N-fixing, AM	Allard *et al.* (2005) ***	pers.comm	Caemmerer *et al.* (2001)	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); pers.comm	Rogers *et al.* (1998)	Schneider *et al.* (2004)	van Kessel *et al.* (2006)
POP	Tuscany, Italy	Forest (deciduous): *Populus alba, P.*	High	ECM + AM	Finzi *et al.* (2007) *	Finzi *et al.* (2007)	Hovenden (2003)	Finzi *et al.* (2007)	Hoosbeek & Scarascia-

		nigra & P. euramericana							Mugnozza (2009)
Jasper FACE	San Mateo, CA, USA	California grassland dominated by annual non-native grasses	Low (ambient) and high (7)	AM	Zhu *et al.* (2016) ***	pers.comm	-	Zhu *et al.* (2016)	pers.comm
GiFACE	Giessen, Germany	Grassland, including legumes (<1% initially)	Medium (4)	AM, N-fixing	Janze (2006) ***	pers.comm	-	Andresen *et al.* (2017)	Lenhart *et al.* (2016)

* Fine-root growth; ** fine-root biomass; *** root growth; **** root biomass

Table 2. List of major gaps in the framework outlined here about the interactions between the 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 mycorrhizae. 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 Minimise the effect of expanding canopies, prioritising mature plants in steady-state Minimum of 5-10 years of eCO_2 fumigation to allow soil dynamics start developing

