IMPERIAL COLLEGE LONDON
Department of Life Sciences

Thesis submitted for the degree of
DOCTOR OF PHILOSOPHY

The Effects of Nitrogen Availability on the Response of Terrestrial Ecosystems to Elevated Carbon Dioxide

Author:
César Terrer-Moreno

Supervisor:
Professor I. Colin Prentice

August 2017

Preface

This Thesis is the result of a three-year PhD project carried out at Department of Life Sciences, Imperial College London.

This research is a contribution to the Imperial College Initiative Grand Challenges in Ecosystems and the Environment and the AXA Chair Programme in Biosphere and Climate Impacts. The work by the author was supported by an Imperial College Ph.D. studentship within this program.

The Thesis consists of an introduction to the field, three chapters based on first-authored scientific manuscripts, and a final chapter of general conclusions.

Declaration of Originality

I hereby declare that all the work presented in this thesis is my own. Datasets used for analysis where from other sources properly acknowledged and referenced.

César Terrer

London, July 2017

Number of pages: 146
Word count: 43941

Copyright Declaration

Abstract

The effects of nitrogen availability on the response of terrestrial ecosystems to elevated carbon dioxide

Human activities are increasing the concentration of carbon dioxide (CO_2) in the atmosphere, warming the planet. Terrestrial ecosystems currently sequester about a quarter of human CO_2 emissions, slowing climate change. The principal mechanism believed to be responsible for this is an increasing rate of plant growth (that is, "CO_2 fertilization"). However, the fate of this ecosystem service is uncertain, as it has been proposed that soil nitrogen (N) availability will limit plants' capacity to continue absorbing increasing quantities of CO_2. Whether N will limit the CO_2 fertilization effect in the future will determine the rate at which human CO_2 emissions will accumulate in the atmosphere, thereby influencing the climate. In this thesis, I have collected and synthesized the large body of information about the N limitation of CO_2 fertilization, using data from experiments in which atmospheric CO_2 concentration is manipulated. I have found that the hypothesis that the increase in the strength of the CO_2 fertilization effect will be eliminated by restricted N availability is simplistic. Based on the experimental data available, I have found evidence supporting a mechanism by which plants under elevated CO_2 can acquire additional N in exchange for carbohydrates via symbiotic fungi. Using this framework, I have quantified the magnitude of the terrestrial CO_2 fertilization effect on plant biomass worldwide, and identified the areas of the global land mass that could potentially experience a greater enhancement in biomass under elevated CO_2. I propose a framework and areas of further research that may help models better simulate the interactions between the carbon and nitrogen cycles under elevated CO_2 using a plant-economics approach, in which nitrogen is a resource that can be acquired by plants in exchange for energy.

Contents

List of papers

I. 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.

II. 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: 358–358.

III. Terrer C, Vicca S, Stocker BD, Hungate BA, Phillips RP, Reich PB, Finzi AC, Prentice IC. Ecosystem responses to elevated CO_2 governed by plant-soil interactions and the cost of nitrogen acquisition.
Manuscript in review in New Phytologist as an invited Tansley review.

IV. Terrer C, Franklin O, Kaiser C, McCallum I, Soudzilovskaia NA, Vicca S, Stocker BD, Hungate BA, Reich PB, Prentice IC. Quantification and distribution of the CO_2 fertilization effect on plant biomass.
Manuscript in preparation

List of Figures

List of Tables

Chapter I: Introduction

1.1. Introduction

The current atmospheric [CO_2] is more than 40% above pre-industrial levels, and the projections are for concentrations to continue to rise during this century (*1*). This increase in atmospheric [CO_2] may be enabling plants to fix more carbon into carbohydrates via photosynthesis, potentially releasing them from limitations to growth imposed by this factor. Plant growth is a major component of carbon sink capacity (*2*), also related to carbon storage. Thus, it is key to assess the response of plant growth to atmospheric [CO_2] under a changing climate (*3*). If elevated CO_2 continues enhancing plant growth in the future, carbon sink activity would increase further, slowing down climate change (*4*).

Various large-scale observations show that the terrestrial biosphere is indeed acting as a carbon sink, taking up on average around 30% of all the CO_2 emitted by human activities (*5-8*). An inventory-based analysis has indicated that observed increases in forest carbon storage could fully account for the terrestrial carbon sink (*4*). Moreover, simple assumptions about the upscaling of CO_2 fertilization at the leaf level to increased biomass growth at the ecosystem level (*9*) lead to predictions of the terrestrial carbon sink that are consistent in magnitude with observations as summarized in the Global Carbon Project (*8*) and IPCC (*1*) carbon budgets. Extending this reasoning suggests that the terrestrial carbon sink should continue to increase throughout the 21st century, albeit with a diminishing return, as CO_2 ceases to be rate-limiting to photosynthesis at high concentrations, and due to the accelerating effect of increased temperature on the rate of heterotrophic respiration per unit of soil C (*10-12*).

On the other hand, biomass growth requires other elements than carbon (C). N in particular is a limiting factor for plant growth in many cool-climate and tropical ecosystems (*13-15*). This knowledge is based on many experiments in which the addition of inorganic N results in increased plant growth. A simple interpretation of ecological stoichiometry theory (*16*) would then lead to a prediction of no CO_2 fertilization effect, or none that could be sustained in the long run (*17*). An influential paper by Hungate *et al.* (*18*) estimated maximum potentials for plants to acquire additional N to support increased growth by two mechanisms: atmospheric deposition and biological fixation, and concluded that projections of increased future C uptake by terrestrial ecosystems (by models that were then state-of-the-art: (*19*, *20*)) were grossly exaggerated. This line of argument has been widely rehearsed, and apparently accepted by modellers who have developed schemes that reduce the magnitude of the CO_2 fertilization effect in response to limited N availability (*21*). It is now widely held that the CO_2 fertilization effect is overestimated in 'carbon-only' models and that it will be necessary to include nutrient limitations on the CO_2 effect in order to reduce its magnitude.

There is a major problem, however. Most of the CMIP5 models already underestimate the total historical CO_2 uptake (by oceans and land combined) (*22*), and the two that include interactive terrestrial N cycle model not only greatly underestimate CO_2 uptake but also fail to simulate the pattern of interannual variability in terrestrial CO_2 uptake (*12*), which is more realistically simulated by several carbon-only models. On the other hand, nutrient limitations are a key control on primary production (*23*) and it would be perverse to ignore them. It is surely reasonable to expect models to represent the most important processes seen in nature, and to be able to simulate large effects on ecosystem processes seen in nutrient addition experiments (*24*); which carbon-only models have no way to capture.

There is therefore a contradiction to be resolved. Models that can account for recent CO_2 uptake ignore nutrient limitations, while models that represent nutrient limitations cannot account for the magnitude of the terrestrial carbon sink. Exceptions (*25-27*) are models that assume that additional demand for N can always be supplied from some source (e.g. geological sources, enhanced N fixation) and thus they sidestep the contradiction, rather than resolving it.

1.1.1. What do we know about the effects of CO_2 on plant growth?

The resolution of this contradiction must take account of experimental results in which CO_2 concentration is elevated under field conditions. Much experimentation has been done since Hungate et al.'s (2003) paper was published. Free-Air Carbon dioxide Enrichment (FACE) technology is a powerful experimental approach that makes it possible to fumigate plants with elevated levels of CO_2 in their natural environment and thereby to study plant growth in future scenarios, avoiding most of the artefacts associated with greenhouses and chambers (see e.g. the review in ref. *28*).

From FACE experiments we know that elevated CO_2 (eCO_2) can initially increase both photosynthesis (*29*) and net primary production (NPP) (*30*, *31*). These responses were consistent across four of the most comprehensive and well-studied forest FACE experiments, leading to the general hypothesis that eCO_2 would increase tree growth in the future by an average of 23% for a *ca* 200 ppm elevation, compared to plants grown at current CO_2 (*31*). However, one of these four experiments showed a decrease in the eCO_2 effect on NPP after a few more years (ORNL in Fig. I.1). The initial growth response increased plant demand for N, which could have led to soil N depletion, constraining or suppressing the eCO_2 fertilization effect (*32*). This hypothesis is known as Progressive Nitrogen Limitation (PNL) (*33*). Fig. I.2 shows the decrease in N uptake in elevated CO_2 plots over time at ORNL. Consequently, the initial hypothesis of a sustained eCO_2 effect was no longer supported by one of the four forest FACE experiments initially analysed in ref. *31*. Some other experiments found similar results (e.g. *34*, *35*), in line with Hungate *et al.*'s (2003) hypothesis. The PNL hypothesis, apparently supported by the conclusions found at ORNL (*32*), gained momentum, with Luo *et al.*'s paper (33) doubling its number of citations in the period 2010-2015 compared to 2005-2010, and a total 820 citations (Google Scholar, July 2017).

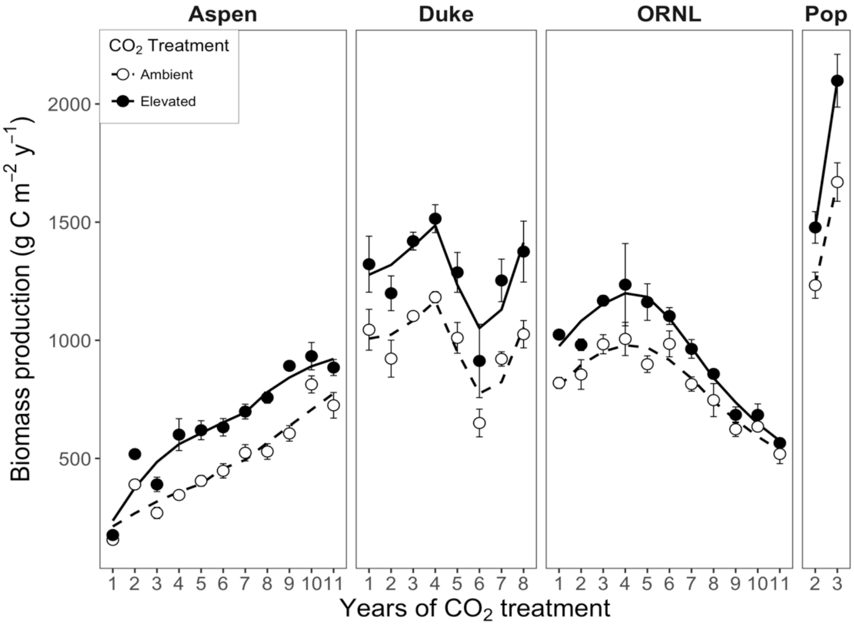


Figure I.1 Total biomass production (NPP) over time for four different FACE experiments in forests, under ambient (white dots, dashed lines) and elevated CO_2 (~550 ppm; solid dots and lines). Methodology from Norby et al. (2005). Data for Aspen, Duke, ORNL and PopFACE (Pop) experiments from Talhelm et al. (2014), McCarthy et al. (2010), Norby et al. (2010) and Norby et al. (2005) respectively. Error bars are standard error of the among-plot means.

As shown in Fig. I.1, however, this negative feedback of N availability on plant growth, has not been observed in the Duke FACE experiment, a pine plantation in North Carolina (*37*, *38*) and Aspen FACE, an aspen and birch forest in Wisconsin (*36*), driven by a sustained increase in N uptake under eCO_2 (Fig. I.2), suggesting that either: i) PNL might develop given enough time (*39*), or ii) some plants can overcome PNL through a cascade of effects triggered by eCO_2, which would involve interactions between the C and N cycles below-ground leading to an increase in plant N availability over time (*40*, *41*).

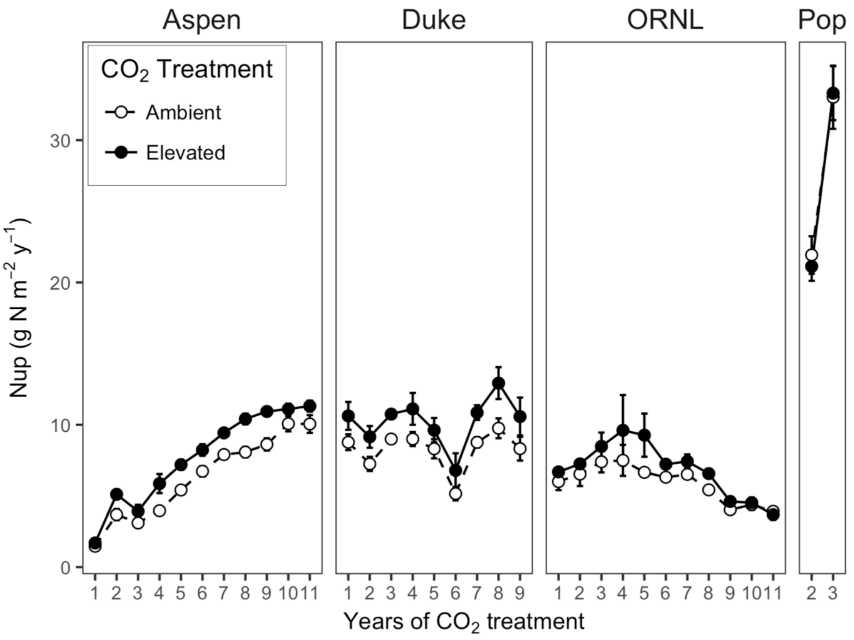


Figure I.2 Nitrogen uptake (N_{up}) over time for four different FACE experiments in forests, under ambient (white dots, dashed lines) and elevated levels of CO_2 (~550 ppm; solid dots and lines). Methodology from Finzi *et al.* (2007). Data for Aspen, Duke, ORNL and PopFACE (Pop) experiments from Talhelm *et al.* (2014), McCarthy *et al.* (2010), Norby *et al.* (2010) and Finzi *et al*. (2007) respectively. Error bars are standard error of the among-plot means.

1.1.2. Plant mechanisms to increase N availability

Current evidence indicates that a CO_2 effect on plant growth should be accompanied by an increase in N acquisition (*43*) (Fig. I.3). Therefore, differences in the capacity of plants to acquire extra N under eCO_2 (Fig. I.2) might be the key to explain the differences in magnitude of the CO_2 fertilisation effect between, for example, Duke and ORNL FACE experiments (Fig. I.1).

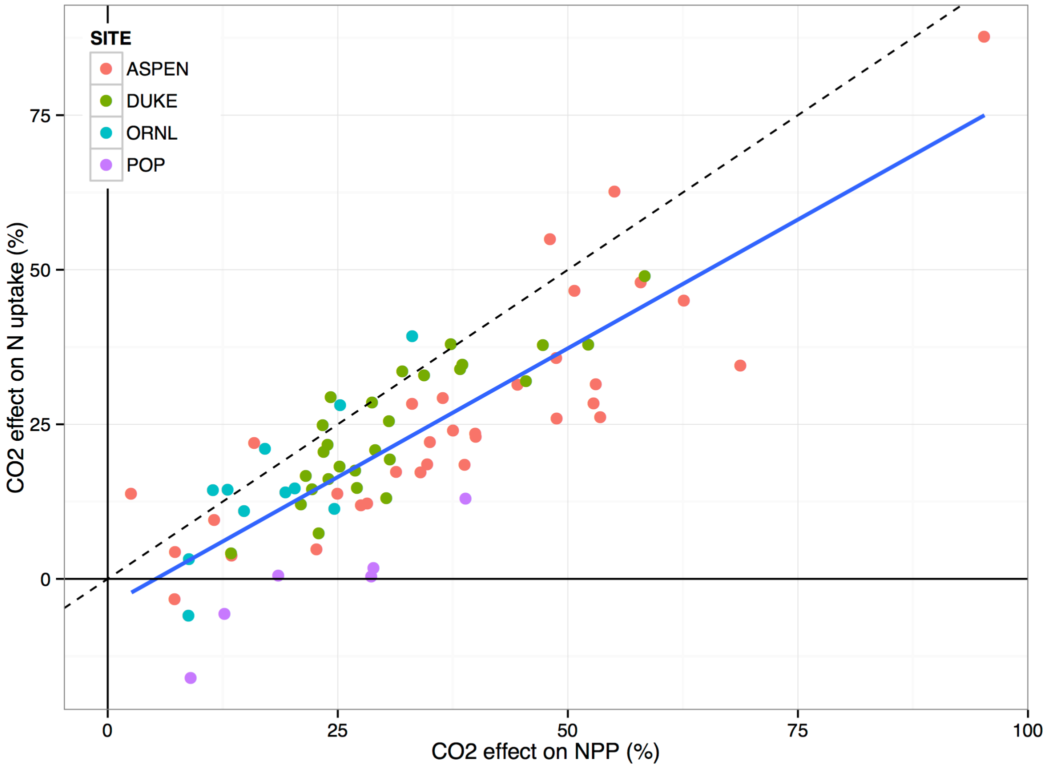


Figure I.3 Relationship between the effects of elevated CO_2 on NPP and N uptake (%) for four different FACE experiments in forests. Data from Figs I.1 and I.2.

New information regarding the processes of nutrient acquisition by plants, including the importance of mycorrhizae, root exudation, priming, and biological control on N fixation rates (Table I.1), may help to shed light on the mechanisms of enhanced N uptake, and their limits. Classically, litterfall has been assumed to be the only important flux of C from plant to soil, in what is now recognized as an oversimplified view of the terrestrial C cycle (*44*, *45*). Litter is decomposed by microorganisms, potentially releasing inorganic N that can be assimilated by plants to sustain their growth. This process creates a feedback loop between the C and N cycles (Fig. I.4). However, a key process not yet represented in most models (*46*) is the allocation of a significant proportion of net primary production (NPP) to mycorrhizal symbionts (*23*), and/or to the rhizosphere (zone of soil influenced by roots) in the form of exudates (C_{ex}, Fig. I.4). This labile C exported as exudates or allocated to mycorrhizal fungi and root symbionts may trigger more complex interactions between the C and N cycles, which potentially can enable plants to increase their access to nutrients, including N.

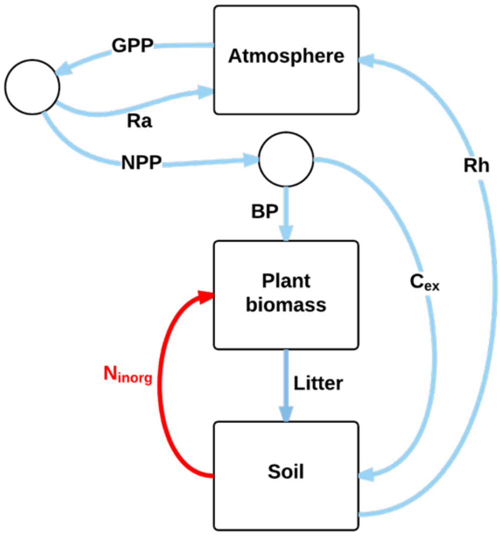


Figure I.4 Representation of the C cycle, including pools (squares), the fluxes of C between pools (blue arrows), and its interaction with the N cycle (red arrow). Circles represent the division of a flux in to two different sub-fluxes. Plants absorb CO_2 from the atmosphere via photosynthesis. Part of the photosynthetically fixed C (gross primary production, GPP) goes back to the atmosphere (Ra, autotrophic respiration). The remaining utilisable C (net primary production, NPP; NPP = GPP – Ra) can be allocated to biomass productivity (BP, growth), and to C_{ex} (C export, exudation into the soil and allocation to mycorrhizal fungi and root symbionts). When plants die, the C in plant biomass is transferred into the soil C pool. Soil microorganisms, fuelled by the C_{ex} released belowground, can decompose the available soil organic matter (SOM): a process in which N is transformed from organic to inorganic form (mineralization), and hence it can become available to plants to support their growth. The decomposition of SOM emits CO_2 back to the atmosphere (Rh, heterotrophic respiration).

The term "allocation of C belowground" found in the literature may refer to three different mechanisms plants can use to increase N availabilty: i) root growth: plants may increase the proportion of NPP allocated to root growth compared to wood or leaves, in order to explore a larger proportion of the soil; ii) root exudates (Table I.1): plants can release, or "exude", C compounds ("exudates") into the rhizosphere; iii) C allocation to symbionts (Table I.1). The last two mechanisms involve root-derived C sources allocated belowground, generally termed rhizodeposition (e.g. *47*). One of the main differences between these two types of rhizodeposition is that root exudates are labile C compounds released into the rhizosphere and used by free-living soil microorganisms, whereas the C allocated preferentially to symbionts is taken up directly by the symbiont from inside the root, and hence not directly accessible to the rest of soil microbiota. Although these pathways are usually unaccounted elements of NPP, they may represent as much as 16% of all photosynthetically fixed carbon in forests with low nutrient availability (*23*). These two strategies can trigger the so-called "priming effect" (Table I.1), which may increase plant N-availability (and in turn, plant biomass) to an extent not yet quantified, nor considered by current experiments and models. It seems plausible that under eCO_2 and N-limitations, plants use part of the extra C to be allocated belowground and increase N availability to sustain biomass enhancement (*38*, *48*). In addition to these direct mechanisms to increase N availability, two further processes may affect C-N interactions and N availability indirectly: i) tissue turnover, which increases SOM availability in the long term; and ii) litter quality (C:N ratio), which influences whether N is mineralized or immobilized by microbes (Table I.1).

Long-term, well-replicated eCO_2 experiments are too few to allow a full understanding of eCO_2 effects, and do not represent all life-form vegetation groups (*30*, *49*). As a consequence, our conceptual model of the effects of eCO_2 on vegetation may not be appropriately represented in models, and current Earth-system models cannot simulate these effects confidently (*50*-*53*), leading to high uncertainty in predictions of the effects of rising atmospheric CO_2 on plant growth. In a data-model intercomparison study between the observations of Duke and ORNL FACE experiments and the simulations of 11 ecosystem models at these two sites, Zaehle *et al.* (*52*) found that models could not correctly simulate the long-term NPP response under eCO_2 at both sites. Indeed, current models simply do not account for any increase in below-ground C allocation under eCO_2 (*52, 53*) and so models cannot simulate the potential of plants to increase N availability – even though this effect has been found in several experiments (*38*, *48*).