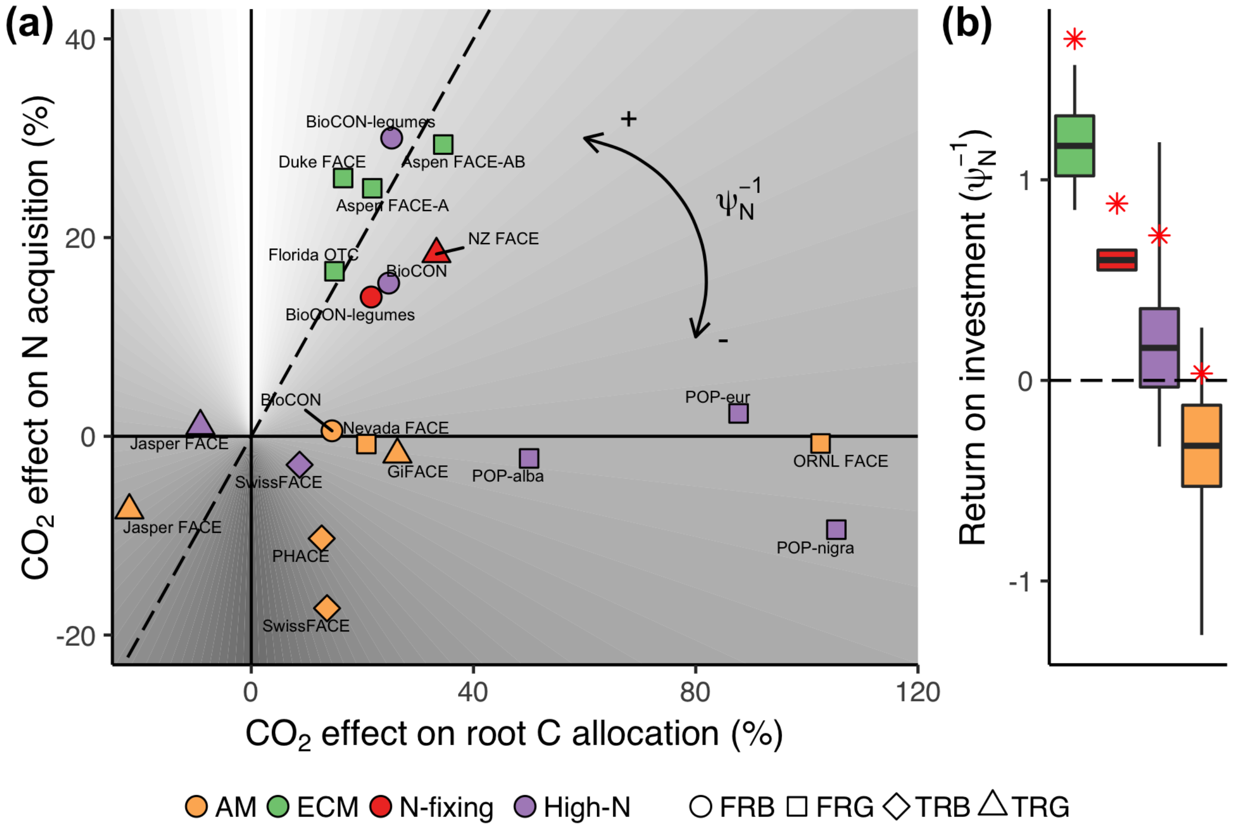


Figure 1. Plant-economics spectrum of the efficiency of plants to acquire additional N under elevated CO$_2$. (a) Relationship between the eCO$_2$-induced relative change (%) in C_{root} ("investments") and aboveground N-acquisition ("returns"). (b) Mean, standard error, max and min return on investment (Ψ_N^{-1}, *Eq. 1*). Colours represent four different N-acquisition strategies characterised by the type of symbiotic association arbuscular mycorrhizae (AM), ectomycorrhizae (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 C_{bg} 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 (*Eq. 2*). N_{acq} is the 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.

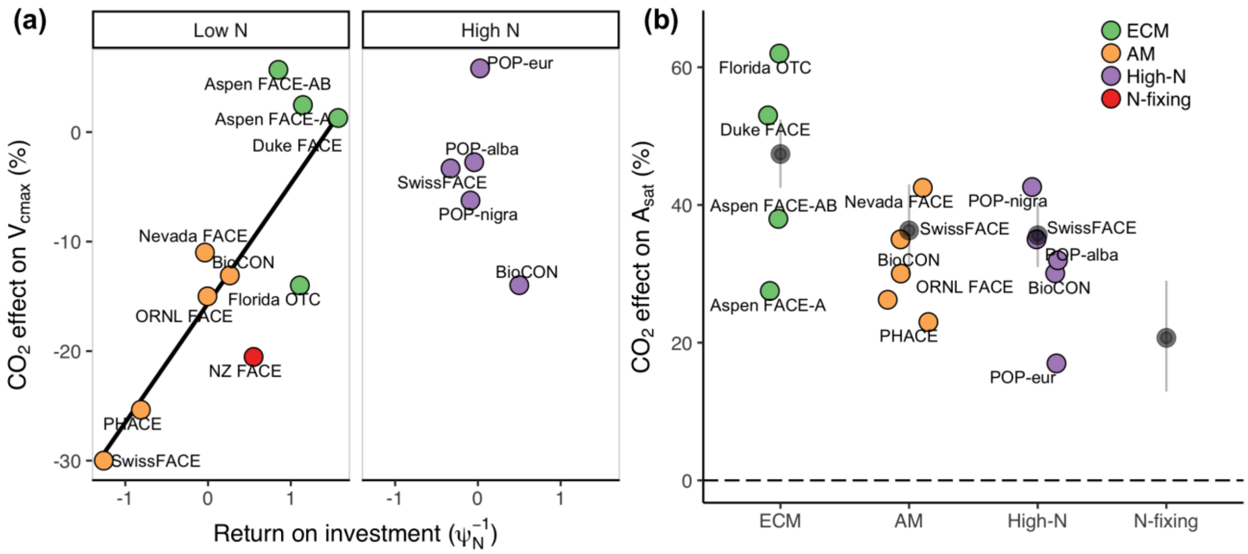


Figure 2. Effects of elevated CO_2 on leaf-level photosynthesis and its modulation by nitrogen acquisition efficiency. (a) Relationship between the effect of elevated CO_2 on maximum rate of carboxylation (V_{cmax}) and the N return on investment (Ψ_N^{-1}, Eq. 1) under low (left panel) and high (right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