Table I.1 Glossary

Root exudates	C compounds released into the rhizosphere by plants. The term "exudation" is sometimes used to describe both the C released into the soil mainly used by bacteria, and the C allocated to symbionts. To avoid confusion about these two processes, we hereafter use the term "exudate" and "exudation" to refer only to the former
Allocation to symbionts	Unlike exudates, these C compounds are not released into the soil, but taken up directly by the symbiont from inside the root
Mycorrhizal symbiosis	Symbiotic relationship between a mycorrhizal fungus and mycorrhizal plant root common in most plants. In exchange for C, the mycorrhizal fungi assist plants in obtaining nutrients and water to maintain plant growth.
Nitrogen mineralization and immobilization	Transformation of organic N to inorganic forms is mediated by microbes. Plant N availability is determined by net mineralization, which is the difference between gross mineralization and immobilization. Microbes are usually C-limited, so root exudates fuel microbial growth, leading to microbial N assimilation to meet their N requirements for protein synthesis, and limiting plant N availability. When microbial C availability drops due to microbial assimilation, microbial mortality occurs and the scarce C is only used for maintenance instead of growth. Under these circumstances microbes do not require N for protein synthesis (no immobilization), and inorganic N is released to the soil, where it can be assimilated by plants. Overall, greater rates of immobilization indicate microbial N-limitations and high C substrate, whereas high rates of net mineralization indicate microbial C-limitations and low C substrate.
Priming effect hypothesis	Short-term change in the decomposition rate of SOM that can result in the release of nutrients in the soil. Priming effects can be triggered by different factors, but in the context of this thesis, we refer to the increase in microbial decomposition of SOM fueled by root exudates. Thereby, the priming effect hypothesis suggest that plants can regulate microbial-driven mineralization of N through the release of organic C compounds via roots, that results in an increase in plant N uptake.
Progressive nitrogen limitation (PNL) hypothesis	Enhanced plant growth under eCO_2 leads to increased N uptake and its sequestration in plant biomass and soil organic matter and a decrease in plant available N. Without any changes in ecosystem N inputs and losses and no adjustment of ecosystem C:N stoichiometry, this leads to a negative feedback on plant growth, suppressing its initial stimulation on longer time scales (years).
Progressive release from nitrogen limitation (PRNL) hypothesis	A common feature of a range of C-N cycle models is that ecosystem N retention is simulated to be enhanced under eCO_2 (higher plant N uptake, smaller inorganic N pools, less N losses). Together with a delayed enhancement of N release by a higher SOM decomposition flux, short-term PNL is alleviated on a longer time scale (decades to centuries) and under gradually increasing CO_2.

1.2. Statement of the problem / knowledge gap

- Long-term, well-replicated eCO_2 experiments are too few, and do not represent all life-form vegetation groups. Consequently, the current understanding of the effects of eCO_2 on plant growth is limited. Experiments in which both N and CO_2 effects are combined are even more scarce, and the role of N availability on the eCO_2 effect is highly uncertain.

- Results from elevated CO_2 experiments are commonly analysed individually, and the general mechanisms that drive the differences among experiments in the magnitude of the CO_2 fertilisation effect under low N availability is still lacking. It is therefore necessary to analyse available results globally to formulate a conceptual framework of the interactions between the C and N cycles that is consistent with observations and experimental results.

- There is some evidence supporting the hypothesis that different levels of N availability at different FACE sites may explain divergent results (*37*, *42*). It has been further discovered that nutrient availability is the key factor determining the efficiency of trees (*23*) and ecosystems as a whole (*54*) to store the assimilated carbon. However, the lack of a standardised metric for nutrient availability currently impedes fully unraveling these global patterns and also hampers the inclusion of functional relationships between carbon cycling and nutrient availability in global models.

As a consequence, two big questions remain open regarding C-N interactions under elevated CO_2:

1. What are the mechanisms that drive an increase in plant N availability under elevated CO_2*?*

2. Why do some ecosystems seem to have the potential to overcome N limitations and show a positive increase in growth under elevated CO_2*, whereas the effect is nonexistent in other ecosystems?*

As the current conceptual model of the effects of eCO_2 on vegetation and N feedback is incomplete, current Earth-system models cannot simulate these effects accurately – leading to high uncertainty about the effects of rising atmospheric CO_2.

1.3. Aims / research questions

The overall aim of this PhD thesis is to improve understanding of the effects that eCO_2 triggers both above- and below-ground, affecting N availability, which in turn feeds back on plant growth.

The work undertaken has the following aims that pertain to C-N interactions under eCO_2. Each aim has some questions that follow from it:

1. *Develop a conceptual model of C-N interactions.* I undertake a comprehensive reassessment and reconciliation of the range of observations relevant to the assessment of CO_2 effects on ecosystems, and the role of nutrients, especially nitrogen. Understanding the interactions that produce divergent experimental results is essential to predict future changes in N availability for plants, and this in turn controls the extent to which future CO_2 will enhance plant growth and atmospheric C sequestration.

Question: *To what extent does nitrogen availability influence the effect of elevated* CO_2 *on plant growth? Can plants increase N availability under elevated* CO_2*? If so, what are the factors that could drive potential differences in the amount of N acquired under elevated* CO_2 *across ecosystems?*

2. *Develop a simple quantitative model to simulate the role of nitrogen availability on the* eCO_2 *effect*, based on the conceptual model previously developed.

Question: *What is the magnitude and distribution of the terrestrial* CO_2 *fertilization effect on plant biomass?*

1.4. References

1. P. Ciais *et al.*, in *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, T. F. Stocker et al., Eds. (Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 2013), pp. 465–570.
2. G. B. Bonan, Forests and climate change: forcings, feedbacks, and the climate benefits of forests. *Science*. **320**, 1444–1449 (2008).
3. J. Canadell *et al.*, Contributions to accelerating atmospheric CO_2 growth from economic activity, carbon intensity, and efficiency of natural sinks. *Proceedings of the National Academy of Sciences*. **104**, 18866–18870 (2007).
4. Y. Pan *et al.*, A Large and Persistent Carbon Sink in the World's Forests. *Science*. **333**, 988–993 (2011).
5. A. P. A. Ballantyne, C. B. C. Alden, J. B. J. Miller, P. P. P. Tans, J. W. C. J. White, Increase in observed net carbon dioxide uptake by land and oceans during the past 50 years. *Nature*. **488**, 70–72 (2012).
6. P. Ciais *et al.*, Current systematic carbon-cycle observations and the need for implementing a policy-relevant carbon observing system. *Biogeosciences*. **11**, 3547–3602 (2014).
7. E. Shevliakova *et al.*, Historical warming reduced due to enhanced land carbon uptake. *Proceedings of the National Academy of Sciences*. **110**, 16730–16735 (2013).
8. C. L. Quéré *et al.*, Global Carbon Budget 2016. *Earth System Science Data*. **8**, 605–649 (2016).
9. T. Keenan *et al.*, Recent pause in the growth rate of atmospheric CO_2 due to enhanced terrestrial carbon uptake. *Nature Communications*. **7**, 13428 (2016).
10. J. Lloyd, J. A. Taylor, On the Temperature Dependence of Soil Respiration. *Functional Ecology*. **8**, 315 (1994).
11. P. M. Cox *et al.*, Sensitivity of tropical carbon to climate change constrained by carbon dioxide variability. *Nature*. **494**, 341–344 (2013).
12. S. Wenzel, P. M. Cox, V. Eyring, P. Friedlingstein, Emergent constraints on climate-carbon cycle feedbacks in the CMIP5 Earth system models. *Journal of Geophysical Research: Biogeosciences*. **119**, 794–807 (2014).
13. P. Vitousek, R. Howarth, Nitrogen limitation on land and in the sea: How can it occur? *Biogeochemistry*. **13** (1991).
14. D. S. LeBauer, K. K. Treseder, Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology*. **89**, 371–379 (2008).
15. D. N. L. Menge, L. O. Hedin, S. W. Pacala, Nitrogen and Phosphorus Limitation over Long-Term Ecosystem Development in Terrestrial Ecosystems. *PLoS ONE*. **7**, e42045 (2012).
16. J. J. Elser *et al.*, Biological stoichiometry from genes to ecosystems. *Ecol Lett*. **3**, 540–550 (2000).
17. C. Körner, Carbon limitation in trees. *Journal of Ecology*. **91**, 4–17 (2003).
18. B. A. Hungate, J. S. Dukes, M. R. Shaw, Y. Luo, C. B. Field, Nitrogen and climate

change. *Science*. **302**, 1512–1513 (2003).

19. W. Cramer *et al.*, Global response of terrestrial ecosystem structure and function to CO_2 and climate change: results from six dynamic global vegetation models. *Global Change Biology*. **7**, 357–373 (2001).

20. C. Prentice, M. Heimann, S. Sitch, The Carbon Balance of the Terrestrial Biosphere: Ecosystem Models and Atmospheric Observations. *Ecological Applications*. **10**, 1553 (2000).

21. W. R. Wieder, C. C. Cleveland, W. K. Smith, K. Todd-Brown, Future productivity and carbon storage limited by terrestrial nutrient availability. *Nature Geosci*. **8**, 441–444 (2015).

22. F. M. Hoffman *et al.*, Causes and implications of persistent atmospheric carbon dioxide biases in Earth System Models. *Journal of Geophysical Research: Biogeosciences*. **119**, 141–162 (2014).

23. S. Vicca *et al.*, Fertile forests produce biomass more efficiently. *Ecol Lett*. **15**, 520–526 (2012).

24. P. B. Reich, B. A. Hungate, Y. Luo, Carbon-Nitrogen Interactions in Terrestrial Ecosystems in Response to Rising Atmospheric Carbon Dioxide. *Annu. Rev. Ecol. Evol. Syst.* **37**, 611–636 (2006).

25. Xu-Ri, I. C. Prentice, Terrestrial nitrogen cycle simulation with a dynamic global vegetation model. *Global Change Biology*. **14**, 1745–1764 (2008).

26. J. Kattge *et al.*, TRY – a global database of plant traits. *Global Change Biology*. **17**, 2905–2935 (2011).

27. B. D. Stocker *et al.*, Multiple greenhouse-gas feedbacks from the land biosphere under future climate change scenarios. *Nature Climate change*. **3**, 666–672 (2013).

28. H. Saxe, D. S. Ellsworth, J. Heath, Tree and forest functioning in an enriched CO_2 atmosphere. *New Phytologist*. **139**, 395–436 (1998).

29. E. A. Ainsworth, S. P. Long, 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 Phytol*. **165**, 351–372 (2005).

30. R. S. Nowak, D. S. Ellsworth, S. D. Smith, 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 (2004).

31. R. Norby *et al.*, Forest response to elevated CO_2 is conserved across a broad range of productivity. *Proceedings of the National Academy of Sciences*. **102**, 18052–18056 (2005).

32. R. J. Norby, J. M. Warren, C. M. Iversen, B. E. Medlyn, R. E. McMurtrie, CO_2 enhancement of forest productivity constrained by limited nitrogen availability. *Proc Natl Acad Sci U S A*. **107**, 19368–19373 (2010).

33. Y. Luo *et al.*, Progressive Nitrogen Limitation of Ecosystem Responses to Rising Atmospheric Carbon Dioxide. *BioScience*. **54**, 731–739 (2004).

34. M. Dawes *et al.*, An alpine treeline in a carbon dioxide-rich world: synthesis of a nine-year free-air carbon dioxide enrichment study. *Oecologia*. **171**, 623–37 (2013).

35. B. A. Newingham *et al.*, 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 (2013).

36. A. F. Talhelm *et al.*, Elevated carbon dioxide and ozone alter productivity and ecosystem carbon content in northern temperate forests. *Global Change Biology*. **20**, 2492–2504 (2014).

37. H. R. McCarthy *et al.*, 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 Phytol*. **185**, 514–528 (2010).

38. J. E. Drake *et al.*, Increases in the flux of carbon belowground stimulate nitrogen uptake and sustain the long-term enhancement of forest productivity under elevated CO_2. *Ecol Lett*. **14**, 349–357 (2011).

39. R. J. Norby, D. R. Zak, Ecological lessons from free-air CO_2 enrichment (FACE) experiments. *Annual Review of Ecology*. **42**, 181–203 (2011).

40. A. C. Finzi *et al.*, Rhizosphere processes are quantitatively important components of terrestrial carbon and nutrient cycles. *Global Change Biology*. **21**, 2082–2094 (2015).

41. R. P. Phillips *et al.*, Roots and fungi accelerate carbon and nitrogen cycling in forests exposed to elevated CO_2. *Ecol Lett*. **15**, 1042–1049 (2012).

42. A. C. Finzi *et al.*, Increases in nitrogen uptake rather than nitrogen-use efficiency support higher rates of temperate forest productivity under elevated CO_2. *Proc Natl Acad Sci U S A*. **104**, 14014–14019 (2007).

43. Z. Feng *et al.*, Constraints to nitrogen acquisition of terrestrial plants under elevated CO_2. *Global Change Biology*. **21**, 3152–3168 (2015).

44. M. Makkonen, M. P. Berg, I. T. Handa, Highly consistent effects of plant litter identity and functional traits on decomposition across a latitudinal gradient. *Ecology*. **15**, 1033–1041 (2012).

45. V. Brovkin *et al.*, Plant-driven variation in decomposition rates improves projections of global litter stock distribution. *Biogeosciences*. **9**, 565–576 (2012).

46. B. E. Medlyn *et al.*, Using ecosystem experiments to improve vegetation models. *Nature Climate change*. **5**, 528–534 (2015).

47. D. L. Jones, C. Nguyen, R. D. Finlay, Carbon flow in the rhizosphere: carbon trading at the soil–root interface. *Plant Soil*. **321**, 5–33 (2009).

48. R. P. Phillips, A. C. Finzi, E. S. Bernhardt, Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO_2 fumigation. *Ecol Lett*. **14**, 187–194 (2011).

49. R. J. Norby *et al.*, Model-data synthesis for the next generation of forest free-air CO_2 enrichment (FACE) experiments. *New Phytol*. **209**, 17–28 (2015).

50. M. G. De Kauwe *et al.*, Forest water use and water use efficiency at elevated CO_2 : a model-data intercomparison at two contrasting temperate forest FACE sites. *Global Change Biology*. **19**, 1759–1779 (2013).

51. A. P. Walker *et al.*, Comprehensive ecosystem model-data synthesis using multiple data sets at two temperate forest free-air CO_2 enrichment experiments: Model performance at ambient CO_2 concentration. *Journal of Geophysical Research:*

Biogeosciences. **119**, 937–964 (2014).

52. S. Zaehle *et al.*, Evaluation of 11 terrestrial carbon–nitrogen cycle models against observations from two temperate Free-Air CO_2 Enrichment studies. *New Phytol.* **202**, 803–822 (2014).

53. M. G. De Kauwe *et al.*, 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 Phytol*. **203**, 883–899 (2014).

54. M. Fernández-Martínez *et al.*, Nutrient availability as the key regulator of global forest carbon balance. *Nature Climate change*. **4**, 471–476 (2014).

Chapter II: Mycorrhizal association as a primary control of the CO_2 fertilization effect

From Terrer, C. et al. “Mycorrhizal association as a primary control of the CO_2 fertilization effect”. Science, vol. 353, issue 6294 (July 2016), p. 72-74. DOI: 10.1126/science.ssf4610. Reprinted with permission from AAAS.

Mycorrhizal association as a primary control of the CO_2 fertilization effect

2.1. Overview

Plants buffer increasing atmospheric CO_2 concentrations through enhanced growth, but the question whether nitrogen availability constrains the magnitude of this ecosystem service remains unresolved. Synthesizing experiments from around the world, we show that CO_2 fertilization is best explained by a simple interaction between nitrogen availability and mycorrhizal association. Plant species that associate with ectomycorrhizal fungi show a strong biomass increase (30 ± 3%, $P<0.001$) in response to elevated CO_2 regardless of nitrogen availability, whereas low nitrogen availability limits CO_2 fertilization (0 ± 5%, $P=0.946$) in plants that associate with arbuscular mycorrhizal fungi. The incorporation of mycorrhizae in global carbon cycle models is feasible, and crucial if we are to accurately project ecosystem responses and feedbacks to climate change.

2.2. Introduction

Terrestrial ecosystems sequester annually about a quarter of anthropogenic CO_2 emissions (*1*), slowing climate change. Will this effect persist? Two contradictory hypotheses have been offered: the first is that CO_2 will continue to enhance plant growth, partially mitigating anthropogenic CO_2 emissions (*1*, *2*), while the second is that nitrogen (N) availability will limit the CO_2 fertilization effect (*3*, *4*), reducing future CO_2 uptake by the terrestrial biosphere (*5*-*7*). Plants experimentally exposed to elevated levels of CO_2 (eCO_2) show a range of responses in biomass, from large and persistent (*8*, *9*) to transient (*6*), to non-existent (*10*), leaving the question of CO_2 fertilization open. Differences might be driven by different levels of plant N availability across experiments (*11*), but N availability alone cannot explain contrasting results based on available evidence (*7*, *12*). For instance, among two of the most studied free-air CO_2 enrichment (FACE) experiments with trees, eCO_2 enhanced biomass production only during the first few years at ORNL-FACE (*6*), whereas trees in the Duke FACE experiment showed a sustained enhancement during the course of the experiment (*8*), despite N limitation. In addition to N limitation, other factors have been suggested as potential drivers of the response of plant biomass to eCO_2: age of the vegetation (*13*), water limitation (*14*), temperature (*15*), type of vegetation (*12*), or even the eCO_2 fumigation technology used (*11*). Although these factors may explain some observations, none has been found to be general, explaining the range of observations globally.

About 94% of plant species form associations with mycorrhizal fungi, an ancient mutualism thought to have facilitated the colonization of land by early plants (*16*). In this mutualism, the fungus transfers nutrients and water to the plant in exchange for carbohydrates, necessary for fungal growth. Mycorrhizal fungi are critical for terrestrial C cycling (*17*), are known to influence plant growth (*18*), nutrient cycling (*19*, *20*), and soil carbon storage (*21*), and respond strongly to elevated CO_2 (*22*, *23*). Yet, their impact on the N-dependence of the CO_2 fertilization effect has not been tested, despite the increasing evidence that N limitation constrains the CO_2 fertilization effect (*5*). Arbuscular mycorrhizae (AM) and ectomycorrhizae (ECM) are, by far, the most widespread types of mycorrhizae (*24*): AM-plants predominate in deserts, grasslands, shrublands and tropical forest ecosystems, whereas ECM-fungi predominate in boreal and many temperate forests (e.g., those dominated by *Pinus*). ECM can transfer N to the host plant under eCO_2 to sustain CO_2 fertilization (*25*), whereas the symbiotic effects of AM fungi in N-limited systems can range from beneficial to parasitic (*19*). Hence, the association of *Liquidambar styraciflua* with AM-fungi at ORNL, and *Pinus taeda* with ECM-fungi at Duke, might explain why only trees in the latter could increase N-uptake and take advantage of eCO_2 to grow faster for a sustained period (*20*, *25*). Here, we tested the hypothesis that the differences in the nutrient economies of ECM and AM fungi influence global patterns of the magnitude of plant biomass responses to elevated CO_2.

2.3. Methods

We synthesized data (overview in Table S-II.1) on total plant biomass (g m^{-2}) from 83 eCO_2 experiments (Fig. S-II.1), separating responses into aboveground biomass (n=83, Fig. S-II.2) and belowground biomass (n=82, Fig. S-II.3) in a mixed effects meta-analysis. As potential drivers of the plant biomass response, we considered the increase in atmospheric CO_2 concentration (ΔCO_2), mean annual precipitation (MAP), mean annual temperature

(MAT), age of the vegetation at the start of the experiment, vegetation type (e.g. grassland, forest), CO_2 fumigation technology (e.g. FACE, growth chamber), length of the study (years), dominant mycorrhizal type (AM or ECM), and N-status (high or low N availability, considering soil characteristics and occasional fertilizer treatments, following the approach by Vicca *et al.* (*17*) and assigning all experiments with indications for some degree of N limitation to the "low N" class and experiments that were unlikely N limited to the "high N" class; Materials and Methods, Table S-II.2).

Data collection

We collected published and unpublished data on total, aboveground and belowground biomass (g m^{-2}) from eCO_2 experiments. We consulted the list of CO_2 experiments from INTERFACE (https://www.bio.purdue.edu/INTERFACE/experiments.php), the Global List of FACE Experiments from the Oak Ridge National Laboratory (http://facedata.ornl.gov/global_face.html), the ClimMani database on manipulation experiments (www.climmani.org), and the database described by Dieleman *et al.* (*35*), and freely available (https://www.researchgate.net/publication/276839560_Database_of_Global_Change_Manipulation_Experiments). We used Google Scholar to locate the most recent publications for each of the previously listed experiments, and collected data on total, aboveground and belowground plant biomass for ambient and elevated CO_2 treatments. When the data were presented in figures we extracted mean values and standard error using GraphClick. Additionally, we collected available data about the vegetation, sample size, soil fertility, land use history, MAP, MAT and the age of the vegetation at the start of the experiment. Some experiments were not included in the meta-analysis if they met any of the following exclusion criteria: i) species did not form associations with AM or ECM; ii) papers did not report biomass data; iii) standard error or standard deviation was not provided; iv) information about the fertility of the site was not reported (e.g. soil type, pH, or qualitative assessments of N-availability); v) duration of the experiment was less than 3 months.

Experimental units

Where possible, data were collected at the species level, and different species within experiments were considered independent when grown in monoculture; when available data were pooled across several species, these were only included in the analysis if all the species were associated with the same type of mycorrhizal fungi. Experiments in which the most abundant species were C_4 or N_2-fixing species were excluded from the main analysis to avoid confounding effects. Different N-fertilization treatments within experiments were considered independent. These selection criteria allowed us to assess N-status and mycorrhizal association in the individual experimental units. Overview of the experiments included in the dataset is in Table S-II.1, and the data included in the meta-analysis in figs. S-II.1-II.3.

Nitrogen classification

N-classification followed a similar approach as refs. *17*, *36*, *37*, but did not consider limitations of nutrients other than N. Experiments were classified as "low" or "high" in terms of N-availability based on the amount of N-fertilizer applied (if so), as well as the original N-availability at the sites, as a function of available data such as soil type, nitrogen and carbon content, pH, land use history, and the assessment of N-availability (reported in the literature or provided by the site principal investigators -PIs-). For example, sandy soils have an inherently low nitrogen retention capacity, and are typically N poor if not fertilized or exposed to high N deposition. The C:N ratio of soil is indicative of the decomposability of soil organic matter. Especially high C:N ratios (>25) suggest low availability of N and potential N immobilization by microorganisms (*38*). For some experiments, the available soil data were scarce. We then requested direct expert assessment by the PIs. More information regarding the classification of each experiment and the underlying reasons is given in Table S-II.2. We classified all sites that had indications of N limitation to "low N"; sites that were unlikely N-limited (e.g., where N fertilization had no effects on plant growth) were designated as "high N". We created an alternative N-classification with an additional "medium" class that grouped all those experiments with intermediate N-availability (e.g. moderately fertile soils with no N-fertilization, or N-poor soils with modest N-fertilization, but in the range in which N-availability limits growth). This alternative classification was used as a sensitivity analysis to test that the observed effects were not driven by sites with intermediate N availability classified as "low N" in the main classification.

Mycorrhizal status classification

We used the check-list in ref. *39*, with additional classifications derived from the literature, to classify plant species as ECM, or AM. Species that form associations with both ECM and AM fungi (e.g. *Populus* spp.) were classified as ECM because these species can potentially benefit from increased N-availability due to the presence of ECM-fungi, as hypothesized. Overall, CO_2 responses from species associated with AM and ECM were similar to strictly ECM species, and their exclusion did not alter the results of the meta-analysis.

N-fixing species.

When the data were presented at the plot level, with specification of the species present in each plot, all plots containing N-fixing species were not included in the main analysis because they might be particularly responsive to eCO_2 (*40*). We analysed the role of N-fixing species in a separate meta-analysis that included AM-species in N-limited ecosystems only, using the same methods as in the main meta-analysis, and including the responses from both N-fixing and their accompanying non N-fixing species. There were three N-limited-AM-dominated experiments that included N-fixing experiments for total biomass and seven for aboveground biomass. Therefore, the analysis of N-fixing species was performed using aboveground biomass only. The list of experiments with N-fixing species included in the analysis is in Table S-II.3.

ΔCO_2

Ambient CO_2 treatments had concentrations ranging from 280 to 400 μmol mol^{-1}, whereas elevated CO_2 treatments had concentrations ranging from 420 to 780 μmol mol^{-1}, with an average of ~650 μmol mol^{-1}. ΔCO_2 was calculated as the natural log of the difference in CO_2 concentrations between elevated and ambient treatments: $\Delta CO_2 = \ln (eC_a/aC_a)$. Results from meta-analysis shown here were normalised for ΔCO_2 from 400 (current) to 650 (average [eCO_2]) μmol mol^{-1}, after including ΔCO_2 400-650 as a variable in a mixed-effects meta-regression.

MAT, MAP and age of the vegetation

MAT and MAP data were collected from the original source or from WorldClim Global Climate Data (*41*). When the experimental units were irrigated we did not use MAP data in the analysis, but instead we assigned the maximum value of MAP in the dataset (1750 mm y^{-1}) to all irrigated experimental units. When the age of the vegetation at the start of the experiment was not specified in the study, we assigned a value of 1 for seedlings, annuals, frequently grazed vegetation, or experiments under controlled burning, and the maximum value in the dataset (50 years) when the site was classified as “intact” or similar.

Calculation of effect sizes

We used the response ratio (*RR*, mean response in elevated to ambient CO_2 plots) to measure effect sizes (*42*). We calculated the natural logarithm of the response ratio (log *RR*) and its variance for each experimental unit to obtain a single response metric (*42*) in a weighted, mixed-effects model using the R package *metafor* (*43*). Measurements across different time-points (e.g. over several years or harvests) were considered non-independent, and we computed a combined effect across time-points so that only one effect size was analysed per experimental unit. The combined variance that takes account of the correlation among the different time-point measurements was calculated following the method described in Borenstein *et al.* (*44*):

Equation 1

$$\operatorname{var}\left(\frac{1}{m}\sum_{i=1}^{m}Y_i\right)=\left(\frac{1}{m}\right)^2\left(\sum_{i=1}^{m}V_i+\sum_{i\neq j}(r_{ij}\sqrt{V_i}\sqrt{V_j})\right)$$

where V_i is the variance of effect size Y_i for several time-points i=1,...,m and r_{ij} as the correlation between Y_i and Y_j, with r=0 equivalent to treating two outcomes as independent, underestimating the error (and overestimating the precision). We used a conservative approach with r=1 (assuming non independence). The outcome was not sensitive to the assumption of r=1, with r=0 (independence) and r=0.5 rendering slightly different SE terms (and *P-value*) that did not alter the conclusions (Table S-II.4).

Weighting functions

Effect size measurements from individual studies in meta-analysis are commonly weighted by the inverse of the variance (*45*) (W_V). For this particular analysis, not only well replicated, but also long-term studies provide more reliable estimates of ecosystem CO_2 responses (*46*). Thus, we weighted the individual effects by both replication and experimental duration by using the function in refs *11*, *47*:

Equation 2

$$W_{NY} = (n_a * n_e)/(n_a + n_e) + (yr * yr)/(yr + yr),$$

with n_a and n_e as the number of replicates under ambient and elevated CO_2, and *yr* as the length of the study in years. Both weighting functions were used, but W_V assigned about half of the total weight to two experiments with very low variance creating a sub-optimal imbalance, and the results using W_V are only shown for comparison purposes in Fig. S-II.6). Results shown in the main report and figures correspond to the meta-analysis using W_{NY} as weights. In all cases, the conclusions were consistent across various weighting functions.

Calculation of the overall true effect

We used the R package *metafor* (*43*) to calculate overall effect sizes and 95% confidence intervals (CI). The mixed-effects meta-regression model was fitted using maximum likelihood for the amount of residual heterogeneity. The Knapp and Hartung method (*48*) was included as an adjustment to the standard errors of the estimated coefficients to control the Type I error rate (*49*). This method leads to an F-test for sets of model predictors (test of moderators) to test their significance to influence the average effect of CO_2. For individual model coefficients, the method leads to t-tests. We inferred CO_2 effects if the calculated 95 % CI did not overlap with zero. The log response ratio was back-transformed and expressed as percentage CO_2 effect ($[e^{\log RR} - 1] \times 100$) to ease interpretation in figures and text.

Model selection

We analysed the plausibility of models containing all potential combinations of the studied predictors in a mixed-effects meta-regression model using maximum likelihood estimation. For this purpose, we used the R packages *gmulti* (*50*) and *metafor* (*43*). Model selection was based on AICc. The relative importance value for a particular predictor was equal to the sum of the Akaike weights (probability that a model is the most plausible model) for the models in which the predictor appears. Hence, a predictor that is included in models with large Akaike weights will receive a high importance value. These values can be regarded as the overall support for each variable across all models. A cut-off of 0.7 was set to differentiate between important and non-essential predictors.

2.4. Results

Model selection analysis, based on corrected Akaike Information Criterion (AICc), showed that the most parsimonious model within 2 AICc units included N-status, mycorrhizal type and ΔCO_2 ($P<0.001$). The relative importance of the predictors (Fig. II.1) supported the removal of climate variables, length of the experiment, age of the vegetation, fumigation technology and system type. Some predictors reduced the CO_2 effect on biomass (e.g. age of the vegetation), whereas others were associated with an increased CO_2 effect (e.g. ECM, ΔCO_2, high N availability) (Fig. S-II.4).

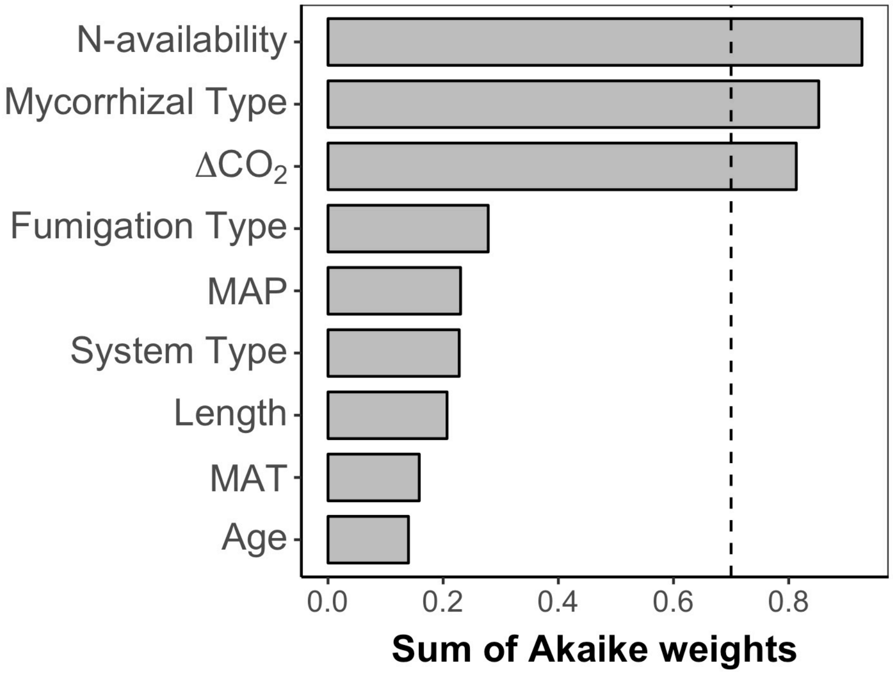


Figure II.1 Model-averaged importance of the predictors of the CO_2 fertilization effect on total biomass. The importance is based on the sum of Akaike weights derived from model selection using AICc (Akaike's Information Criteria corrected for small samples). Cutoff set at 0.7 (dashed line) to differentiate among the most important predictors.

The response of total biomass to an increase of CO_2 from 400 to 650 μmol mol^{-1} was larger ($P<0.001$) in ECM (30 ± 3%, $P<0.001$) than in AM-dominated (7 ± 4%, $P=0.089$) ecosystems (mean ± SE, mixed effects meta-regression). The overall response of total biomass was 20 ± 3% ($P<0.001$), similar to previous meta-analyses (e.g., *15*), with a larger effect under high (27 ± 4%, $P<0.001$) than low N availability (15 ± 4%, $P<0.001$), as expected (*5*, *7*, *11*). Furthermore, we found a strong interaction between mycorrhizal type and N-status ($P<0.001$): under low N availability, eCO_2 had no effect on total biomass of AM-dominated species (0 ± 5%, $P=0.946$) but increased biomass by 28 ± 5% in ECM-dominated species ($P<0.001$) (Fig. II.2A). Under high N availability, the CO_2 effect on total biomass in both AM- and ECM-dominated species was significant: 20 ± 6% ($P=0.002$) for AM and 33 ± 4% ($P<0.001$) for ECM (Fig. II.2A), with no significant differences between the two groups ($P=0.139$). Hence, high N availability significantly increased the CO_2 effect in AM (Post-hoc, Tukey's HSD: adj-$P=0.038$) but not in ECM-associated species (adj-$P=0.999$).

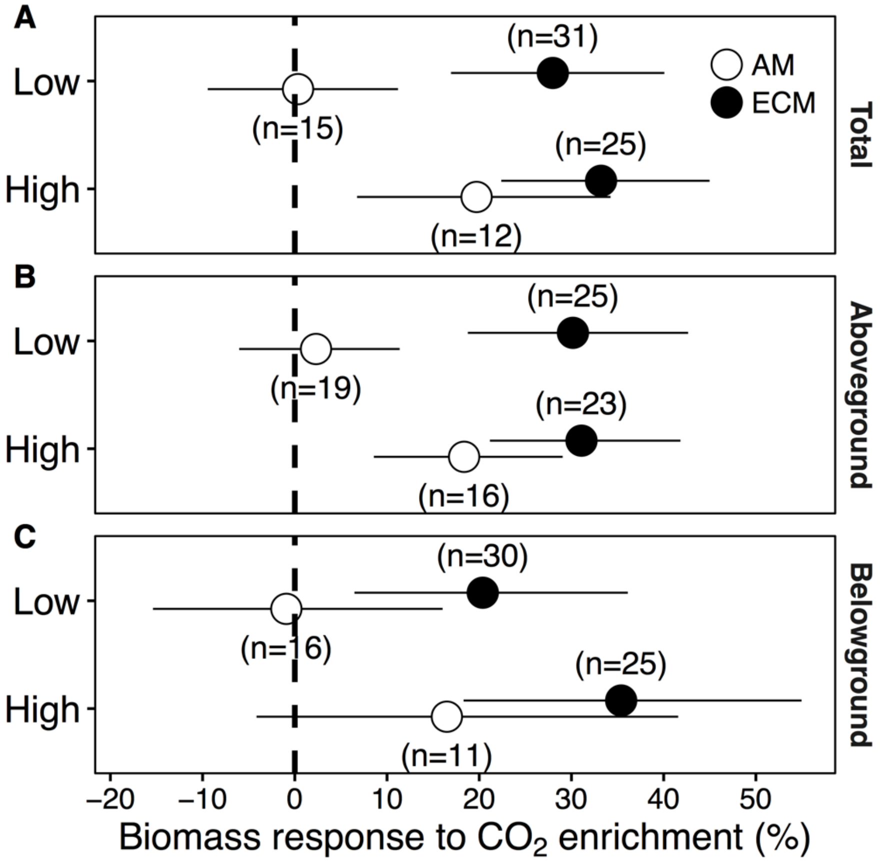


Figure II.2 Overall effects of CO_2 on plant biomass. Effects on **(A)** total, **(B)** aboveground, and **(C)** belowground biomass for two types of mycorrhizal plants species (AM: arbuscular mycorrhizae and ECM: ectomycorrhizae) in N limited experiments (low N) or experiments that are unlikely N limited (high N). Overall means and 95% confidence intervals are given; we interpret CO_2 effects when the zero line is not crossed.

The patterns observed for total biomass were reflected in both aboveground and belowground biomass. Under low N availability, eCO_2 stimulated aboveground biomass significantly in ECM plants (*P*<0.001), with no effect in AM plants (*P*=0.584) (Fig. II.2B). Similarly, eCO_2 enhanced belowground biomass in ECM plants at low N (*P*=0.003), but not in AM plants (*P*=0.907) (Fig. II.2C).

We conducted a sensitivity analysis to ensure the findings were robust. First, we added an intermediate level of N availability (Table S-II.2) by assigning some ecosystems that were initially classified as “low” to a “medium” class (e.g. Duke, Aspen, ORNL) (Fig. S-II.5). This enabled testing whether the large CO_2 stimulation in ECM plants was driven by experiments with intermediate N availability. Second, we weighted individual experiments by the inverse of the mixed-model variance (Fig. S-II.6), to ensure that the weights of the meta-analysis did not affect the outcome. Third, we ran a separate meta-analysis with the subset of experiments with trees only (Fig. S-II.7). Previous meta-analysis have reported that trees are more responsive to eCO_2 than grasslands (*12*); as such, our findings could reflect differences of plant growth form rather than mycorrhizal association *per se*. Since trees are the only type of vegetation that can associate with ECM and AM (or both), an analysis of tree responses to eCO_2 can thus be used to isolate the influence of mycorrhizal type from that of vegetation growth form. These three sensitivity analyses confirmed that the CO_2 stimulation of total and aboveground plant biomass was significant and large in ECM plants regardless of N availability, whereas the effect was not significant in AM plants under low N availability. The trend was consistent for belowground biomass in ECM plants, although with high variance and low sample size, the effect was not significant (P=0.244) under low N when the “medium” class was included.

Plant N uptake can be enhanced through mycorrhizal associations, or through associations with N fixing microbes. Some of the CO_2 experiments in our study contained N-fixing species, which might have increased N availability (Table S-II.3). eCO_2 stimulated aboveground biomass in AM species under low N by 8 ± 3% (P=0.019) in this subgroup of experiments that included N-fixing species, whereas the remaining AM experiments under low N availability showed no biomass response to eCO_2 (1 ± 10%, P=0.893). But even with the additional N input from N_2 fixation, the 8% biomass increase in AM plants under low N was considerably smaller than the 28 ± 5% increase found for ECM plants.

2.5. Discussion

Most CO_2 experiments have been carried out in the Northern Hemisphere (Fig. S-II.8, where N, rather than phosphorus (P), is limiting. AM fungi transfer large quantities of P to the plant, and hence are more likely mutualistic in P-limited ecosystems (*19*). Tropical forests are typically associated with P limitations and dominated by AM-fungi, and could potentially show enhanced biomass under eCO_2. The role of nutrients on the CO_2 fertilization effect in these P-limited forests has yet to be explored (*26*).

Responses of plants to rising CO_2 are thus well explained by a simple interaction between nitrogen (N) and microbial mutualists: when N availability is limited, only plant species that associate with ECM-fungi show an overall biomass increase due to eCO_2. Several mechanisms could explain these responses. First, ECM-associated plants typically allocate more C to support mycorrhizae than AM plants, particularly under eCO_2 (*23*). Moreover, because some ECM fungi, unlike AM fungi, produce extracellular enzymes that degrade organic N compounds (*27*), increased allocation to ECM fungi under eCO_2 may supply host plants with the N needed to sustain their growth response to eCO_2. This may explain why eCO_2 often stimulates priming effects in ECM-dominated ecosystems (*28*, *29*). Second, differences in litter quality between ECM and AM plants may influence how much N is available to be primed or decomposed. Several studies have reported that AM plants produce litters that decompose faster than ECM plants (*20*, *30*). Given emerging evidence that fast decomposing litters promote the formation of stable mineral-associated organic matter (*31*, *32*), much of the organic N in AM-dominated ecosystems may be inaccessible to AM plants or their associated mycorrhizae (*20*). And while slow-degrading ECM litters may reduce N availability in the short-term, most of the N exists in particulate forms, which should be accessible to most microbes (including ECM fungi). Therefore, AM fungi are equipped with less specialized enzymes for N acquisition than ECM and occur in soils were N is more tightly protected. Both factors would presumably limit the enhancement of AM plant growth in response to eCO_2.

Mycorrhizal symbioses are not accounted for in most global vegetation models (but see ref. *24*). Thus, the projected CO_2 fertilization effect by "carbon-only models" (*1*) is likely overestimated for AM-dominated ecosystems, which cover ~65% of the global vegetated area (*24*), albeit only when N limited. On the other hand, global models that consider N limitation to constrain the CO_2 fertilization effect (*4*) likely underestimate responses of ECM plants to eCO_2, an area that encompasses ~35% of the vegetated area of the earth (*24*), most of which is considered N limited by these models. Our framework reconciles the apparent discrepancy between widespread N limitation (*3*) assumed to limit C sequestration on land (*4*), and the observed increase over time of the terrestrial C sink (*1*, *2*), thought to be driven primarily by CO_2 fertilization (*33*). These results may also partly explain past findings that forests (commonly ECM) show stronger responses to eCO_2 compared to grasslands (AM) (*12*). We propose that the CO_2 fertilization effect be quantified based on mycorrhizal type and soil nitrogen status, and that large-scale ecosystem models incorporate mycorrhizal types to account for the differences in biomass enhancement by eCO_2. Mycorrhizae are ubiquitous, and sort predictably with plant functional type (*24*, *34*), making feasible their inclusion in models to capture this microbial influence on global biogeochemistry. Accounting for the influence of mycorrhizae will improve representation of the CO_2 fertilization effect in vegetation models, critical for projecting ecosystem responses and feedbacks to climate change.

2.6. Supplementary Material

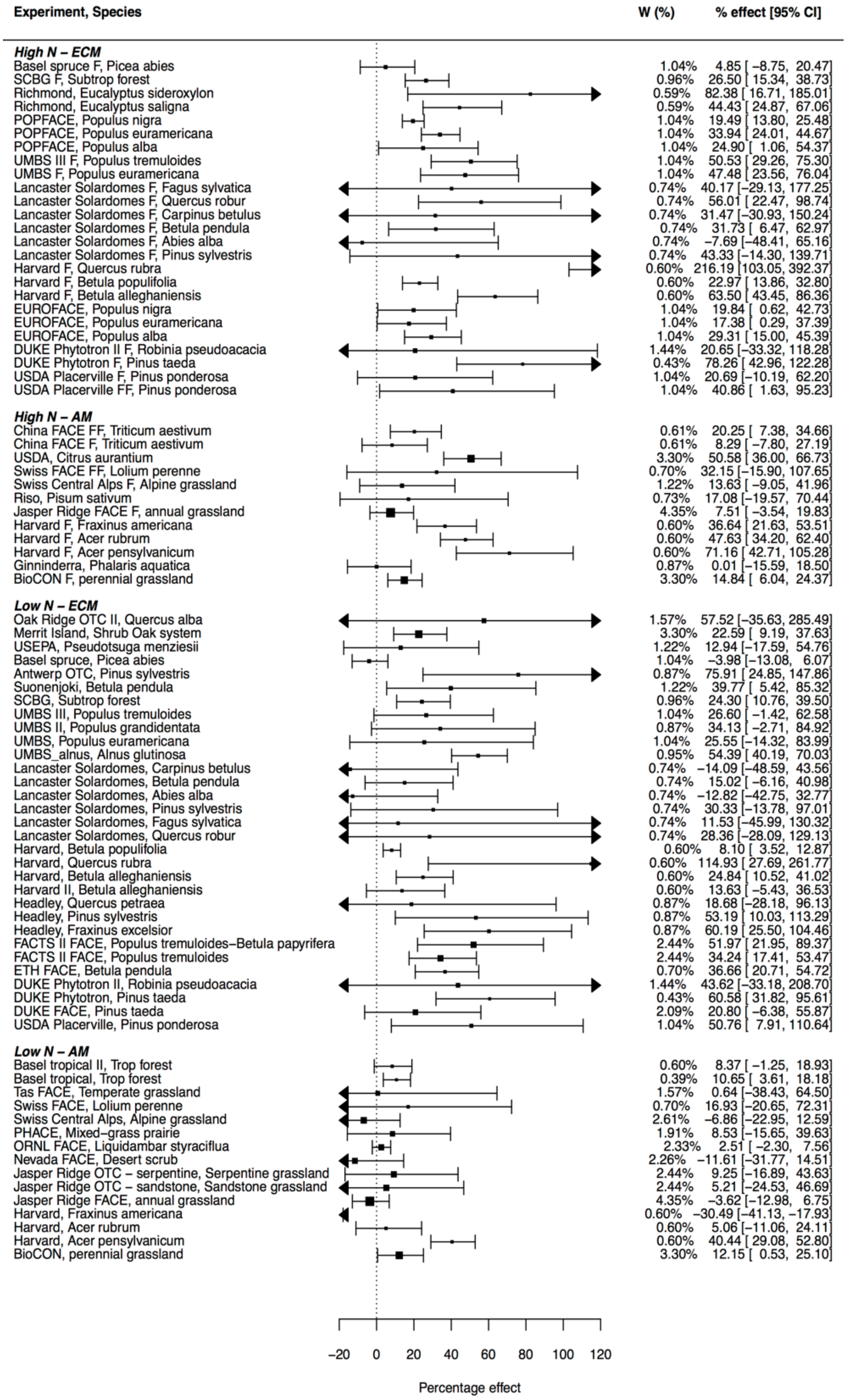


Figure S-II.1 Total biomass data included in meta-analysis in Fig. II.2A. W (%) are the weights used in the meta-analysis, based on the number of replicates and the length (years) of the studies.