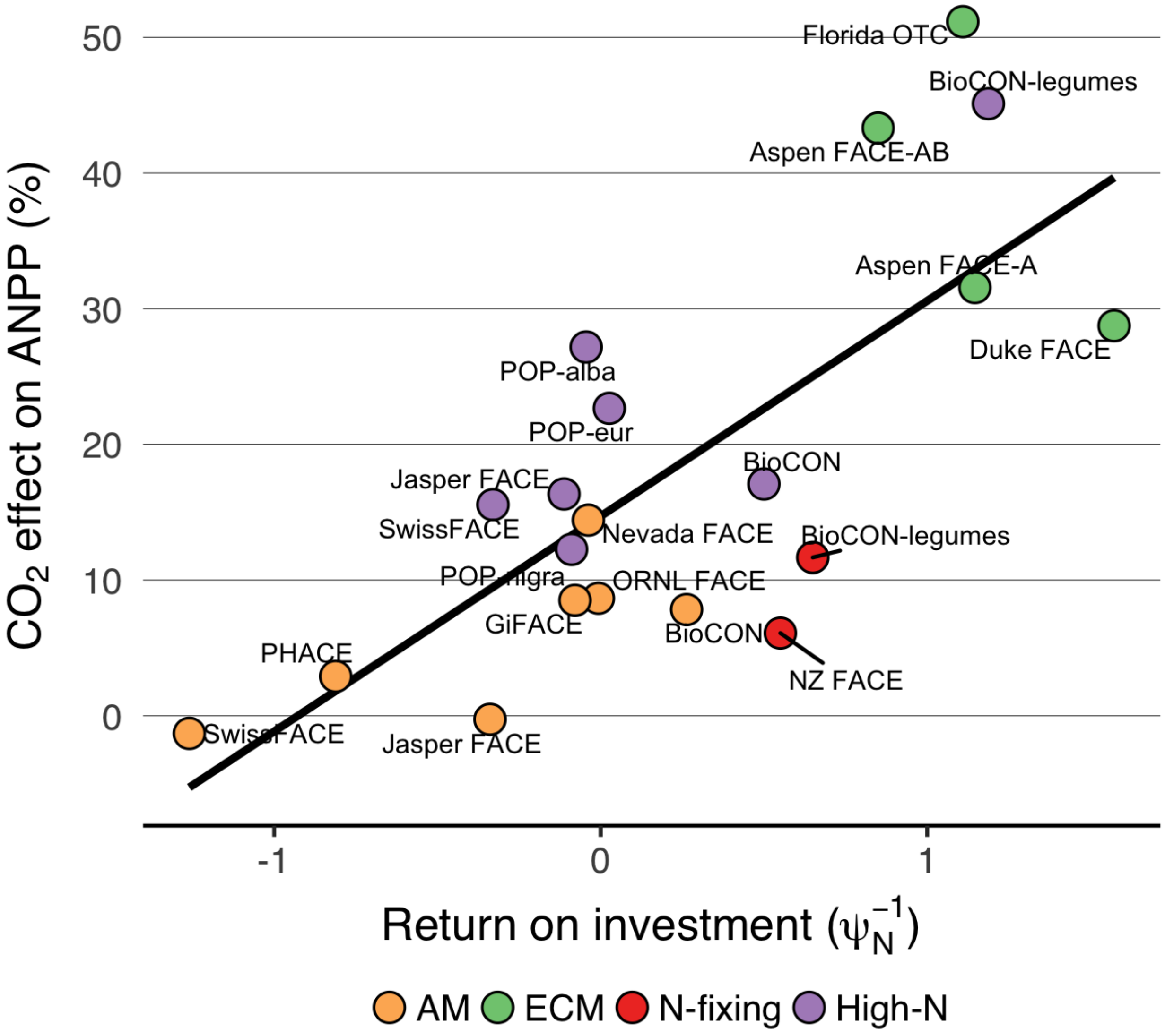


Figure 3. Relationship between the effect of elevated CO_2 on aboveground biomass production (ANPP) and the N return on investment (Ψ_N^{-1}, Eq. 1). Sources of site-level data are given in Table 1.