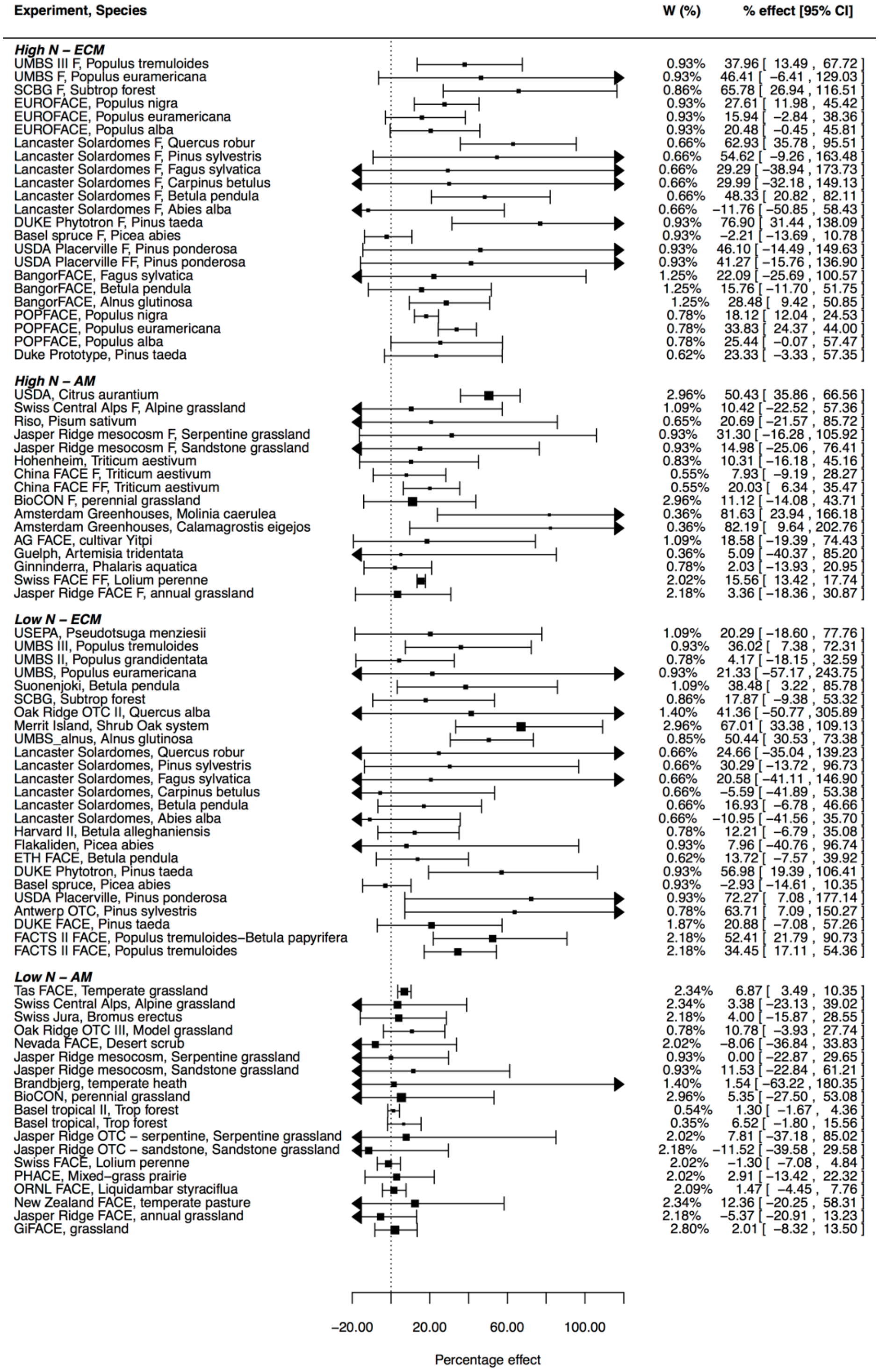


Figure S-II.2 Aboveground biomass data included in meta-analysis in Fig. II.2B. W (%) are the weights used in the meta-analysis, based on the number of replicates and the length (years) of the studies.

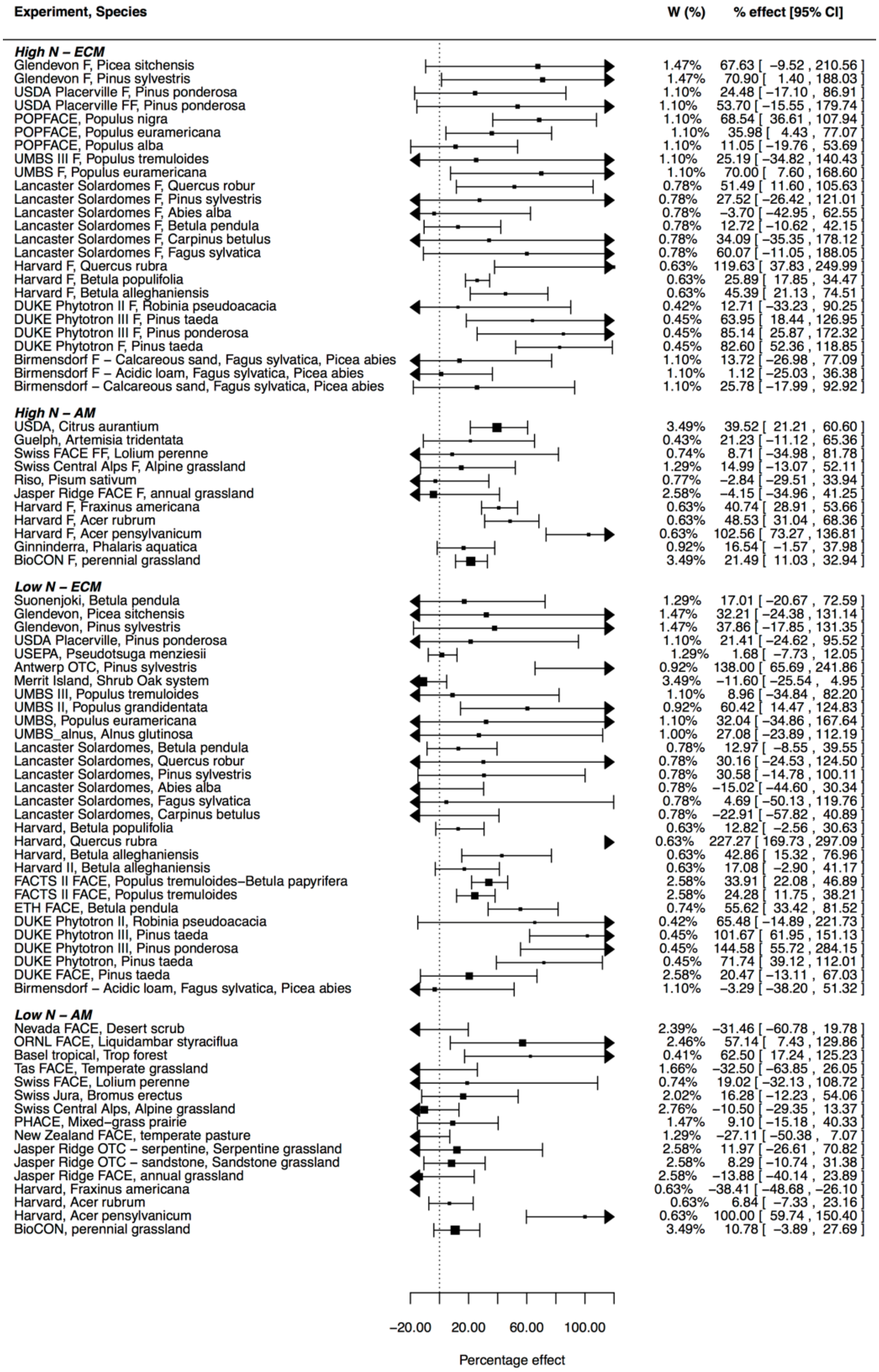


Figure S-II.3 Belowground biomass data included in meta-analysis in Fig. II.2C. W (%) are the weights used in the meta-analysis, based on the number of replicates and the length (years) of the studies.

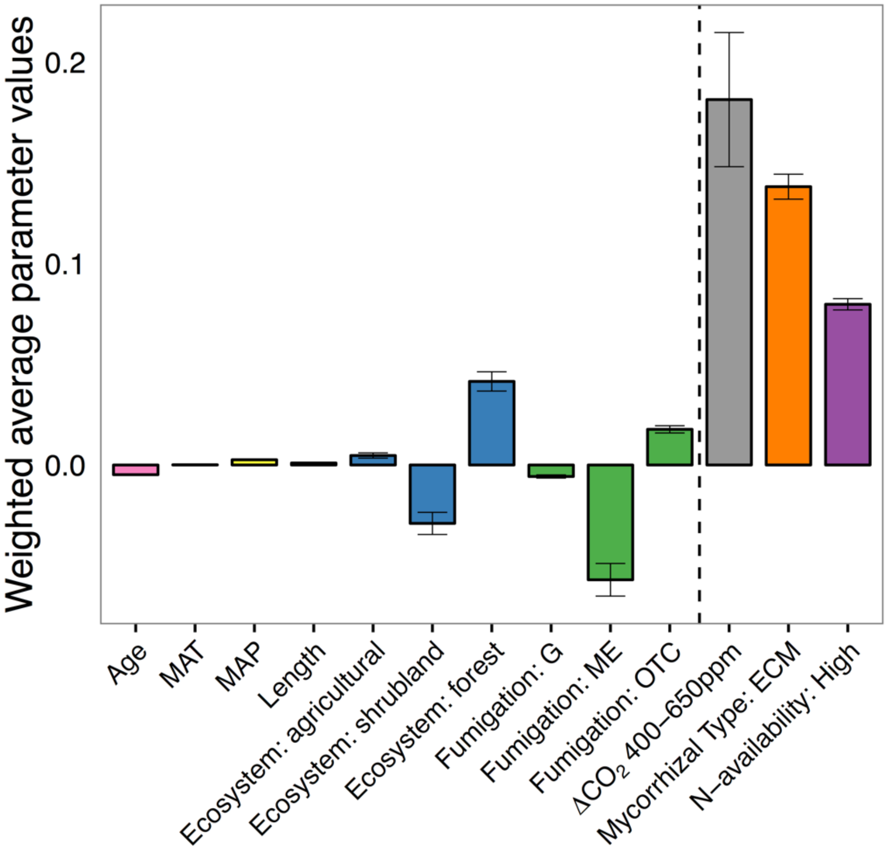


Figure S-II.4 Weighted average parameter values of model coefficients. Weights equal to the model probabilities. Error bars are weighted SE. Model parameters in increasing relative importance, with predictors on the right side of the dashed line as the terms included in the AICc-selected best model and sum of Akaike weights > 0.7. G = Greenhouse/Growth chamber, ME = Model ecosystem, OTC = Open Top Chamber, ΔCO_2 = $[CO_2]$ increment from 400 to 650 ppm. Reference parameters for qualitative factors are Fumigation: FACE, Ecosystem: grassland, Mycorrhizal type: AM, N-availability: Low.

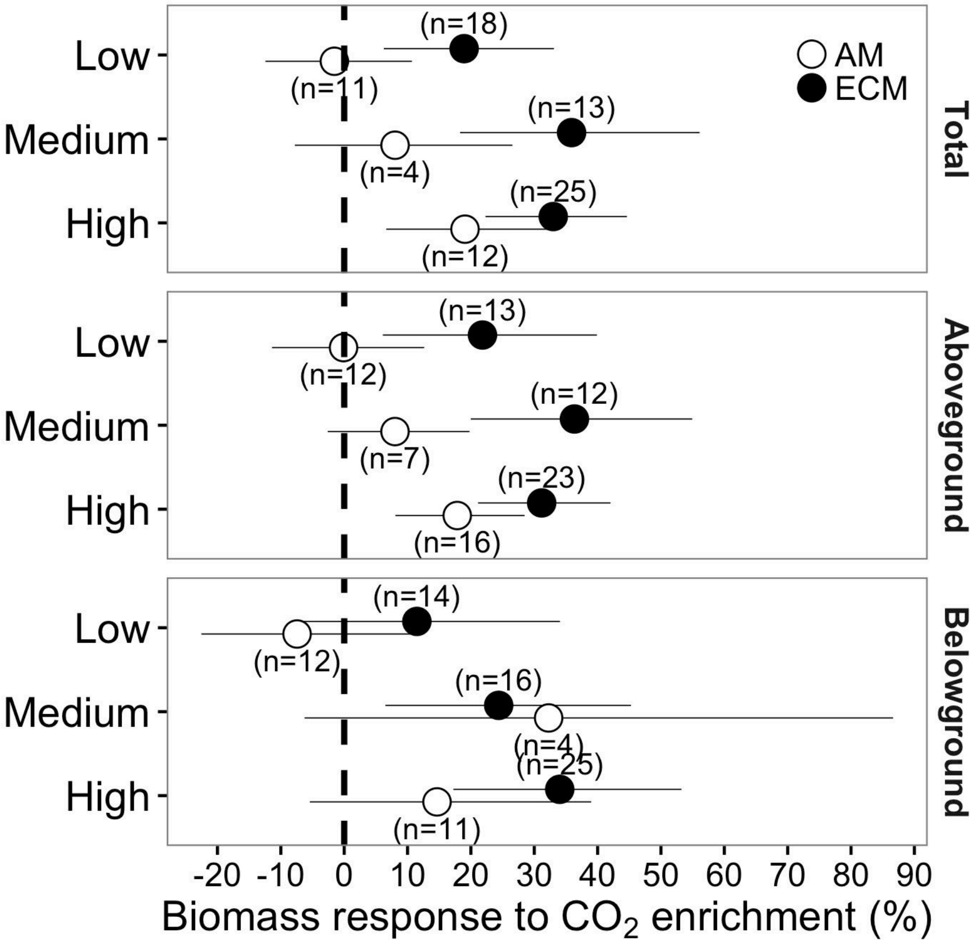


Figure S-II.5 Overall effects of CO_2 on total, aboveground, and belowground biomass for two types of mycorrhizal plants species (AM: arbuscular mycorrhizae and ECM: ectomycorrhizae) in strongly N limited experiments (low N), moderately N limited experiments (medium N) or experiments that are unlikely N limited (high N). Overall means and 95% confidence intervals are given; we interpret CO_2 effects when the zero line is not crossed.

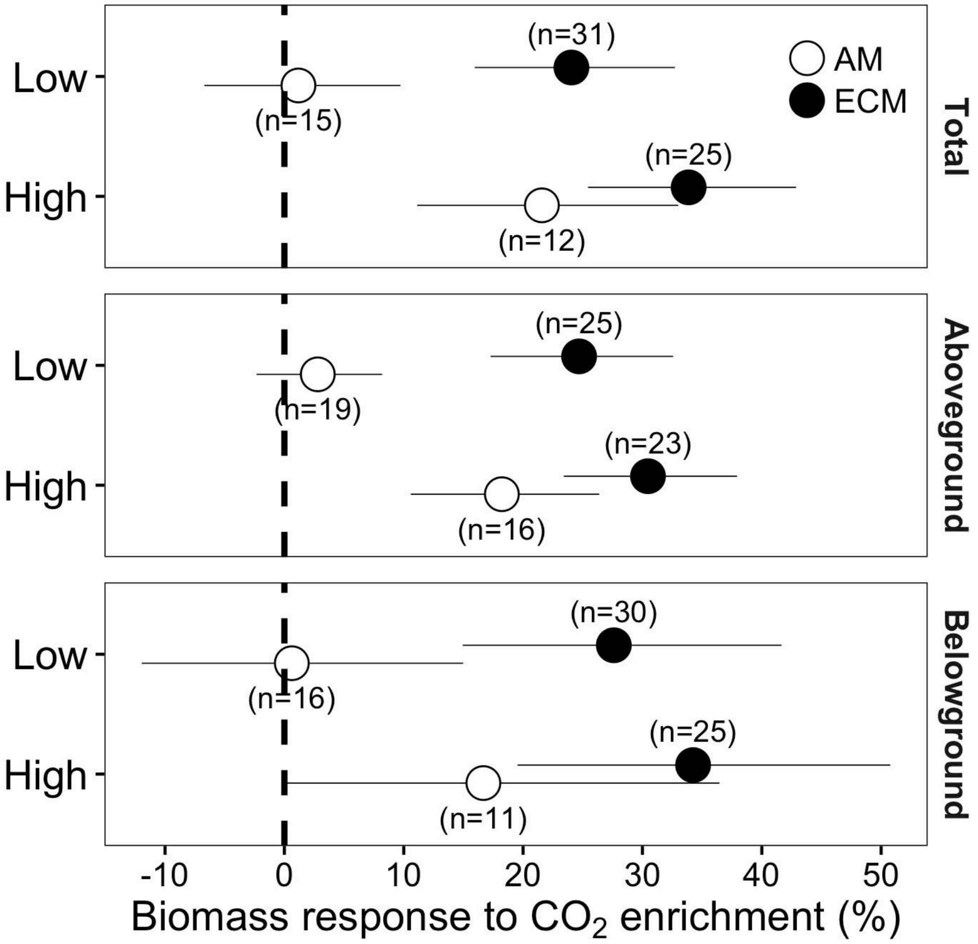


Figure S-II.6 Overall effects of CO_2 on total, aboveground, and belowground biomass for two types of mycorrhizal plants species (AM: arbuscular mycorrhizae and ECM: ectomycorrhizae) in N limited experiments (low N) or experiments that are unlikely N limited (high N). Experiments in this meta-analysis are weighted by the inverse of the variance, whereas weights in main meta-analysis in Fig. II.2 are based on sample size and length (years) of the experiments. Overall means and 95% confidence intervals are given; we interpret CO_2 effects when the zero line is not crossed.

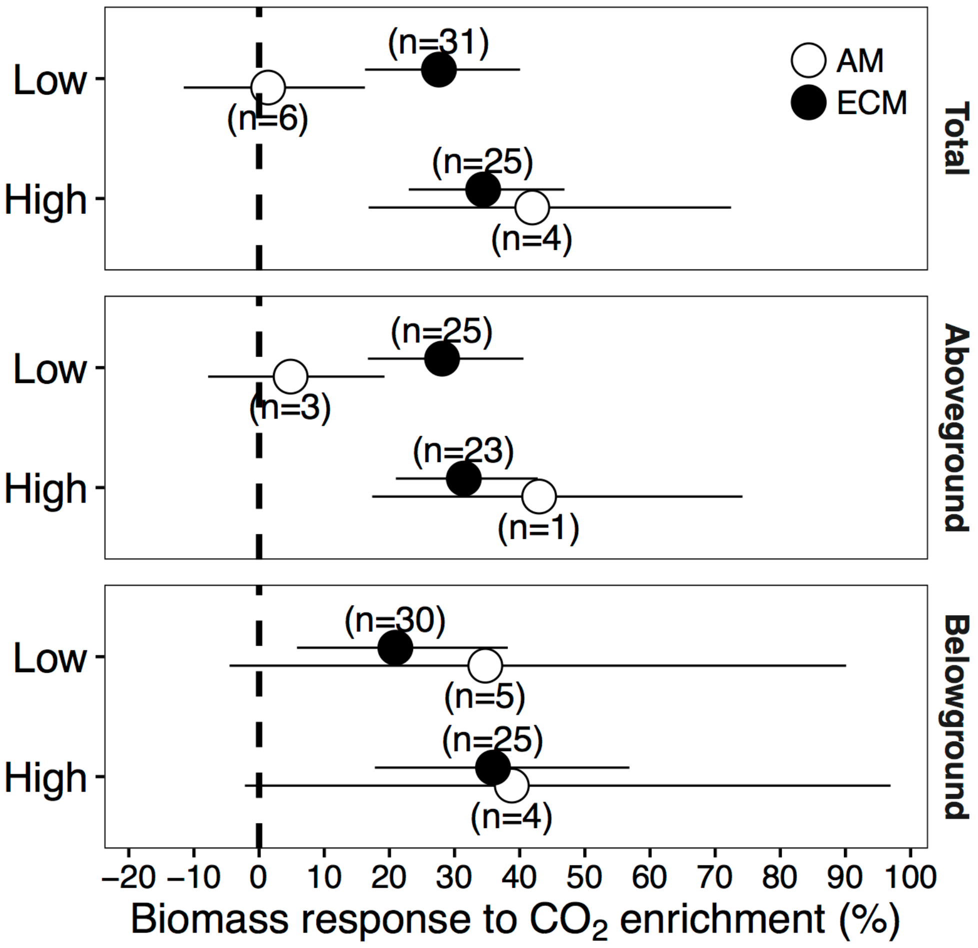


Figure S-II.7 Meta-analysis output for the subset of experiments with tree species, showing the effects of CO_2 on total, aboveground, and belowground biomass for two types of mycorrhizal plants species (AM: arbuscular mycorrhizae and ECM: ectomycorrhizae) in N limited experiments (low N) or experiments that are unlikely N limited (high N). Overall means and 95% confidence intervals are given; we interpret CO_2 effects when the zero line is not crossed.

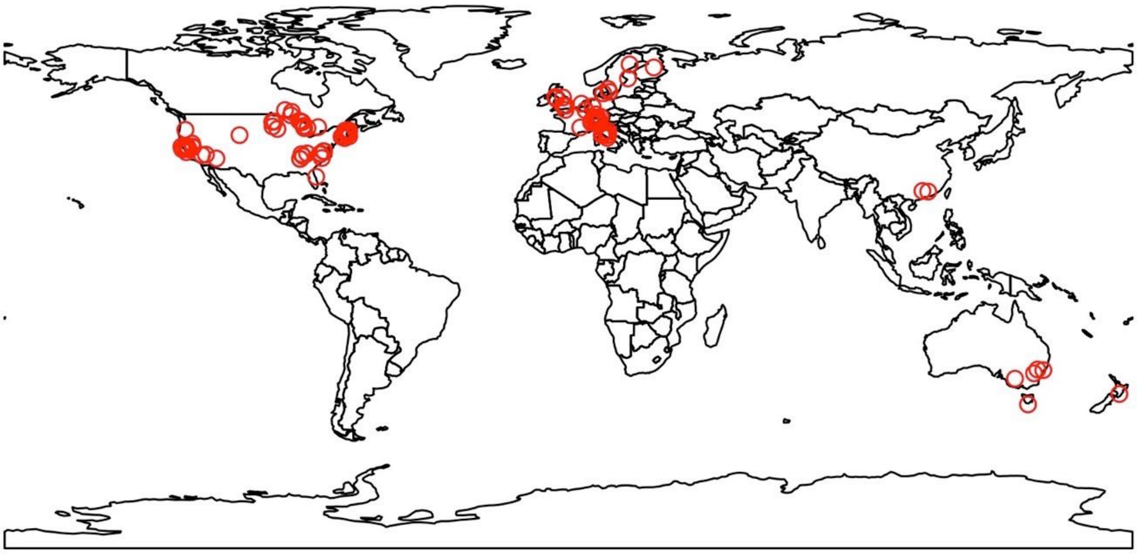


Figure S-II.8 Location of elevated CO_2 experiment with total biomass data included in the dataset (Fig. S-II.1). Experiments from the same site are spaced to avoid overlapping.

Table S-II.1 Overview of CO$_2$ enrichment experiments included in our analysis. Abbreviations: Myc: mycorrhizal type (AM: arbuscular mycorrhizae, ECM: ectomycorrhizae); N-class: main soil N availability classification (L: low, H: high); N-class2: alternative N-availability classification (L: low, M: medium, H: high); TB = Total Biomass, AB = Aboveground Biomass, BB = Belowground Biomass, FACE = Free Air Carbon Dioxide Enrichment, G = Greenhouse/Growth chamber, ME = Model ecosystem, OTC = Open Top Chamber.

Site	**Species**	**Country**	**Myc**	**N-class**	**N-class2**	**Facility**	**References**		
							TB	**AB**	**BB**
AG FACE	Yitpi	Australia	AM	H	H	FACE		*51*	
Amsterdam Greenhouses	*Calamagrostis eigejos*	The Netherlands	AM	H	H	G		*52*	
Amsterdam Greenhouses	*Molinia caerulea*	The Netherlands	AM	H	H	G		*52*	
Antwerp OTC	*Pinus sylvestris*	Belgium	ECM	L	L	OTC	*53*	*53*	*53*
BangorFACE	*Alnus glutinosa*	UK	ECM	H	H	FACE		*54*	
BangorFACE	*Betula pendula*	UK	ECM	H	H	FACE		*54*	
BangorFACE	*Fagus sylvatica*	UK	ECM	H	H	FACE		*54*	
Basel spruce	*Picea abies*	Switzerland	ECM	L	L	ME	*55*	*55*	
Basel spruce F	*Picea abies*	Switzerland	ECM	H	H	ME	*55*	*55*	
Basel tropical	Mix. Trop forest	Switzerland	AM	L	M	ME	*56*	*56*	*56*
Basel tropical II	Mix. Trop forest	Switzerland	AM	L	M	ME	*57*	*57*	
BioCON	perennial grassland	USA	AM	L	L	FACE	*5*	*5*	*5*
BioCON F	perennial grassland	USA	AM	H	H	FACE	*5*	*5*	*5*
Birmensdorf - Acidic loam	*Fagus sylvatica, Picea abies*	Switzerland	ECM	L	M	OTC			*58*
Birmensdorf - Calcareous sand	*Fagus sylvatica, Picea abies*	Switzerland	ECM	H	H	OTC			*58*
Birmensdorf F - Acidic loam	*Fagus sylvatica, Picea abies*	Switzerland	ECM	H	H	OTC			*58*
Birmensdorf F - Calcareous sand	*Fagus sylvatica, Picea abies*	Switzerland	ECM	H	H	OTC			*58*
Brandbjerg	temperate heath	Denmark	AM	L	L	FACE		*59*	
China FACE F	*Triticum aestivum*	China	AM	H	H	FACE	*60*	*60*	
China FACE FF	*Triticum aestivum*	China	AM	H	H	FACE	*60*	*60*	
DUKE FACE	*Pinus taeda*	USA	ECM	L	M	FACE	*25*	*25*	*25*
DUKE Phytotron	*Pinus taeda*	USA	ECM	L	M	G	*61*	*61*	*61*
DUKE Phytotron F	*Pinus taeda*	USA	ECM	H	H	G	*61*	*61*	*61*
DUKE Phytotron II	*Pinus ponderosa*	USA	ECM	L	M	G			*62*
DUKE Phytotron II	*Pinus taeda*	USA	ECM	L	M	G			*62*
DUKE Phytotron II F	*Pinus ponderosa*	USA	ECM	H	H	G			*62*
DUKE Phytotron II F	*Pinus taeda*	USA	ECM	H	H	G			*62*
Duke Prototype	*Pinus taeda*	USA	ECM	H	H	FACE		*8*	

ETH FACE	*Betula pendula*	Switzerland	ECM	L	M	FACE	*63*	*63*	*63*
EUROFACE	*Populus alba*	Italy	ECM	H	H	FACE	*64*	*64*	
EUROFACE	*Populus euramericana*	Italy	ECM	H	H	FACE	*64*	*64*	
EUROFACE	*Populus nigra*	Italy	ECM	H	H	FACE	*64*	*64*	
FACTS II FACE	*Populus tremuloides*	USA	ECM	L	M	FACE	*9*	*9*	*9*
FACTS II FACE	*Populus tremuloides-Betula papyrifera*	USA	ECM	L	M	FACE	*9*	*9*	*9*
Flakaliden	*Picea abies*	Sweden	ECM	L	L	OTC		*65*	
GiFACE	Mix grassland	Germany	AM	L	M	FACE		*66*	
Ginninderra	*Phalaris aquatica*	Australia	AM	H	H	G	*67*	*67*	*67*
Glendevon	*Pinus sylvestris*	UK	ECM	L	M	OTC			*68*
Glendevon	*Picea sitchensis*	UK	ECM	L	M	OTC			*68*
Glendevon F	*Pinus sylvestris*	UK	ECM	H	H	OTC			*68*
Glendevon F	*Picea sitchensis*	UK	ECM	H	H	OTC			*68*
Guelph	*Artemisia tridentata*	Canada	AM	H	H	G		*69*	*69*
Harvard	*Acer pensylvanicum*	USA	AM	L	L	G	*70*		*70*
Harvard	*Acer rubrum*	USA	AM	L	L	G	*70*		*70*
Harvard	*Betula alleghaniensis*	USA	ECM	L	L	G	*70*		*70*
Harvard	*Fraxinus americana*	USA	AM	L	L	G	*70*		*70*
Harvard	*Quercus rubra*	USA	ECM	L	L	G	*70*		*70*
Harvard	*Betula populifolia*	USA	ECM	L	L	G	*70*		*70*
Harvard F	*Acer pensylvanicum*	USA	AM	H	H	G	*70*		*70*
Harvard F	*Acer rubrum*	USA	AM	H	H	G	*70*		*70*
Harvard F	*Betula alleghaniensis*	USA	ECM	H	H	G	*70*		*70*
Harvard F	*Betula populifolia*	USA	ECM	H	H	G	*70*		*70*
Harvard F	*Fraxinus americana*	USA	AM	H	H	G	*70*		*70*
Harvard F	*Quercus rubra*	USA	ECM	H	H	G	*70*		*70*
Harvard II	*Betula alleghaniensis*	USA	ECM	L	L	G	*71*	*71*	*71*
Headley	*Fraxinus excelsior*	UK	ECM	L	L	OTC	*72*		
Headley	*Pinus sylvestris*	UK	ECM	L	L	OTC	*72*		
Headley	*Quercus petraea*	UK	ECM	L	L	OTC	*72*		
Hohenheim	*Triticum aestivum*	Germany	AM	H	H	FACE		*73*	
Jasper Ridge FACE	annual grassland	USA	AM	L	L	FACE	*74*	*74*	*74*
Jasper Ridge	annual grassland	USA	AM	H	H	FACE	*74*	*74*	*74*

FACE F									
Jasper Ridge OTC	Sandstone grassland	USA	AM	L	L	OTC	*75, 76*	*75-77*	*75, 76*
Jasper Ridge OTC	Serpentine grassland	USA	AM	L	L	OTC	*75*	*75, 77*	*75*
Jasper Ridge mesocosm	Sandstone grassland	USA	AM	L	L	G		*78*	
Jasper Ridge mesocosm	Serpentine grassland	USA	AM	L	L	G		*78*	
Jasper Ridge mesocosm F	Sandstone grassland	USA	AM	H	H	G		*78*	
Jasper Ridge mesocosm F	Serpentine grassland	USA	AM	H	H	G		*78*	
Lancaster Solardomes	*Quercus robur*	UK	ECM	L	L	G	*79*	*79*	*79*
Lancaster Solardomes	*Fagus sylvatica*	UK	ECM	L	L	G	*79*	*79*	*79*
Lancaster Solardomes	*Pinus sylvestris*	UK	ECM	L	L	G	*79*	*79*	*79*
Lancaster Solardomes	*Abies alba*	UK	ECM	L	L	G	*79*	*79*	*79*
Lancaster Solardomes	*Betula pendula*	UK	ECM	L	L	G	*79*	*79*	*79*
Lancaster Solardomes	*Carpinus betulus*	UK	ECM	L	L	G	*79*	*79*	*79*
Lancaster Solardomes F	*Pinus sylvestris*	UK	ECM	H	H	G	*79*	*79*	*79*
Lancaster Solardomes F	*Abies alba*	UK	ECM	H	H	G	*79*	*79*	*79*
Lancaster Solardomes F	*Betula pendula*	UK	ECM	H	H	G	*79*	*79*	*79*
Lancaster Solardomes F	*Carpinus betulus*	UK	ECM	H	H	G	*79*	*79*	*79*
Lancaster Solardomes F	*Quercus robur*	UK	ECM	H	H	G	*79*	*79*	*79*
Lancaster Solardomes F	*Fagus sylvatica*	UK	ECM	H	H	G	*79*	*79*	*79*
Merrit Island	Shrub-*Quercus* system	USA	ECM	L	L	OTC	*80*	*80*	*80*
Nevada FACE	Desert scrub	USA	AM	L	L	FACE	*81*	*81*	*81*
New Zealand FACE	temperate pasture	New Zealand	AM	L	M	FACE		*82*	*83*
Oak Ridge OTC	*Liriodendron tulipifera*	USA	AM	-	-	OTC	*84*	*84*	*84*
Oak Ridge OTC	*Acer saccharum, Acer rubrum*	USA	AM	-	-	OTC			*85*
Oak Ridge OTC II	*Quercus alba*	USA	ECM	L	M	OTC	*86*	*86*	
Oak Ridge OTC	Model grassland	USA	AM	L	M	OTC		*87*	

III									
ORNL FACE	*Liquidambar styraciflua*	USA	AM	L	M	FACE	*6*	*6*	*88*
PHACE	Mixed-grass prairie	USA	AM	L	L	FACE	*89*	*89*	*89, 90*
POPFACE	*Populus alba*	Italy	ECM	H	H	FACE	*91*	*91*	*91*
POPFACE	*Populus euramericana*	Italy	ECM	H	H	FACE	*91*	*91*	*91*
POPFACE	*Populus nigra*	Italy	ECM	H	H	FACE	*91*	*91*	*91*
Richmond	*Eucalyptus saligna*	Australia	ECM	H	H	G	*92*		
Richmond	*Eucalyptus sideroxylon*	Australia	ECM	H	H	G	*92*		
Riso	*Pisum sativum*	Denmark	AM	H	H	G	*93*	*93*	*93*
SCBG	Subtrop forest	China	ECM	L	M	OTC	*94*	*94*	
SCBG F	Subtrop forest	China	ECM	H	H	OTC	*94*	*94*	
Suonenjoki	*Betula pendula*	Finland	ECM	L	M	OTC	*95*	*95*	*96*
Swiss Central Alps	Alpine grassland	Switzerland	AM	L	L	OTC	*97*	*97*	*97*
Swiss Central Alps F	Alpine grassland	Switzerland	AM	H	H	OTC	*97*	*97*	*97*
Swiss FACE	*Lolium perenne*	Switzerland	AM	L	M	FACE	*98*	*99*	*98*
Swiss FACE FF	*Lolium perenne*	Switzerland	AM	H	H	FACE	*98*	*99*	*98*
Swiss Jura	*Bromus erectus*	Switzerland	AM	L	L	G		*100*	*101*
Tas FACE	Temperate grassland	Australia	AM	L	L	FACE	*102*	*14*	*102*
UMBS	*Populus euramericana*	USA	ECM	L	L	OTC	*103*	*103*	*103*
UMBS F	*Populus euramericana*	USA	ECM	H	H	OTC	*103*	*103*	*103*
UMBS II	*Populus grandidentata*	USA	ECM	L	L	OTC	*104*	*104*	*104*
UMBS III	*Populus tremuloides*	USA	ECM	L	M	OTC	*105*	*105*	*105*
UMBS III F	*Populus tremuloides*	USA	ECM	H	H	OTC	*105*	*105*	*105*
UMBS_alnus	*Alnus glutinosa*	USA	ECM	L	M	OTC	*106*	*106*	*106*
USDA	*Citrus aurantium*	USA	AM	H	H	OTC	*107*	*107*	*108*
USDA Placerville	*Pinus ponderosa*	USA	ECM	L	M	OTC	*109*	*109*	*109*
USDA Placerville F	*Pinus ponderosa*	USA	ECM	H	H	OTC	*109*	*109*	*109*
USDA Placerville FF	*Pinus ponderosa*	USA	ECM	H	H	OTC	*109*	*109*	*109*
USEPA	*Pseudotsuga menziesii*	USA	ECM	L	M	G	*110*	*110*	*110*

Table S-II.2 Justification for the soil nitrogen (N) availability classification. N-class: main soil N availability classification (L: low, H: high); N-class2: alternative N-availability classification (L: low, M: medium, H: high); N-fert: fertilized site (yes or no) and indication of the amount of N fertilizer in g N m-2 y-1, unless other units are specified; %N: soil N content (%); %C: soil carbon content (%); pH: when available pH in CaCl2 was reported, otherwise from water solution; C:N: C:N ratio; Report: N-availability or soil fertility assessment of the site found in the literature or confirmed by the site PI. Lack of information on N-availability in some experiments did not allow to assess them in N-class, but were classified as “medium” in N-class2

Site	**N-class**	**N-class2**	**N-fert.**	**Extra fert.**	**Soil type**	**Soil texture**	**%N**	**%C**	**pH**	**C:N**	**Ref**	**Remarks**
AG FACE	H	H	0 -13.8	P, S		clay (60%)	0.03 - 0.10	1.24	8.4	12	*51*	1
Amsterdam Greenhouses	H	H	yes	P, K					5.5		*52*	2
Antwerp OTC	L	L	no		poor forest soil	sandy	0.12		4.3		*53*	3
BangorFACE	H	H	no		Dystric Cambisol	Fine loamy brown earth over gravel; 62.2 sand, 28.5 silt, 9.3 clay	2.6		4.6	10.5	*111*	4
Basel spruce	L	L	no		podzol				4.5		*55*	5
Basel spruce F	H	H	9		podzol				4.5		*55*	6
Basel tropical	L	M	13.3	fertilizer pellets	fresh tropical soil						*56*	7
Basel tropical II	L	M	11.8	Osmocote and OM	fresh tropical soil						*57*	8
BioCON	L	L	no		Nymore series, subgroup Typic Upidsamment, suborder Psamments, Order Entisols	93% sand, 3% silt, and 4% clay	0.001				*112*	9
BioCON F	H	H	4		Nymore series, subgroup Typic Upidsamment, suborder Psamments, Order Entisols	93% sand, 3% silt, and 4% clay	0.001				*112*	10
Birmensdorf - Acidic loam	L	M	0.5 - 0.7		Haplic Halisol	acidic sandy loamy; 55% sand, 29% silt, 16% clay		12.9 mg kg^{-1}	4.1		*113*	11
Birmensdorf -	H	H	0.5 -		Fluvisol	calcareous loamy		13.1	7.2		*113*	12

Calcareous sand			0.7			sandy; 84% sand, 10% silt, 6% clay		mg kg^{-1}				
Birmensdorf F - Acidic loam	H	H	5 - 7		Haplic Halisol	acidic sandy loamy; 55% sand, 29% silt, 16% clay		12.9 mg kg^{-1}	4.1		*113*	13
Birmensdorf F - Calcareous sand	H	H	5 - 7		Fluvisol	calcareous loamy sandy; 84% sand, 10% silt, 6% clay		13.1 mg kg^{-1}	7.2		*113*	14
Brandbjerg	L	L	no			sandy deposit hill			5		*59*	15
China FACE F	H	H	15	P, K	Shajiang Aquic Cambisol	sandy-loamy ; total porosity: 54%; clay 13.6%, silt 28.5%, sand 57.8%	0.145	1.84	7.2		*60*	16
China FACE FF	H	H	25	P, K	Shajiang Aquic Cambiosol	sandy-loamy ; total porosity: 54%; clay 13.6%, silt 28.5%, sand 57.8%	0.145	1.84	7.2		*60*	17
DUKE FACE	L	M	no		Ultic Hapludalfs	Clay loam; well-developed soil horizons with mixed clay mineralogy.	0.079		5.75	18.9	*114*	18
DUKE Phytotron	L	M	1.75 mM	Hoagland solution	mixture of Turface, vermiculite, gravel and soil (4:2:2:1)						*61*	19
DUKE Phytotron F	H	H	5.5 mM	Hoagland solution	mixture of Turface, vermiculite, gravel and soil (4:2:2:1)						*61*	20
DUKE Phytotron II	L	M	1 mM	Hoagland solution	sterilized sand						*62*	21
DUKE Phytotron II F	H	H	5 mM	Hoagland solution	sterilized sand						*62*	22
Duke Prototype	H	H	11.2		Ultic Hapludalfs	Clay loam			5.75	18.9	*8*	23

ETH FACE	L	M	no		from an agricultural site, used for maize cultivation since 1962	5.6% clay, 17.7% loam, 76.8% sand	0.08		5.05		*63*	24
EUROFACE	H	H	21.2-29	P, K	Xeric Alfisol	heavy clay loam; 37% sand, 44% silt, 19% clay	0.13	1.06-1.13	4.89-5.18	9.31	*64*	25
FACTS II FACE	L	M	no		Alfic Haplorthods	Mixed, frigid, coarse loamy ; 56% sand, 36% silt, 8% clay	0.12		5.5	12.9-13.5	*115*	26
Flakaliden	L	L	no		Typic Haplocryods	silty-sandy till; O-layer average depth is 3cm			4.4		*65*	27
GiFACE	L	M	4		stagno-fluvic gleysol	porosity 60 - 65%; loamy-sandy sediments over clay	0.45	4.7	5.9	10.5	*116*	28
Ginninderra	H	H	10	P, K							*67*	29
Glendevon	L	M	no		brown forest soil 40-60 cm deep.	loam of shallow brown earth, locally podzolized	NO3: 0.49; NH4: 0.26		4.7		*117*	30
Glendevon F	H	H	7	other nutrients	brown forest soil 40-60 cm deep.	loam of shallow brown earth, locally podzolized	NO3: 0.54; NH4: 0.22		4.7		*117*	31
Guelph	H	H	400 ml	Hoagland solution		Turface					*69*	32
Harvard	L	L	0.18 g	P, K + micronutrients	pots with a 1:1:1 mixture of sand:perlite:peat			5			*70*	33
Harvard F	H	H	1.8 g	P, K + micronutrients	pots with a 1:1:1 mixture of sand:perlite:peat			5			*70*	34
Harvard II	L	L	no		Canton	low density O2 horizon; stony to sandy loams					*71*	35

Headley	L	L	no		humo-ferric podzol	Sandy					*72*	36
Hohenheim	H	H	14		slightly stagnic luvisol						*73*	37
Jasper Ridge FACE	L	L	no		Typic Haploxeralfs						*74*	38
Jasper Ridge FACE F	H	M	7		Typic Haploxeralfs						*74*	39
Jasper Ridge OTC - serpentine	L	L	no		Lithic Haploxerolls	Clay loam	0.16	1.8	6.6	11.2	*118*	40
Jasper Ridge OTC - sandstone	L	L	no		Lithic Xerochrepts	Loamy	0.12	1.2	5.5	10	*76*	41
Jasper Ridge mesocosm	L	L	no		0.8 m subsoil from serpentine quarry and 0.15 m serpentine topsoil						*78*	42
Jasper Ridge mesocosm F	H	H	20	P, K	0.8 m subsoil from serpentine quarry and 0.15 m serpentine topsoil						*78*	43
Lancaster Solardomes	L	L	no		Udertic Paleustoll	silt loam or silty clay loam (clay 26-34%)					*79*	44
Lancaster Solardomes F	H	H	2.5 g L^{-1}	P, K, Mg and trace elements	Udertic Paleustoll	silt loam or silty clay loam (clay 26-34%)					*79*	45
Merrit Island	L	L	no		Pomello (Arenic Haplahumod) and Poala sands (Spodic Quartzipsamment)	moderately well drained sandy soils		2-7	3.9-4.1		*119*	46
Nevada FACE	L	L	no		Aridosols derived from calcareous alluvium	Loamy and coarse sand; well-drained	0.01-0.08	0.18-1.8	7-8		*120*	47
New Zealand FACE	L	M	no	P, S, K	Mollic Psammaquent	fine sand; 0.25m black loamy top horizon underlain by	0.37-0.41	4.52-5.02	5.9-6	12.4	*82*, *121*	48

						grayish-brown horizon						
Oak Ridge OTC	-	-	no								*84*	49
Oak Ridge OTC II	L	M	no			silt loam					*86*	50
Oak Ridge OTC III	L	M	no		Typic Fragiudult	well-drained; fine-silty, siliceous, mesic					*87*	51
ORNL FACE	L	M	no		Aquic Hapludult	silty clay loam, moderately well drained; 21% sand, 55% silt, 24% clay	0.112	1.08	5.7		*114*	52
PHACE	L	L	no		Aridic Argiusstoll	fine-loamy, mixed mesic			7.9		*89*	53
POPFACE	H	H	no		Xeric Alfisol	loam; 37% sand, 44% silt, 19% clay	0.11-0.14	0.9 - 1.13	4.9-5.18	8.7-9.9	*91*	54
Richmond	H	H	0.2 g N L^{-1}	P, K, S, Fe, Mn, B		loamy sand	<1 mg kg^{-1}	0.7	5.5		*92*	55
Riso	H	H	20 mg N kg^{-1}		from an arable layer	49.9% sand, 31.8% silt, 16% clay	0.14	1.36			*122*	56
SCBG	L	M	no		from an evergreen broadleaved forest						*94*	57
SCBG F	H	H	10		from an evergreen broadleaved forest						*94*	58
Suonenjoki	L	M	2.2 - 4.1		soil composed of sand and clay; no humus layer on top of the mineral soil	0.046			21		*123*	59
Swiss Central Alps	L	L	no		alpine stagnic pseudo-gleysols				4		*97*	60
Swiss Central Alps F	H	H	4	P, K	alpine stagnic pseudo-gleysols				4		*97*	61