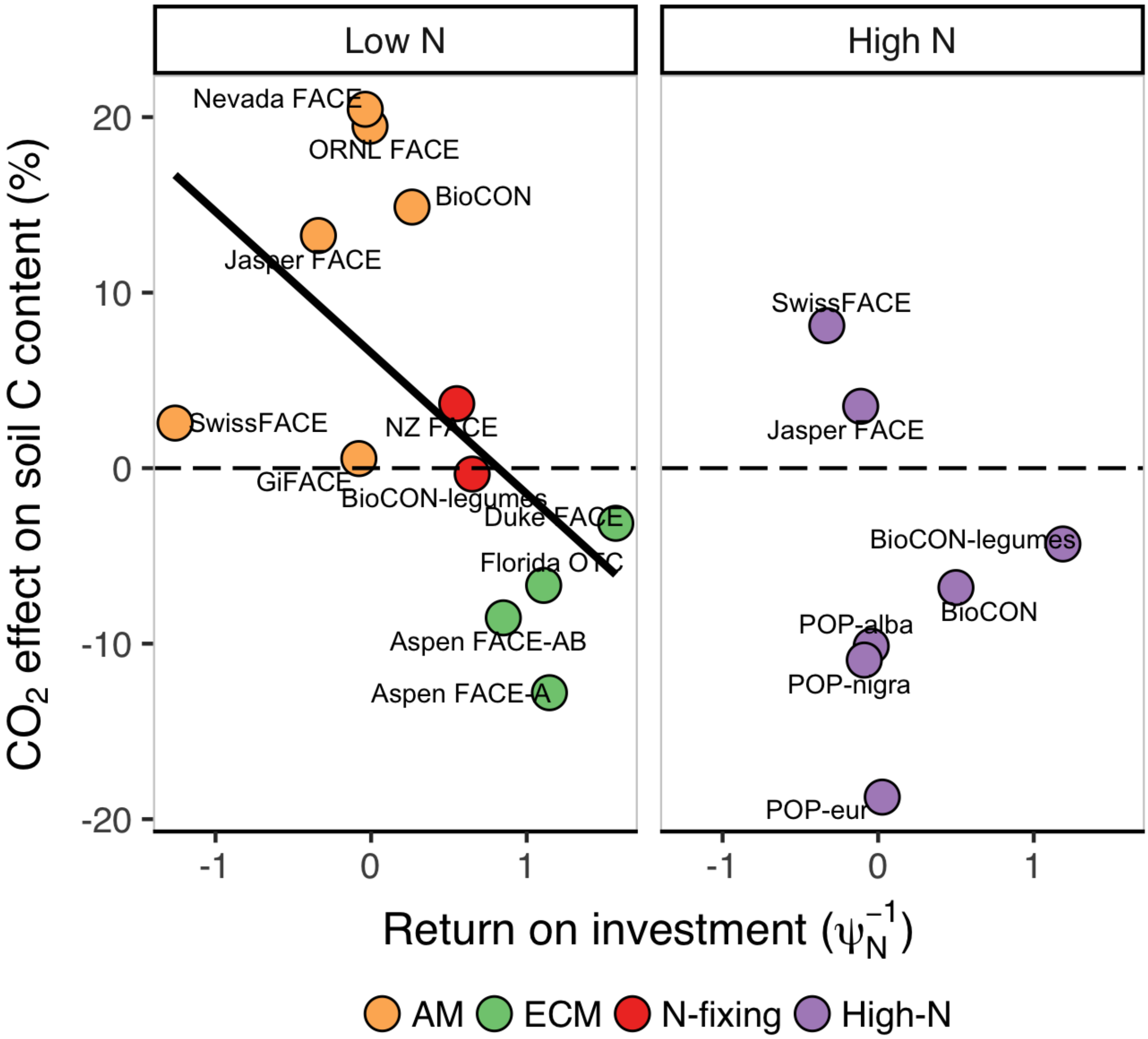
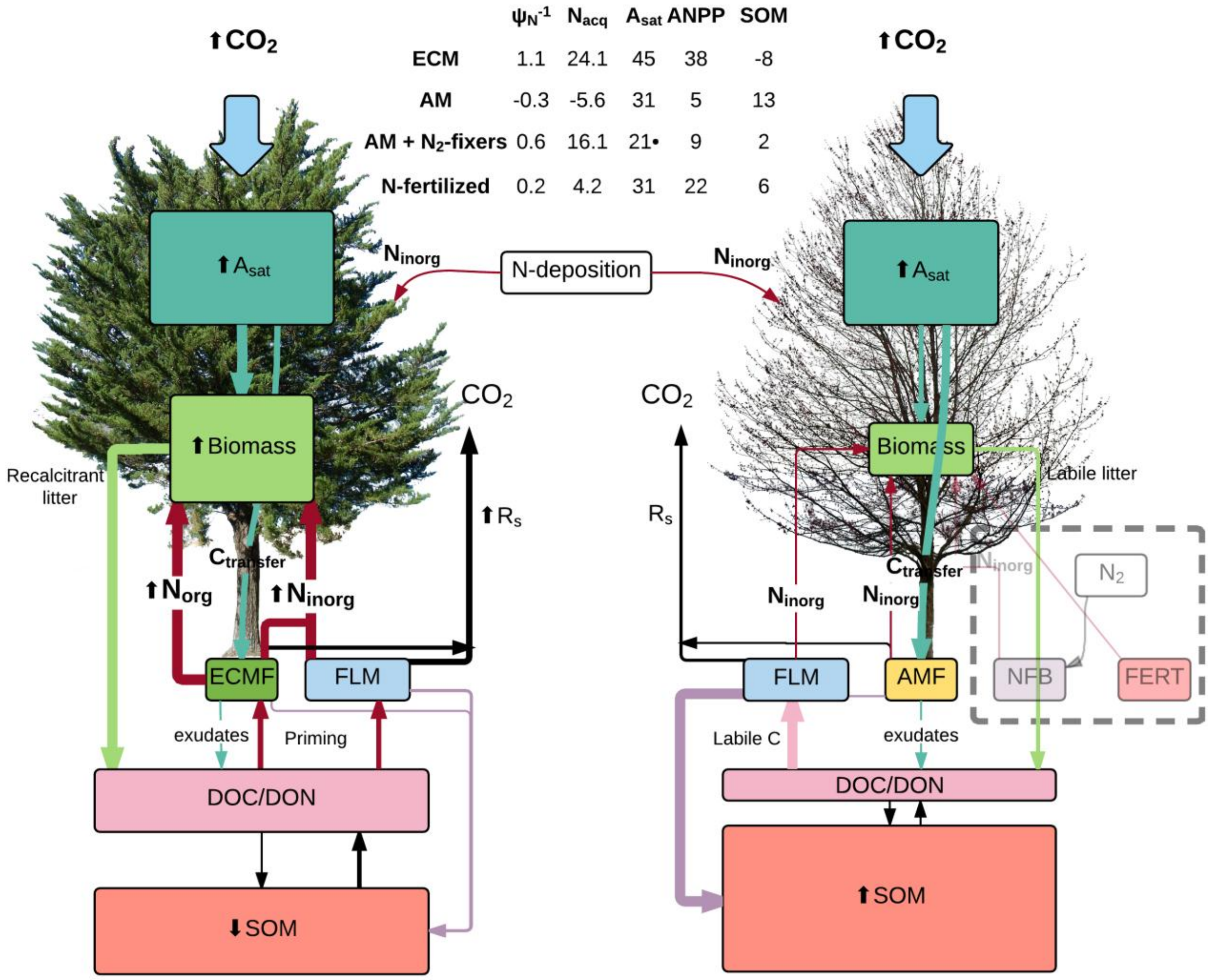


Figure 4. Relationship between the effect of elevated CO_2 on soil C content (%) and the N return on investment (Ψ_N^{-1}, Eq. 1). Sources of site-level data are given in Table 1



	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

Figure 5. Conceptual framework, representing the effects of elevated CO_2 under low 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 C cost of N-acquisition (Ψ_N^{-1}, Eq. 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. Other Abbreviations: $C_{transfer}$= C exported to mycorrhizae, 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. TO BE REDRAWN BY ILLUSTRATOR.

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