Swiss FACE	L	M	10 - 14		eutric Cambisol	clay loam; 28% clay, 33% silt, 36% sand	0.28-0.46	2.9-5.1	6.5-7.6		*124*	62
Swiss FACE FF	H	H	40 -56		eutric Cambisol	clay loam; 28% clay, 33% silt, 36% sand	0.28-0.46	2.9-5.1	6.5-7.6		*124*	63
Swiss Jura	L	L	no	P		silty clay-loam underlain with calcareous debris.	0.33		7-8		*100*, *125*	64
Tas FACE	L	L	no		black Vertisol	formed of basaltic clay	0.2		6		*126*	65
UMBS	L	L	no		Rubicon sand + Kalkaska series topsoil		0.45-0-46	1			*127*	66
UMBS F	H	H	no		Kalkaska series topsoil		1.5-1.52	2.7			*127*	67
UMBS II	L	L	4.5		Entic Haplorthod	sandy, mixed, frigid	0.0079-0.01				*104*	68
UMBS III	L	L	no		Rubicon sand + Kalkaska series topsoil	93% sand, 2.5% clay	0.021		6.74	14.8	*105*	69
UMBS III F	H	H	no		Kalkaska series topsoil	72% sand, 10.1% clay	0.097		6.08	13.3	*105*	70
UMBS_alnus	L	M	no		Rubicon sand + Kalkaska series topsoil		0.016-0.020				*106*	71
USDA	H	H	ample	ample	Avondale	loam					*107*	72
USDA Placerville	L	L	no		Aiken clay loam		0.09		5.1 - 5.5	24-25	*109*	73
USDA Placerville F	H	M	yes		Aiken clay loam		0.1		5.1 - 5.5	24-25	*109*	74
USDA Placerville FF	H	H	yes		Aiken clay loam		0.11		5.1 - 5.5	24-25	*109*	75
USEPA	L	M	no		Typic Hapludand	coarse, loamy, mixed, frigid	0.06-0.11		6.2-6.3		*110*	76

1. Large soil mineral N content (~300 kg N ha^{-1}) at the site precluded any significant effect of applied N, indicating the site was initially N-rich.
2. The experiment simulates conditions of mesotrophic soils, thereby, inherently fertile.
3. Sandy soils with low pH, classified by the authors as "poor forest soils".
4. Former agricultural field, fertile soil type and low C:N, therefore high nitrogen availability. Analysis of P-availability indicates that plants in this site are P-limited, but not N-limited (pers. comm).
5. Authors reported the soil is "natural nutrient-poor montane soil".
6. N-fertilization in the site increased fertility from "nutrient-poor" to "medium-high" N availability, as reported by the authors.
7. Low-fertility litter compost mix was added to the soil to simulate nutrient cycling, but no fertilizer was applied. Nutrients were kept low, and plants showed visual signs of nutrient limitations in CO_2-fumigated plots as seen by the yellowish appearance of the vegetation. PI described the soils as N-low to moderately fertile (pers. comm).
8. Low-fertility litter compost mix was added to the soil to simulate nutrient cycling, but no fertilizer was applied. Nutrients were kept low, and plants showed visual signs of nutrient limitations in CO_2-fumigated plots as seen by the yellowish appearance of the vegetation. PI described the soils as N-low to moderately fertile (pers. comm).
9. Authors reported that plants in this low SOM (1.4%), low N (10 µg g^{-1}) and high P content (46.5 µg g^{-1}) sandy soil were "N-limited". In addition, N-availability constrained the CO_2 biomass response (*128*).
10. Same soils as in 9, but N-amended with 4 g N m^{-2}, corresponding to high N deposition rates.
11. Authors reported this acidic soil as "nutrient-poor", with low SOC content in the subsoil (2.3 g kg^{-1}). N-addition simulated "low levels of N deposition", and higher levels of N-fertilization in adjacent plots increased growth further, indicating N-limitations in these plots, therefore N-class2=M.
12. This Fluvisol is reported as "nutrient-rich" by the authors, with high SOC content, pH and CEC (127 mmol/kg soil), therefore N-class=H. Although N-addition addition levels simulated "low levels of N deposition", increasing N-fertilization in adjacent plots did not increase biomass further, therefore N-class2=H.
13. Soils in 11 with higher levels of N-fertilization.
14. Soil in 12 with higher levels of N-fertilization.

15. Sandy soils reported as “nutrient-poor”.
16. Soil type and texture indicate intermediate fertility, but fertilization is high.
17. Soils in 16 with even higher fertilization.
18. Soil type and high C:N ratio indicate low N-availability. The soil is classified as “moderately low fertile” by the authors (*8*), and forest production showed a substantial response to N fertilization (*129*), indicating N-limitations. However, plants initially had not yet fully explored soil resources due to high initial spacing among seedlings (expanding systems), which may increase N availability at the individual plant level (*130*), therefore N-class2=M.
19. Artificial soil with modest N-fertilization. The authors reported that “N is believed to be the primary limiting factor”. Based on the scarce soil data, the soil was classified as L-M despite N-fertilization, because fertilization with higher amount of N in soil 20 increased biomass by 20%.
20. Same soil as 19 with higher N fertilization.
21. Available soil data scarce, but artificial soil (sand) with modest N-fertilization.
22. Same soil as 21 with higher N fertilization.
23. Same soil as 18 with N amendments.
24. CEC is low and the site was not N-fertilized, but it was formerly a maize field, reason we assumed it was fertilized in the past and we assigned N-class2=M.
25. Fertile soils (Alfisol) with good texture (loam) and former agriculture land. The site was classified as “nutrient-rich” by the authors. N-fertilization in the second rotation of the experiment did not enhance plant growth, indicating high N availability.
26. According to the authors N-availability is medium due to previous agricultural use prior to 1972, hence N-class2=M. N-class=L because the soil is sandy, SOM is relatively low (pers. comm) and it is not fertilized.
27. Boreal forest, classified as “strongly nutrient limited” (*131*). Long term (25 years) fertilization of experimental plots in this forest quadrupled productivity (pers. comm.).
28. Classified as “nitrogen limited” by the authors (pers. comm). The fertilization rate is smaller than what is removed by the harvest, so the site is considered N limited even though it is fertilized (pers. comm), hence N-class=L. However, the soil is moderately fertile based on soil texture and intermediate C:N, therefore N-class2=M.
29. No soil information was available, but N and other nutrients are supplied in abundance.
30. The soil was classified as “intermediate nutrient status” by the authors, hence N-class2=M. Based on the lack of fertilization, N-class=L.

31. Same soil as 30 with intermediate nutrient availability with extra N and other nutrients.

32. Soil was sterilized Turface, low-nutrient calcined clay (AM fungi inoculation), but plants were fertilized frequently with Hoagland’s solution.

33. The soil “simulated poor-nutrient forest soil at Harvard Forest”. They further showed that nitrogen mineralization rates were low in this forest (34 kg ha^{-1} y^{-1}) (*132*), and higher N supply in adjacent plots greatly increased plant growth, therefore N-class=L.

34. The nutrient treatment simulated high N deposition and organic matter mineralization rates (400 kg N ha^{-1} yr^{-1}).

35. They used a 1:1:1 mix of coarse sand, peat and field soil (from a nutrient poor forest soil). No fertilized was supplied, therefore N availability was low.

36. Sandy soils classified as “nutrient-poor” and “low soil N content” by the authors.

37. Soil type typically nutrient-rich, and very high N-fertilization.

38. Soil classified as “nutrient-poor” by the authors, and N addition increased plant growth significantly.

39. Same soil as 38 but N-fertilized. N-class2=H because even though Haploxeralfs soils are N-poor, the supply of N is high.

40. Soil reported as “low nutrient availability”, and “low N availability” (pers. comm). Serpentine grasslands at Jasper Ridge consistently respond to N and P additions, with N almost doubling growth (*133*). CEC=0.7 mmhos cm^{-1}, SOM=7.5%.

41. This sandstone-derived soil had lower CEC (0.1 mmhos cm^{-1}), N content and SOM (5.2%) than soil 40.

42. Same soil as 40.

43. Same soil as 40 and 42, but highly fertilized.

44. Authors reported this soil was characterised by “low organic matter content” and “low nitrogen availability” (pers. comm), as also observed by the increase in growth upon fertilization.

45. Same as 44 but fertilized with N and other nutrients.

46. Sandy soils with nutrient content. Reported “infertile sandy soils”.

47. Calcareous soil with very high C:N ratio. Authors reported “low N concentration”.

48. Sheep create N-rich urine patches with larger CO_2 response, which indicates that the site is N-limited in general (pers. comm). Classified as “N-limited” (*134*). N-class2=M because C:N ratio is moderate, and sheep excrete and N_2-fixing species may increase N-availability.

49. Not included in the meta-analysis due to the lack of available soil information.

50. "Low in available P and estimated annual N availability of 50 µg g^{-1}". N-class2=M because it was not possible to assign N availability with certainty based on available information.

51. Soil type and low C:N indicate intermediate N-availability, but given the lack of fertilization we classified this soil as L-M.

52. Plant productivity is N-limited at this site (*6*), N-class=L. Moderately fertile soil type, low C:N ratio and evidence for nitrogen fixation (*135*, *136*), therefore N-class2=M.

53. The high pH suggests low availability of P and some other nutrients. Reported as "nutrient-poor", and N-availability limits plant growth.

54. Same soil as in 25, except fertilizer was not used. Nevertheless, these soils were "nutrient-rich" given past agricultural use and soil type. N-fertilization did not enhance plant growth, indicating high N-availability.

55. Even though soil organic matter content was low, we classified these soils as high due to fertilization with N and other nutrients.

56. Soil type is fertile with low C:N ratio, and was also N-fertilized. Reported as "nutrient rich".

57. N-fertilization enhanced plant growth in the experiment, suggesting N-limitations, therefore we classified the soil as L-M.

58. Same soil as 57 but heavily fertilized with N.

59. N fertilization was kept modest so trees would not become totally deficient of it, but plants were N-limited (pers. comm).

60. Very nutrient-poor soils, in situ, very old, late successional system (pers. comm).

61. Same soil as in 60, amended with NPK.

62. Soil type characterized by high fertility. However, the authors reported that the "reduced availability of N constantly limited the response of harvestable biomass to elevated CO_2 throughout the experiment". These plots were fertilized with 15 g N m^{-2}, and yet, fertilization with 45 g N m^{-2} in adjacent plots produced more yield (*137*), suggesting that 15 g N m^{-2} fertilization is in the range of N-limitations (*138*), classifying plants in these plots as moderately N-limited (pers. comm).

63. Same soil as in 62 with high levels of N-fertilization.

64. "Very nutrient poor despite high rates of N deposition" (pers. comm), with P probably at least as limiting as N.

65. Many Vertisols are N-deficient, in line with low SOM, and have low available P (http://www.fao.org/docrep/003/y1899e/y1899e06.htm#P381_59788). Authors reported "low total N and extractable P".

66. Sandy soils, low in organic matter content and %N. N Mineralization = 45 $\mu g\ N\ g^{-1}\ day^{-1}$. Authors reported "low soil N" and "P not limiting".

67. N Mineralization = 348 $\mu g\ N\ g^{-1}\ day^{-1}$. Authors reported "high soil N". Since plants were well watered and and P was not limiting, the major difference between soils 67 and 66 was N content, therefore, we classified it as H.

68. Nutrient-poor sandy soil, despite modest N-fertilization.

69. Equivalent to soil 66. N Mineralization = 89 $\mu g\ N\ g^{-1}\ day^{-1}$. Plants received an initial dose of N-fertilizer, and for that reason N-class2=M.

70. Equivalent to soil 67. N Mineralization = 333 $\mu g\ N\ g^{-1}\ day^{-1}$.

71. Similar to soils 66 and 69, and authors reported "nutrient-poor" and "low soil N". However, Alnus spp. is a N_2-fixing species, therefore N-class2=M.

72. Avondale are very fertile soils used for growing cultivated crops and pasture under irrigation. Ample nutrients were added.

73. The low N treatment consisted of unamended soil which had a total N concentration of approximately 900 $\mu g\ g^{-1}$, that we assume as low to moderate, therefore N-class2=M. N-fertilization in adjacent plots increased growth, therefore plants were N-limited and N-class=L.

74. "Intermediate soil N fertility treatment" was imposed by supplying soil 73 with sufficient $(NH_4)_2\ SO_4$ to increase total soil N by 100 $\mu g\ g^{-1}$ N. Higher levels of N-fertilization in soil 75 did not significantly increase growth, suggesting plants in this soils were not N-limited, therefore N-class2=H.

75. "High soil N fertility treatment" was imposed by supplying soil 73 with sufficient $(NH_4)_2\ SO_4$ to increase total soil N by 200 $\mu g\ g^{-1}$ N.

76. Typic Hapludand soils are usually moderately fertile, and pH is good, therefore N-class2=M. Authors reported that the soil was "nutrient-poor", with "soil N concentration lower than optimum for highly productive Douglas-fir forest in Oregon", hence N-class=L.

Table S-II.3 Experiments with arbuscular mycorrhizal plant species under low N-availability grown with N-fixing species. es: effect size, var: varianze, W_{NY}: weights used for the meta-analysis, based on the number of replicates and the duration (years) of the experiment. The data used for the analysis is aboveground biomass for all the species sampled (i.e. non N-fixing and N-fixing species).

Site	**Species**	**Data source**	**es**	**var**	W_{NY}
GiFACE	grassland	*66*	0.0198	0.0029	9
New Zealand FACE	temperate pasture	*82*	0.1165	0.0305	7.5
ORNL FACE	*Liquidambar styraciflua*	*6*	0.0146	0.0009	6.7
Swiss Central Alps	Alpine grassland	*97*	0.0331	0.0228	7.5
Tas FACE	Temperate grassland	*14*	0.0664	0.0002	7.5
Swiss Jura	Calcareous grassland	*100*	0.1870	0.0158	7
BioCON	perennial grassland	*5*	0.0892	0.0013	9.5

GIFACE: legumes (mainly *Lathyrus pratensis*) contribute less than 0.5% to the total plant biomass (*116*); New Zealand FACE: mixture of plant species including legumes, principally *Trifolium repens L.* and *Trifolium subterraneum L.* (*139*); ORNL FACE: evidence for nitrogen fixation, and an increasing presence of *Elaeagnus umbellata* (an invasive actinorhizal N fixing shrub) (*135*, *136*); Swiss Central Alps: *Trifolium alpinum L.* is the only legume species and comprises less than 2% of the total phanerogam biomass; Tas FACE: N fixing forbs, including *Trifolium subterraneum* and *T. striatum*, form an extremely small fraction (0.01%) of the biomass. The community also contains the N-fixing woody twining species *Bossiaea prostrata*, that forms only a small fraction of the total biomass (1%) (*126*); Swiss Jura: data pooled across all species.

Table S-II.4 Meta-analysis output with three different correlation factors (*r*) to aggregate repeated measurements over time. %es=effect size (%), se=standard error, Myc=mycorrhizal status, N=nitrogen availability.

			r=1			***r=0.5***			***r=0***		
Biomass	**Myc**	**N**	**%es**	**se**	***P*-value**	**%es**	**se**	***P*-value**	**%es**	**se**	***P*-value**
Total	AM	High	19.71	5.92	0.002	19.71	5.72	0.001	19.71	5.55	0.001
		Low	0.35	5.29	0.946	0.35	5.13	0.945	0.35	4.98	0.943
	ECM	High	33.21	4.35	0	33.21	4.34	0	33.21	4.34	0
		Low	27.98	4.64	0	27.98	4.65	0	27.98	4.68	0
Aboveground	AM	High	18.36	4.44	0	18.04	4.49	0	18.36	4.49	0
		Low	2.3	4.36	0.595	3.55	5.59	0.523	2.3	4.45	0.425
	ECM	High	31.09	4.03	0	31.14	3.75	0	31.09	3.5	0
		Low	30.16	4.71	0	29.84	4.73	0	30.16	4.72	0
Belowground	AM	High	16.49	10.29	0.123	16.49	10.11	0.117	16.49	9.95	0.111
		Low	-0.92	8.25	0.907	-0.92	8.11	0.906	-0.92	7.99	0.905
	ECM	High	35.39	7.01	0	35.39	6.74	0	35.39	6.47	0
		Low	20.38	6.36	0.003	20.38	6.3	0.003	20.38	6.27	0.003

2.7. References

1. P. Ciais *et al.*, in *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, T. F. Stocker et al., Eds. (Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 2013), pp. 465–570.
2. C. L. Quéré *et al.*, Global Carbon Budget 2015. *Earth System Science Data*. **7**, 349–396 (2015).
3. B. A. Hungate, J. S. Dukes, M. R. Shaw, Y. Luo, C. B. Field, Nitrogen and climate change. *Science*. **302**, 1512–1513 (2003).
4. W. R. Wieder, C. C. Cleveland, W. K. Smith, K. Todd-Brown, Future productivity and carbon storage limited by terrestrial nutrient availability. *Nature Geosci*. **8**, 441–444 (2015).
5. P. B. Reich, P. B. Reich, S. E. Hobbie, S. E. Hobbie, Decade-long soil nitrogen constraint on the CO_2 fertilization of plant biomass. *Nature Climate change*. **3**, 278–282 (2013).
6. R. J. Norby, J. M. Warren, C. M. Iversen, B. E. Medlyn, R. E. McMurtrie, CO_2 enhancement of forest productivity constrained by limited nitrogen availability. *Proc Natl Acad Sci U S A*. **107**, 19368–19373 (2010).
7. P. B. Reich, B. A. Hungate, Y. Luo, Carbon-Nitrogen Interactions in Terrestrial Ecosystems in Response to Rising Atmospheric Carbon Dioxide. *Annu. Rev. Ecol. Evol. Syst.* **37**, 611–636 (2006).
8. H. R. McCarthy *et al.*, 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 Phytol*. **185**, 514–528 (2010).
9. A. F. Talhelm *et al.*, Elevated carbon dioxide and ozone alter productivity and ecosystem carbon content in northern temperate forests. *Global Change Biology*. **20**, 2492–2504 (2014).
10. B. A. Newingham *et al.*, 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 (2013).
11. M.-A. de Graaff, K. J. van Groenigen, J. Six, B. Hungate, C. van Kessel, Interactions between plant growth and soil nutrient cycling under elevated CO_2: a meta-analysis. *Global Change Biology*. **12**, 2077–2091 (2006).
12. E. A. Ainsworth, S. P. Long, 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 Phytol*. **165**, 351–372 (2005).
13. C. Körner *et al.*, Carbon flux and growth in mature deciduous forest trees exposed to elevated CO_2. *Science*. **309**, 1360–1362 (2005).
14. M. J. Hovenden, P. C. D. Newton, K. E. Wills, Seasonal not annual rainfall determines grassland biomass response to carbon dioxide. *Nature*. **511**, 583–586 (2014).

15. S. Baig, B. E. Medlyn, L. M. Mercado, S. Zaehle, Does the growth response of woody plants to elevated CO_2 increase with temperature? A model-oriented meta-analysis. *Global Change Biology*. **21**, 4303–4319 (2015).

16. M. C. Brundrett, Coevolution of roots and mycorrhizas of land plants. *New Phytol.* **154**, 275–304 (2002).

17. S. Vicca *et al.*, Fertile forests produce biomass more efficiently. *Ecol Lett.* **15**, 520–526 (2012).

18. M. G. A. van der Heijden *et al.*, Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature*. **396**, 69–72 (1998).

19. N. C. Johnson, G. W. T. Wilson, J. A. Wilson, R. M. Miller, M. A. Bowker, Mycorrhizal phenotypes and the Law of the Minimum. *New Phytol.* **205**, 1473–1484 (2015).

20. R. P. Phillips, E. Brzostek, M. G. Midgley, The mycorrhizal-associated nutrient economy: a new framework for predicting carbon–nutrient couplings in temperate forests. *New Phytol.* **199**, 41–51 (2013).

21. C. Averill, B. L. Turner, A. C. Finzi, Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. *Nature*. **505**, 543–545 (2015).

22. K. K. Treseder, A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO_2 in field studies. *New Phytol.* **164**, 347–355 (2004).

23. O. Alberton, T. W. Kuyper, A. Gorissen, Taking mycocentrism seriously: mycorrhizal fungal and plant responses to elevated CO_2. *New Phytol.* **167**, 859–868 (2005).

24. M. Shi, J. B. Fisher, E. R. Brzostek, R. P. Phillips, Carbon cost of plant nitrogen acquisition: global carbon cycle impact from an improved plant nitrogen cycle in the Community Land Model. *Global Change Biology*. **22**, 1299–1314 (2016).

25. J. E. Drake *et al.*, Increases in the flux of carbon belowground stimulate nitrogen uptake and sustain the long-term enhancement of forest productivity under elevated CO_2. *Ecol Lett.* **14**, 349–357 (2011).

26. R. J. Norby *et al.*, Model-data synthesis for the next generation of forest free-air CO_2 enrichment (FACE) experiments. *New Phytol.* **209**, 17–28 (2015).

27. J. Perez-Moreno, D. J. Read, Mobilization and transfer of nutrients from litter to tree seedlings via the vegetative mycelium of ectomycorrhizal plants. *New Phytol.* **145**, 301–309 (2000).

28. R. P. Phillips, A. C. Finzi, E. S. Bernhardt, Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO_2 fumigation. *Ecol Lett.* **14**, 187–194 (2011).

29. B. A. Hungate *et al.*, Fire, hurricane and carbon dioxide: effects on net primary production of a subtropical woodland. *New Phytol.* **200**, 767–777 (2013).

30. J. Cornelissen, R. Aerts, B. Cerabolini, M. Werger, M. van der Heijden, Carbon cycling traits of plant species are linked with mycorrhizal strategy. *Oecologia*. **129**, 611–619 (2001).

31. M. F. Cotrufo *et al.*, Formation of soil organic matter via biochemical and physical pathways of litter mass loss. *Nature Geosci.* **8**, 776–779 (2015).

32. B. N. Sulman, R. P. Phillips, A. C. Oishi, E. Shevliakova, S. W. Pacala, Microbe-driven turnover offsets mineral-mediated storage of soil carbon under elevated CO_2. *Nature Climate change*. **4**, 1099–1102 (2014).

33. Z. Zhu *et al.*, Greening of the Earth and its drivers. *Nature Climate change*, advance online publication (2016), doi:10.1038/nclimate3004.

34. V. B. Chaudhary *et al.*, MycoDB, a global database of plant response to mycorrhizal fungi. *Sci. Data*. **3**, 160028 (2016).

35. W. I. J. Dieleman *et al.*, Simple additive effects are rare: a quantitative review of plant biomass and soil process responses to combined manipulations of CO_2 and temperature. *Global Change Biology*. **18**, 2681–2693 (2012).

36. M. Fernández-Martínez *et al.*, Nutrient availability as the key regulator of global forest carbon balance. *Nature Climate change*. **4**, 471–476 (2014).

37. M. Campioli *et al.*, Biomass production efficiency controlled by management in temperate and boreal ecosystems. *Nature Geosci*. **8**, 843–846 (2015).

38. R. G. McLaren, K. C. Cameron, *Soil science: an introduction to the properties and management of New Zealand soils* (Oxford University Press, Auckland, N.Z., 1990).

39. B. Wang, Y. L. Qiu, Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza*. **16**, 299–363 (2006).

40. K. E. Mueller, S. E. Hobbie, D. Tilman, P. B. Reich, 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 (2013).

41. R. J. Hijmans, S. E. Cameron, J. L. Parra, P. G. Jones, A. Jarvis, Very high resolution interpolated climate surfaces for global land areas. *Int. J. Climatol.* **25**, 1965–1978 (2005).

42. L. V. Hedges, J. Gurevitch, P. S. Curtis, The meta-analysis of response ratios in experimental ecology. *Ecology*. **80**, 1150 (1999).

43. W. Viechtbauer, Conducting Meta-Analyses in R with the metafor Package. *Journal of Statistical Software*. **36** (2010).

44. M. Borenstein, L. V. Hedges, J. P. T. Higgins, H. R. Rothstein, in *Introduction to Meta-Analysis* (John Wiley & Sons, Ltd, Chichester, UK, 2009), pp. 225–238.

45. C. W. Osenberg, O. Sarnelle, S. D. Cooper, R. D. Holt, Resolving ecological questions through meta-analysis: goals, metrics, and models. *Ecology*. **80**, 1105–1117 (1999).

46. B. A. Hungate, R. B. Jackson, C. B. Field, F. S. Chapin III, Detecting changes in soil carbon in CO_2 enrichment experiments. *Plant Soil*. **187**, 135–145 (1996).

47. K. J. van Groenigen *et al.*, Element interactions limit soil carbon storage. *Proceedings of the National Academy of Sciences*. **103**, 6571–6574 (2006).

48. G. Knapp, J. Hartung, Improved tests for a random effects meta-regression with a single covariate. *Statistics in Medicine*. **22**, 2693–2710 (2003).

49. W. Viechtbauer, J. A. López-López, J. Sánchez-Meca, F. Marín-Martínez, A comparison of procedures to test for moderators in mixed-effects meta-regression models. *Psychological Methods*. **20**, 360–374 (2015).

50. V. Calcagno, C. de Mazancourt, glmulti: an R package for easy automated model selection with (generalized) linear models. *Journal of Statistical Software*. **34** (2010).

51. G. J. O'Leary *et al.*, Response of wheat growth, grain yield and water use to elevated CO_2 under a Free-Air CO_2 Enrichment (FACE) experiment and modelling in a semi-arid environment. *Global Change Biology*. **21**, 2670–2686 (2015).

52. B. Hoorens, R. Aerts, M. Stroetenga, Is there a trade-off between the plant's growth response to elevated CO_2 and subsequent litter decomposability? *Oikos*. **103**, 17–30 (2003).

53. I. A. Janssens *et al.*, Carbon budget of Pinus sylvestris saplings after four years of exposure to elevated atmospheric carbon dioxide concentration. *Tree Physiol*. **25**, 325–337 (2005).

54. A. R. Smith *et al.*, Elevated CO_2 enrichment induces a differential biomass response in a mixed species temperate forest plantation. *New Phytol*. **198**, 156–168 (2013).

55. S. Hättenschwiler, C. Körner, Biomass allocation and canopy development in spruce model ecosystems under elevated CO_2 and increased N deposition. *Oecologia*. **113**, 104–114 (1998).

56. C. Körner, J. A. Arnone III, Responses to Elevated Carbon Dioxide in Artificial Tropical Ecosystems. *Science*. **257**, 1672–1675 (1992).

57. J. A. Arnone III, C. Körner, Soil and biomass carbon pools in model communities of tropical plants under elevated CO_2. *Oecologia*. **104**, 61–71 (1995).

58. D. Spinnler, P. Egli, C. Körner, Four-year growth dynamics of beech-spruce model ecosystems under CO_2 enrichment on two different forest soils. *Trees*. **16**, 423–436 (2002).

59. L. C. Andresen, A. Michelsen, S. Jonasson, C. Beier, P. Ambus, Glycine uptake in heath plants and soil microbes responds to elevated temperature, CO_2 and drought. *Acta Oecologica*. **35**, 786–796 (2009).

60. T. Kou, J. Zhu, Z. Xie, T. Hasegawa, K. Heiduk, Effect of elevated atmospheric CO_2 concentration on soil and root respiration in winter wheat by using a respiration partitioning chamber. *Plant Soil*. **299**, 237–249 (2007).

61. A. Larigauderie, J. F. Reynolds, B. R. Strain, Root response to CO_2 enrichment and nitrogen supply in loblolly pine. *Plant Soil*. **165**, 21–32 (1994).

62. J. S. King, R. B. Thomas, B. R. Strain, Growth and carbon accumulation in root systems of Pinus taeda and Pinus ponderosa seedlings as affected by varying CO_2, temperature and nitrogen. *Tree Physiol*. **16**, 635–642 (1996).

63. P. Ineson, M. F. Cotrufo, R. Bol, D. D. Harkness, H. Blum, Quantification of soil carbon inputs under elevated CO_2: C3 plants in a C4 soil. *Plant Soil*. **187**, 345–350 (1996).

64. M. Liberloo *et al.*, 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 (2006).

65. K. Kostiainen *et al.*, Stem wood properties of mature Norway spruce after 3 years of continuous exposure to elevated [CO_2] and temperature. *Global Change Biology*. **15**, 368–379 (2009).

66. J. Kongstad *et al.*, High Resilience in Heathland Plants to Changes in Temperature, Drought, and CO_2 in Combination: Results from the CLIMAITE Experiment. *Ecosystems*. **15**, 269–283 (2011).

67. A. Volder, R. M. Gifford, J. R. Evans, Effects of elevated atmospheric CO_2, cutting frequency, and differential day/night atmospheric warming on root growth and turnover of Phalaris swards. *Global Change Biology*. **13**, 1040–1052 (2007).

68. P. G. Jarvis, A. M. Aitken, *European Forests and Global Change* (Cambridge University Press, 1998).

69. J. N. Klironomos, M. Ursic, M. Rillig, M. F. Allen, Interspecific differences in the response of arbuscular mycorrhizal fungi to Artemisia tridentata grown under elevated atmospheric CO_2. *New Phytol*. **138**, 599–605 (1998).

70. F. A. Bazzaz, S. L. Miao, Successional Status, Seed Size, and Responses of Tree Seedlings to CO_2, Light, and Nutrients. *Ecology*. **74**, 104 (1993).

71. G. M. Berntson, P. M. Wayne, F. A. Bazzaz, Below-ground architectural and mycorrhizal responses to elevated CO_2 in Betula alleghaniensis populations. *Functional Ecology*. **11**, 684–695 (1997).

72. M. S. J. Broadmeadow, S. B. Jackson, Growth responses of Quercus petraea, Fraxinus excelsior and Pinus sylvestris to elevated carbon dioxide, ozone and water supply. *New Phytol*. **146**, 437–451 (2000).

73. P. Högy, M. Keck, K. Niehaus, J. Franzaring, A. Fangmeier, Effects of atmospheric CO_2 enrichment on biomass, yield and low molecular weight metabolites in wheat grain. *Journal of Cereal Science*. **52**, 215–220 (2010).

74. J. Dukes *et al.*, Responses of Grassland Production to Single and Multiple Global Environmental Changes. *PLoS Biology*. **3**, e319 (2005).

75. B. A. Hungate *et al.*, The fate of carbon in grasslands under carbon dioxide enrichment. *Nature*. **388**, 576–579 (1997).

76. P. A. T. Higgins, R. B. Jackson, J. M. Des Rosiers, C. B. Field, Root production and demography in a california annual grassland under elevated atmospheric carbon dioxide. *Global Change Biology*. **8**, 841–850 (2002).

77. C. B. Field, F. S. Chapin III, N. R. Chiariello, E. A. Holland, H. A. Mooney, in *Carbon Dioxide and Terrestrial Ecosystems* (Academic Press, San Diego CA, 1996), pp. 121–145.

78. N. R. Chiariello, C. B. Field, in *Carbon Dioxide, Populations, and Communities* (Elsevier, 1996), pp. 139–157.

79. J. Heath *et al.*, Rising atmospheric CO_2 reduces sequestration of root-derived soil carbon. *Science*. **309**, 1711–1713 (2005).

80. B. A. Hungate *et al.*, Cumulative response of ecosystem carbon and nitrogen stocks to chronic CO_2 exposure in a subtropical oak woodland. *New Phytol*. **200**, 753–766 (2013).

81. R. D. Evans *et al.*, Greater ecosystem carbon in the Mojave Desert after ten years exposure to elevated CO_2. *Nature Climate change*. **4**, 394–397 (2014).

82. P. Newton, M. Lieffering, W. Bowatte, S. C. Brock, 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 Soil*. **336**, 433–441 (2010).

83. V. Allard *et al.*, 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 Soil*. **276**, 49–60 (2005).

84. R. J. Norby, C. A. Gunderson, S. D. Wullschleger, E. G. O'neill, Productivity and compensatory responses of yellow-poplar trees in elevated CO_2. *Nature*. **357**, 322–324 (1992).

85. S. Wan, R. J. Norby, K. S. Pregitzer, J. Ledford, E. G. O’Neill, CO_2 enrichment and warming of the atmosphere enhance both productivity and mortality of maple tree fine roots. *New Phytol*. **162**, 437–446 (2004).

86. R. J. Norby, S. D. Wullschleger, C. A. Gunderson, C. T. Nietch, Increased growth efficiency of Quercus alba trees in a CO_2-enriched atmosphere. *New Phytol*. **131**, 91–97 (1995).

87. P. Kardol *et al.*, Climate change effects on plant biomass alter dominance patterns and community evenness in an experimental old-field ecosystem. *Global Change Biology*. **16**, 2676–2687 (2010).

88. C. M. Iversen, J. K. Keller, C. T. Garten Jr, R. J. Norby, 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 (2012).

89. J. A. Morgan *et al.*, C4 grasses prosper as carbon dioxide eliminates desiccation in warmed semi-arid grassland. *Nature*. **476**, 202–205 (2011).

90. F. A. Dijkstra *et al.*, Contrasting effects of elevated CO_2 and warming on nitrogen cycling in a semiarid grassland. *New Phytol*. **187**, 426–437 (2010).

91. B. Gielen *et al.*, Net carbon storage in a poplar plantation (POPFACE) after three years of free-air CO_2 enrichment. *Tree Physiol*. **25**, 1399–1408 (2005).

92. O. Ghannoum *et al.*, Exposure to preindustrial, current and future atmospheric CO_2 and temperature differentially affects growth and photosynthesis in Eucalyptus. *Global Change Biology*. **16**, 303–319 (2010).

93. M. E. Gavito, P. Schweiger, I. Jakobsen, P uptake by arbuscular mycorrhizal hyphae: effect of soil temperature and atmospheric CO_2 enrichment. *Global Change Biology*. **9**, 106–116 (2003).

94. Q. Deng *et al.*, Responses of soil respiration to elevated carbon dioxide and nitrogen addition in young subtropical forest ecosystems in China. *Biogeosciences*. **7**, 315–328 (2010).

95. J. Riikonen *et al.*, Silver birch and climate change: variable growth and carbon allocation responses to elevated concentrations of carbon dioxide and ozone. *Tree Physiol*. **24**, 1227–1237 (2004).

96. A. Kasurinen *et al.*, Below-ground responses of silver birch trees exposed to elevated CO_2 and O_3 levels during three growing seasons. *Global Change Biology*. **11**, 1167–1179 (2005).

97. B. Schäppi, C. Körner, Growth responses of an alpine grassland to elevated CO_2. *Oecologia*. **105**, 43–52 (1996).

98. S. Bazot, L. Ulff, H. Blum, C. Nguyen, C. Robin, 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 (2006).

99. M. K. Schneider *et al.*, 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 (2004).

100. P. A. Niklaus, C. Körner, Synthesis of a six-year study of calcareous grassland responses to in situ CO_2 enrichment. *Ecological Monographs*. **74**, 491–511 (2004).

101. P. W. Leadley, P. A. Niklaus, R. Stocker, C. Körner, A field study of the effects of elevated CO_2 on plant biomass and community structure in a calcareous grassland. *Oecologia*. **118**, 39–49 (1999).

102. E. Pendall, Y. Osanai, A. L. Williams, M. J. Hovenden, Soil carbon storage under simulated climate change is mediated by plant functional type. *Global Change Biology*. **17**, 505–514 (2011).

103. K. S. Pregitzer *et al.*, Atmospheric CO_2, soil nitrogen and turnover of fine roots. *New Phytol*. **129**, 579–585 (1995).

104. D. R. Zak *et al.*, Elevated atmospheric CO_2 and feedback between carbon and nitrogen cycles. *Plant Soil*. **151**, 105–117 (1993).

105. C. J. Mikan, D. R. Zak, M. E. Kubiske, K. S. Pregitzer, Combined effects of atmospheric CO_2 and N availability on the belowground carbon and nitrogen dynamics of aspen mesocosms. *Oecologia*. **124**, 432–445 (2000).

106. C. S. Vogel, P. S. Curtis, R. B. Thomas, Growth and nitrogen accretion of dinitrogen-fixing Alnus glutinosa (L.) Gaertn. under elevated carbon dioxide. *Plant Ecology*. **130**, 63–70 (1997).

107. B. A. Kimball, S. B. Idso, S. Johnson, M. C. Rillig, Seventeen years of carbon dioxide enrichment of sour orange trees: final results. *Global Change Biology*. **13**, 2171–2183 (2007).

108. S. A. Prior, G. B. Runion, H. A. Torbert, S. B. Idso, B. A. Kimball, Sour orange fine root distribution after seventeen years of atmospheric CO_2 enrichment. *Agricultural and Forest Meteorology*. **162-163**, 85–90 (2012).

109. R. F. Walker, D. R. Geisinger, D. W. Johnson, J. T. Ball, Elevated atmospheric CO_2 and soil N fertility effects on growth, mycorrhizal colonization, and xylem water potential of juvenile ponderosa pine in a field soil. *Plant Soil*. **195**, 25–36 (1997).

110. D. M. Olszyk *et al.*, Whole-seedling biomass allocation, leaf area, and tissue chemistry for Douglas-fir exposed to elevated CO_2 and temperature for 4 years. *Can. J. For. Res.* **33**, 269–278 (2003).

111. A. R. Smith, M. Lukac, M. Bambrick, F. Miglietta, D. L. Godbold, Tree species diversity interacts with elevated CO_2 to induce a greater root system response. *Global Change Biology*. **19**, 217–228 (2013).

112. A. Antoninka, P. B. Reich, N. C. Johnson, Seven years of carbon dioxide enrichment, nitrogen fertilization and plant diversity influence arbuscular mycorrhizal fungi in a grassland ecosystem. *New Phytol*. **192**, 200–214 (2011).

113. F. Hagedorn, D. Spinnler, M. Bundt, P. Blaser, R. Siegwolf, The input and fate of new C in two forest soils under elevated CO_2. *Global Change Biology*. **9**, 862–872 (2003).

114. D. R. Zak, W. E. Holmes, A. C. Finzi, R. J. Norby, W. H. Schlesinger, Soil nitrogen cycling under elevated CO_2: a synthesis of forest face experiments. *Ecological Applications*. **13**, 1508–1514 (2003).

115. R. E. Dickson *et al.*, Forest atmosphere carbon transfer and storage (FACTS-II) the aspen Free-air CO_2 and O_3 Enrichment (FACE) project: an overview. General Technical Report NC-214. St. Paul, MN: U.S. Dept. of Agriculture, Forest Service, North Central Forest Experiment Station (2000).

116. H. J. Jäger *et al.*, The University of Giessen free-air carbon dioxide enrichment study: description of the experimental site and of a new enrichment system. *Journal of Applied Botany*. **77**, 117–127 (2003).

117. V. M. Temperton *et al.*, Effects of elevated carbon dioxide concentration on growth and nitrogen fixation in Alnus glutinosa in a long-term field experiment. *Tree Physiol*. **23**, 1051–1059 (2003).

118. L. F. Huenneke, S. P. Hamburg, R. Koide, H. A. Mooney, P. M. Vitousek, Effects of Soil Resources on Plant Invasion and Community Structure in Californian Serpentine Grassland. *Ecology*. **71**, 478–491 (1990).

119. P. Dijkstra *et al.*, Elevated atmospheric CO_2 stimulates aboveground biomass in a fire-regenerated scrub-oak ecosystem. *Global Change Biology*. **8**, 90–103 (2002).

120. D. N. Jordan *et al.*, Biotic, abiotic and performance aspects of the Nevada Desert Free-Air CO_2 Enrichment (FACE) Facility. *Global Change Biology*. **5**, 659–668 (1999).

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

122. M. E. Gavito, P. S. Curtis, T. N. Mikkelsen, I. Jakobsen, Atmospheric CO_2 and mycorrhiza effects on biomass allocation and nutrient uptake of nodulated pea (Pisum sativum L.) plants. *Journal of Experimental Botany*. **51**, 1931–1938 (2000).

123. A. Kasurinen, P. Kokko Gonzales, J. Riikonen, E. Vapaavuori, T. Holopainen, Soil CO_2 efflux of two silver birch clones exposed to elevated CO_2 and O_3 levels during three growing seasons. *Global Change Biology*. **10**, 1654–1665 (2004).

124. T. Hebeisen *et al.*, Growth response of Trifolium repens L. and Lolium perenne L. as monocultures and bi-species mixture to free air CO_2 enrichment and management. *Global Change Biology*. **3**, 149–160 (1997).

125. P. A. Niklaus, M. Wohlfender, R. Siegwolf, C. Körner, Effects of six years atmospheric CO_2 enrichment on plant, soil, and soil microbial C of a calcareous grassland. *Plant Soil*. **233**, 189–202 (2001).

126. M. J. Hovenden *et al.*, Warming prevents the elevated CO_2-induced reduction in available soil nitrogen in a temperate, perennial grassland. *Global Change Biology*. **14**, 1018–1024 (2008).

127. J. Lussenhop, A. Treonis, P. S. Curtis, J. A. Teeri, C. S. Vogel, Response of soil biota to elevated atmospheric CO_2 in poplar model systems. *Oecologia*. **113**, 247–251 (1998).

128. P. Reich *et al.*, Nitrogen limitation constrains sustainability of ecosystem response to CO_2. *Nature*. **440**, 922–925 (2006).

129. R. Oren *et al.*, Soil fertility limits carbon sequestration by forest ecosystems in a CO_2-enriched atmosphere. *Nature*. **411**, 469–472 (2001).

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

131. P. Jarvis, S. Linder, Botany: Constraints to growth of boreal forests. *Nature*. **405**, 904–905 (2000).

132. P. S. Curtis *et al.*, Biometric and eddy-covariance based estimates of annual carbon storage in five eastern North American deciduous forests. *Agricultural and Forest Meteorology*. **113**, 3–19 (2002).

133. S. N. Turitzin, Nutrient Limitations to Plant Growth in a California Serpentine Grassland. *American Midland Naturalist*. **107**, 95 (1982).

134. T. Rütting, L. C. Andresen, Nitrogen cycle responses to elevated CO_2 depend on ecosystem nutrient status. *Nutr Cycl Agroecosyst*. **101**, 285–294 (2015).

135. R. J. Norby, D. R. Zak, Ecological lessons from free-air CO_2 enrichment (FACE) experiments. *Annual Review of Ecology*. **42**, 181–203 (2011).

136. A. P. Walker *et al.*, Predicting long-term carbon sequestration in response to CO_2 enrichment: How and why do current ecosystem models differ? *Global Biogeochem. Cycles*. **29**, 476–495 (2015).

137. D. Nyfeler *et al.*, Strong mixture effects among four species in fertilized agricultural grassland led to persistent and consistent transgressive overyielding. *Journal of Applied Ecology*. **46**, 683–691 (2009).

138. D. Nyfeler, O. Huguenin-Elie, M. Suter, E. Frossard, A. Lüscher, Grass-legume mixtures can yield more nitrogen than legume pure stands due to mutual stimulation of nitrogen uptake from symbiotic and non-symbiotic sources. *Agriculture, Ecosystems & Environment*. **140**, 155–163 (2011).

139. P. C. D. Newton *et al.*, Selective grazing modifies previously anticipated responses of plant community composition to elevated CO_2 in a temperate grassland. *Global Change Biology*. **20**,

From Terrer C. *et al.* Response to Comment on 'Mycorrhizal association as a primary control of the CO_2 fertilization effect'. Science vol. 355, issue 6323 (Jan 2017), p. 358. DOI: 10.1126/science.aai8242. Reprinted with permission from AAAS.

2.8. Addendum

The following section is based on the response to a comment by Norby *et al*. (DOI: 10.1126/science.aai7976) on the conclusions from Chapter II

Norby *et al.* centre their critique on the design of the dataset and the response variable used. I address these criticisms here, reinforcing the conclusion that plants that associate with ectomycorrhizal (ECM) fungi exhibit larger biomass and growth responses to elevated CO_2 compared to plants that associate with arbuscular mycorrhizae (AM).

Results and Discussion

In their Comment, Norby *et al.* question the robustness of the conclusions from Chapter II (*1*). I hope that answering their queries reinforces the conclusions in the original paper:

First, Norby *et al*'s assertion that we included entries "not relevant to the question at hand" is unfounded: Terrer *et al.* (*1*) evaluated factors that influence plant biomass responses to elevated CO_2, so we used a database of experiments that measured plant biomass responses to elevated CO_2. Norby *et al.* suggest that we intentionally excluded experiments, but this is not so and in fact we included as many as possible. They also recommend the exclusion of pot studies; but *a priori* assessment and exclusion of experiments is ill advised in meta-analysis (*2*). Instead, confounding factors should be postulated and tested quantitatively, as we did through mixed-effects meta-regression models and found no evidence that growth chamber studies underestimate the CO_2 response (see Fig S II.4) Regarding additional experiments that should be included in our dataset, Norby et al. point out Flakaliden; but this study was included in our original dataset of *aboveground* biomass responses (Fig. S-II.2), and did not alter the conclusions. Nevertheless, here we conduct a validation test by excluding all pot experiments, and including not one, but three, nonexistent (hypothetical) ECM experiments under low N with a 0% CO_2 effect. The results of this validation test (n=72) were: AM-lowN: 1.6% (*P*=0.7367) and ECM-lowN: 25.8% (*P*<.0001), with significant differences in AM-lowN versus ECM-lowN (*P*=0.0010 with Bonferroni´s correction). Thus, we are confident that our main finding - CO_2 stimulation of biomass under low N is greater in ECM than AM ecosystems - is robust and unbiased.

Second, we agree that productivity is a more powerful metric than biomass, in part because biomass responses are cumulative, and experiments varied in duration. Relatively few data on productivity have been published from CO_2 experiments. Nonetheless, here we have performed a meta-analysis of aboveground productivity (ANPP) responses to CO_2 in N-limited studies (Fig. Addendum-II.1). Despite the small sample size, results support our original conclusions (Fig. II.2). Norby *et al.* argue that leaf area normalization should be used to control for CO_2 effects on leaf area, but Norby *et al.*'s Fig. 1 represents a special case, showing a pattern that is far from universal. For example, at Duke and Aspen FACE, ECM trees responded positively to elevated CO_2 even when excluding all years before “canopy development was complete” (*3*), while at ORNL, AM trees did not (*4*). Furthermore, if the primary interest is in biomass accumulation, factoring out leaf area effects is inappropriate. On the contrary, as rising CO_2 and N additions affect both leaf area and growth efficiency (*5*), both need to be included in evaluating effects on biomass or productivity.

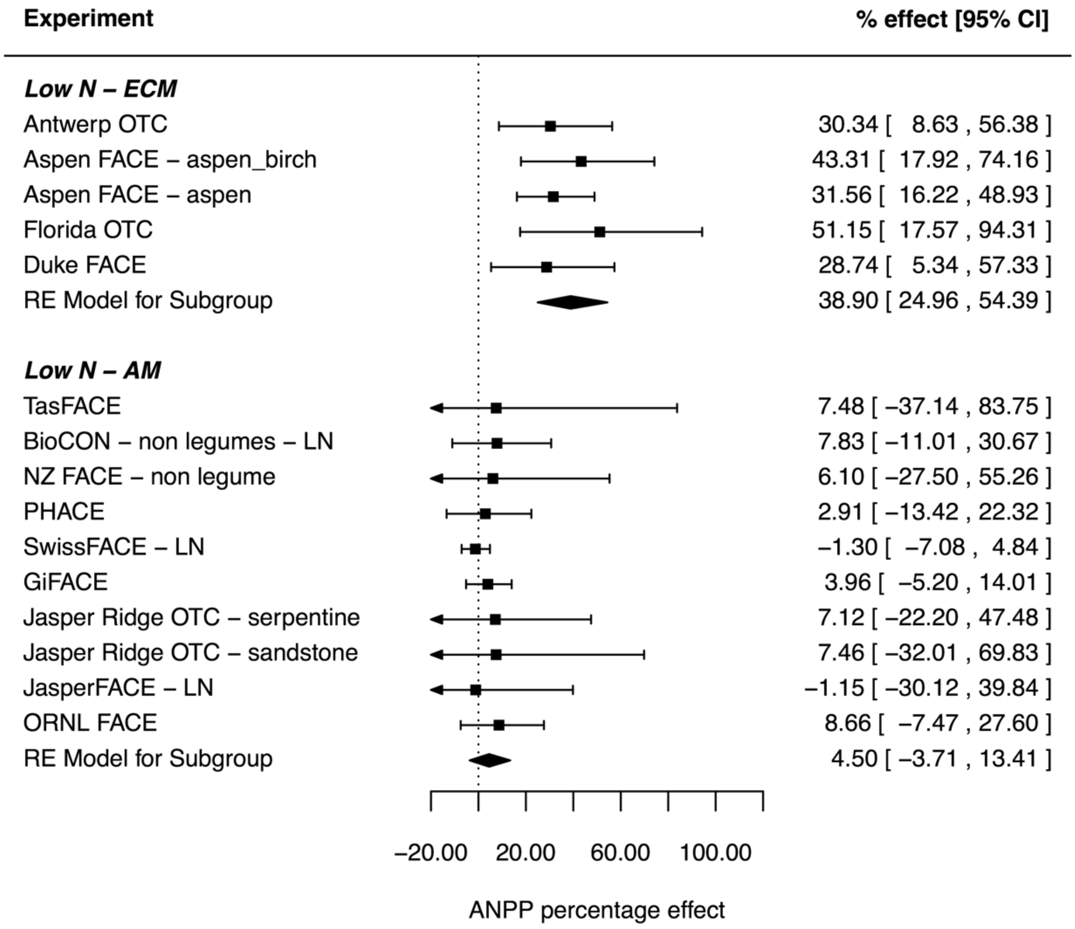


Figure Addendum-II.1 Meta-analysis of CO_2 effects on aboveground net primary productivity (ANPP) for two types of mycorrhizal plants species (AM and ECM) in N-limited experiments (low N). Results for the individual studies (squares) and overall effects for the subgroups (diamonds) are given. We interpret CO_2 effects when the zero line is not crossed. Note that standing crop is the standard proxy for ANPP for grasslands, therefore productivity responses in grasslands were implicitly already considered in the original paper. References and information about the individual experiments in Table S-II.1.

Third, Norby *et al*. suggested that the observed AM versus ECM response difference might simply reflect the differences between grasses and trees. When taking all studies and predictors into account we found that plant functional type and vegetation age were not among the most important predictors (Fig. II.1). Therefore, i) the conclusions are not the result of a comparison of grasses versus trees, and ii) there are no grounds to exclude studies with seedlings, as suggested by Norby *et al*. Nevertheless, we fully agree that more enhanced CO_2 studies in AM forests are merited.

Fourth, in contrast to ECM, AM fungi have no known saprotrophic capability to access N in complex organic forms (*6*). And while differences in enzyme activity among ECM fungal taxa have been reported, most ECM fungi possess the ability to synthesize enzymes that can degrade soil organic matter (*7*). By synthesizing available data from 10 CO_2 experiments under low N (5 ECM, 5 AM), we found that the CO_2 effect on N uptake was four times higher in ECM than AM plants (16.30 vs 4.13%). Since N has been suggested as the most common limiting factor on growth responses to CO_2, the much larger capacity of ECM than AM plants to increase N uptake in response to elevated CO_2 likely helps explain the observed difference in growth responses to elevated CO_2.

Fifth, Norby *et al.* isolated the responses in two particular studies (in which they were involved), and invoke the progressive nitrogen limitation (PNL) hypothesis, which predicts a decreasing CO_2 effect over time, to explain the observed differences. Such comparison between 2 studies cannot be directly compared to the outcome of a meta-analysis with 83 studies. Clearly, various factors are likely at work, but as we show here and in (*1*), mycorrhizal type and nitrogen availability play key roles in explaining CO_2 responses across the full range of enhanced CO_2 experiments. Furthermore, we showed that the length of the treatment was not among the most important predictors (Fig. II.1) indicating that CO_2 responses do not generally decrease, at least over the time scale typical of experiments.

Plants typically allocate a considerable amount of C to their mycorrhizal symbionts (*8*), and this quantity varies with mycorrhizal type (*9*) and nutrient availability (*10*). Model developers are trying to improve representations of the N cycle (*11*), and there have been efforts to include better representations of roots (*12*), microbes, and root-microbe interactions (*13*). Why then should mycorrhizal fungi, which serve as both extensions of the root system (AM, ECM) and mineralizers of organic N (ECM), not be modelled explicitly? In fact, one of the co-authors of the critique specifically recommended including mycorrhizal associations into models (*12*), forming the foundation of our recommendation, which Norby *et al.* now challenge. Given emerging evidence for mycorrhizae as trait integrators (*14*), that mycorrhizal associations may be detectable from space (*15*), and the evidence we have presented here and in our original analysis about the role of mycorrhizae in shaping plant responses to elevated CO_2, we maintain that there is a substantial foundation for including mycorrhizal associations in biogeochemical models. Doing so will accelerate development of the models and, over time, improve their simulations of the future biosphere.

References

1. C. Terrer, S. Vicca, B. A. Hungate, R. P. Phillips, I. C. Prentice, Mycorrhizal association as a primary control of the CO_2 fertilization effect. *Science*. **353**, 72–74 (2016).
2. D. F. Stroup *et al.*, Meta-analysis of Observational Studies in Epidemiology: A Proposal for Reporting. *JAMA*. **283**, 2008–2012 (2000).
3. R. Norby *et al.*, Forest response to elevated CO_2 is conserved across a broad range of productivity. *Proceedings of the National Academy of Sciences*. **102**, 18052–18056 (2005).
4. R. J. Norby, J. M. Warren, C. M. Iversen, B. E. Medlyn, R. E. McMurtrie, CO_2 enhancement of forest productivity constrained by limited nitrogen availability. *Proc Natl Acad Sci U S A*. **107**, 19368–19373 (2010).
5. R. Hyvönen *et al.*, The likely impact of elevated [CO_2], nitrogen deposition, increased temperature and management on carbon sequestration in temperate and boreal forest ecosystems: a literature review. *New Phytol*. **173**, 463–480 (2007).
6. S. E. Smith, D. J. Read, *Mycorrhizal symbiosis* (Academic press, Cambridge, UK, 2008).
7. J. M. Talbot, F. Martin, A. Kohler, B. Henrissat, K. G. Peay, Functional guild classification predicts the enzymatic role of fungi in litter and soil biogeochemistry. *Soil Biology and Biochemistry*. **88**, 441–456 (2015).
8. E. A. Hobbie, Carbon allocation to ectomycorrhizal fungi correlates with belowground allocation in culture studies. *Ecology*. **87**, 563–569 (2006).
9. M. Shi, J. B. Fisher, E. R. Brzostek, R. P. Phillips, Carbon cost of plant nitrogen acquisition: global carbon cycle impact from an improved plant nitrogen cycle in the Community Land Model. *Global Change Biology*. **22**, 1299–1314 (2016).
10. S. Vicca *et al.*, Fertile forests produce biomass more efficiently. *Ecol Lett*. **15**, 520–526 (2012).
11. J. B. Fisher, D. N. Huntzinger, C. R. Schwalm, S. Sitch, Modeling the Terrestrial Biosphere. *Annual Review of Environment and Resources*. **39**, 91–123 (2014).
12. J. M. Warren *et al.*, Root structural and functional dynamics in terrestrial biosphere models--evaluation and recommendations. *New Phytol*. **205**, 59–78 (2015).
13. B. N. Sulman, R. P. Phillips, A. C. Oishi, E. Shevliakova, S. W. Pacala, Microbe-driven turnover offsets mineral-mediated storage of soil carbon under elevated CO_2. *Nature Climate change*. **4**, 1099–1102 (2014).
14. G. Lin, M. L. McCormack, C. Ma, D. Guo, Similar below-ground carbon cycling dynamics but contrasting modes of nitrogen cycling between arbuscular mycorrhizal and ectomycorrhizal forests. *New Phytol* (2016), doi:10.1111/nph.14206.
15. J. B. Fisher *et al.*, Tree-mycorrhizal associations detected remotely from canopy spectral properties. *Global Change Biology*. **22**, 2596–2607 (2016).

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

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

3.1. Overview

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 correlated 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 efficiencies depending on plant interactions with microbial symbionts.

3.2. 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 (1) even under the most ambitious climate-change mitigation scenarios (2). While it is well established that elevated CO_2 (eCO_2) stimulates photosynthesis at the leaf level (3), 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 (4) – 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 (5). Relatively tight stoichiometric constraints imply that if the nutrient requirements to increase plant growth are not met (6), nutrient availability will inevitably limit the terrestrial C sink (7). Nitrogen (N) availability, in particular, appears to limit plant productivity in many terrestrial ecosystems (8-10), and N availability is widely considered to be among the most important factors limiting the productivity response of ecosystems to eCO_2 (7, 11-13). 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 (12, 14-18). Low N availability can limit the positive growth response to eCO_2, resulting in a transient, small or non-existent CO_2 fertilization (19-22). In other cases, plant production can increase with eCO_2 despite apparent N limitation (23-25), suggesting enhanced N availability via natural processes such as biological N2-fixation (BNF) or accelerated SOM decomposition ("priming"). Consequently, most reviews have concluded that the magnitude of the CO_2 effect varies on a site-by-site basis, leaving two important questions open: for how long can eCO_2 enhance plant growth and carbon storage under low N availability, and ii) what are the underlying mechanisms that are responsible for the observed inter-site variation?

One recent hypothesis predicts that the N limitation on plant responses to eCO_2 is modulated by symbiotic plant-microbial interactions (13, 26-28). In Chapters II, using meta-analysis, we 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 (13). 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 N2-fixers (~8%). These conclusions proved consistent for aboveground productivity as well as biomass (29). Although more long-term eCO_2 experiments with both AM and ECM trees are needed to further test this hypothesis (30), 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 (28, 31). 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.

The conclusions of Chapters II (13, 29), however, raise additional hypotheses: i) do ECM plants and N2-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, or by an allocation shift, or both? And iii) how do changes in N availability under eCO_2 affect soil C stocks and 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 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. In section 4.4 we review the ecosystem-level effects triggered by eCO_2 from above to belowground using the efficiency in N acquisition as a common link driving these processes. In section 4.5 we discuss the conclusions and propose a conceptual framework, with indications of productive directions for model and experimental improvements.

3.3 Methods

The return on investment approach

We define *N_{acq}-efficiency* as a "return on investment" that quantifies the marginal relative increase in N_{acq} as a ratio of 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:

Equation 3

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

Ψ_N can be interpreted as the "cost of 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 measures the degree to which aboveground growth is limited by N.

While N_{acq} is often measured in eCO_2 experiments (e.g. 43), estimating the C investment in N_{acq} remains a conceptual and methodological challenge. Net primary productivity (NPP) is often assumed to be equal to biomass productivity, but it is important to highlight in this context that NPP also includes C transferred to root exudates, mycorrhizal fungi and symbiotic N-fixing bacteria ($C_{transfer}$; see *40*). $C_{transfer}$ represents a fraction of 10-

40% of NPP (*32*, *33*). Thereby, allocation of assimilated C to C_{transfer} implies a cost by reducing the C available for biomass productivity (BP):

Equation 4

$$BP = NPP - C_{transfer}$$

Several lines of evidence suggest that plants increase allocation to C_{transfer} as soil resources decrease in availability (*26*, *34-39*), and that such shifts in allocation can decrease plant (*40*) and ecosystem (*41*) production. This may explain why mycorrhizal abundance is typically increased by eCO_2 (by ~47%) but decreased by N-fertilization (by ~15%), indicating that plants invest in C_{transfer} to meet increased N demand caused by eCO_2 (*35*). Moreover, differences in the C cost of nutrient acquisition may also explain why the proportion of fixed C allocated to roots (and by extension C_{transfer}) is inversely related to N availability at global scales (*42*), with greater belowground investment in boreal relative to tropical regions.

The parameter most directly relevant for the plant C budget and reflective of "investments" for N uptake (or nutrient uptake in general) is the total belowground C allocation ($C_{\text{bg}} = C_{\text{transfer}}$ + root production + root respiration) (*42*). However, there have been few measurements of C allocation to fungi and exudates (C_{transfer}) in eCO_2 experiments (*37*). Therefore, we use the relative change in fine-root production, fine-root biomass, or root biomass as a surrogate for ∂C_{bg} (Eq. 3, proxy for C "investment" in N_{acq}) together with aboveground N_{acq} ("return" on the investment) (Fig. III.1). We included data from previous syntheses on eCO_2-driven N_{acq} (*43*, *44*), and searched from the Web of Science for belowground data, to include recent additional years and additional field experiments (Free-Air CO_2 enrichment (FACE) and open top chamber (OTC)) with available data on both N_{acq} and C allocation belowground, necessary to calculate Ψ_N^{-1} (Eq. 3). In total, we use observations from 20 grassland and forest ecosystem experiments corresponding to 12 different sites (Table III.1). We only included observations from experiments with closed canopies, as described elsewhere (*45*).

Table III.1 Meta-data of some of the most commonly discussed CO_2 experiments in the review and references for the data used in Figs. IV.1- IV.4. The amount of N-fertilization applied is indicated in parenthesis (units in g m-2 yr-1). The type of root data used is indicated by an asterisk below.

Site	**Location**	**Ecosystem, species**	**N**	**Symbiont**	**Roots**	**N_{acq}**	**V_{cmax}, A_{sat}**	**ANPP**	**soil C**
Aspen FACE	Rhinelander, WI, USA	Forest (deciduous): *Populus tremuloides & Betula papyrifera*	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, Switzerlan	Ryegrass dominated by *Lolium perenne*	Medium (14) and	AM	(Bazot *et al.*, 2006) ****	(Schneider *et al.*, 2004)	(Rogers *et al.*, 1998)	(Schneider *et al.*, 2004)	(van Kessel *et al.*, 2006)

	d		high (56)			pers.comm			
POP-FACE	Tuscany, Italy	Forest (deciduous): *Populus alba, P. nigra & P. euramericana*	High	ECM	(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 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

3.4 Results

Here we summarize findings regarding the role of N acquisition (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.

3.4.1. CO_2 effects on N acquisition

In the absence of N fertilization, N_{acq} significantly increased by 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. III.1A, the slope represents N_{acq}-efficiency (Ψ_N^{-1}), with lighter shading representing higher “returns”. Most ECM experiments plot close to the 1:1 line, suggesting proportionality between the relative changes in investment and acquisition (1% increase in C investment belowground translates into a 1% increase in N_{acq}). Systems where N_2-fixers are present exhibit 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 (*20*), 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 (*46*). On the other hand, for a given amount of C invested belowground, AM plants achieve a much lower enhancement in N_{acq} than ECM plants, and may even acquire less N than under ambient CO_2 despite increasing C investment (Fig. III.1A). This relates to results by (*43*). 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. III.1B).

The data in Fig. III.1 is limited by the lack of $C_{transfer}$ data, but assuming that changes in $C_{transfer}$ are proportional to changes in root biomass or production (*36*) circumvents this limitation. However, a potential larger investment in ECM- than AM-fungi under eCO_2 (*27*) might slightly underestimate investments in ECM plants in Fig. III.1. We can compare if the patterns observed with Ψ_N^{-1} can also be found using C_{bg} and $C_{transfer}$ estimates calculated via mass-balance approach (*47*) in a few experiments. For example, in the Duke FACE experiment (ECM), plants under eCO_2 invested 82.5 g of C_{bg} per g of N, including 12 g of $C_{transfer}$ (the latter being a better indicator of the non-biomass investment in N acquisition)

(*26*). At BioCON, the cost of N_{acq} under eCO_2 and low N was almost 10 times higher than for ECM-trees at Duke (810 g C_{bg} g^{-1} N). In N_2-fixing legumes, however, eCO_2 stimulated N_{acq} at a rate of 97 g C_{bg} g^{-1} N under low N, and 84 g C_{bg} g^{-1} N under high N (*48*), similar to ECM-trees at Duke. These patterns indicate that the cost of N_{acq} through C_{bg} varies across N_{acq}-strategies.

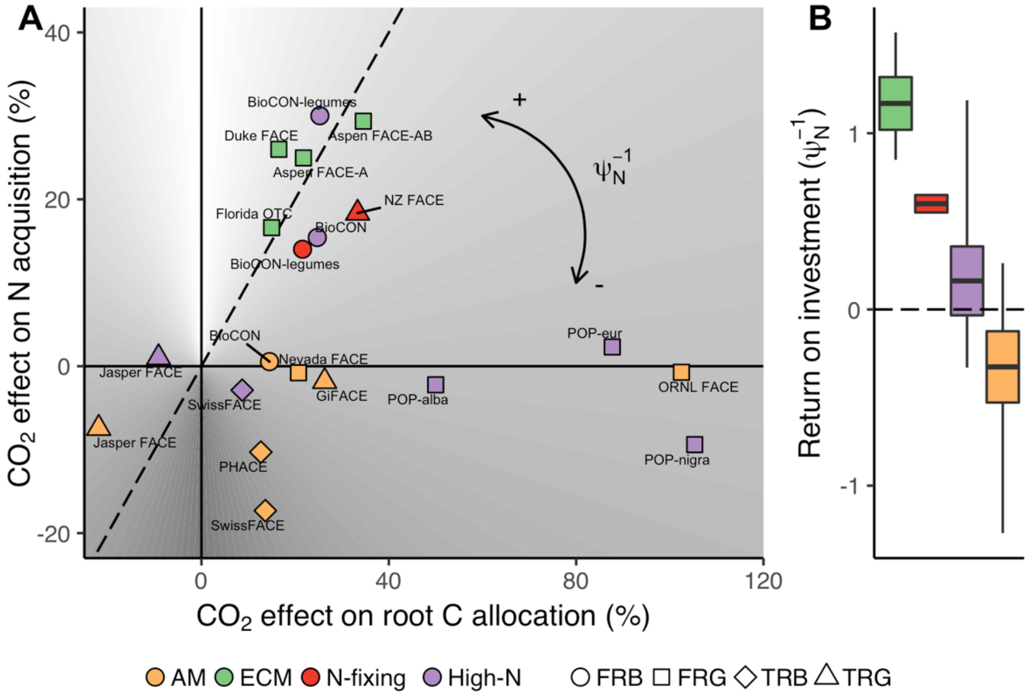


Figure III.1 **A)** Relationship between the eCO_2-induced relative change (%) in belowground C allocation and aboveground N acquisition (*Eq. 3*) evaluated from CO_2 experiments dominated by three main different types of symbiotic associations (arbuscular mycorrhizae (AM), ectomycorrhizae (ECM) and N-fixing species) and N-fertilization (High-N). Point shapes indicate the type of root data used: fine-root biomass (FRB), fine-root growth (FRG), total-root biomass (TRB) or total-root growth (TRG). Black dashed line is the 1:1 line. The slope of the grey lines in the background represents the return on investment (Ψ_N^{-1}, *Eq. 3*), with lower returns (higher costs) as dark grey. N-acquisition is the product of total or aboveground biomass production and N concentration. **B)** Mean, standard error, max and min Ψ_N^{-1}. References in Table III.1.

Indeed, 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 (*49*), with ectomycorrhizal (ECM) fungi generally associated with more beneficial effects on their plant host's fitness than arbuscular mycorrhizal (AM) fungi (*50*, *51*). 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 N when in low supply (*52*, *53*). eCO_2 did not commonly enhance aboveground N_{acq} in AM plants in this dataset (Fig. III.1A), whereas root investment was increased, leading to a negative mean Ψ_N^{-1} (Fig. III.1B). This is consistent with the hypothesis of AM-parasitism under low N availability (*52*, *53*). On the other hand, AM fungi are commonly associated with enhanced plant N_{acq} when N availability is moderate or high (*52*, *54*). By contrast, many ECM fungal species produce extracellular enzymes that break down SOM and transfer organic and inorganic forms of N to the host plant (*55*, *56*).

In the following sections, we explore how the efficiency of plants to acquire additional N influences eCO_2 effects on photosynthesis, plant productivity and SOM decomposition and storage.

3.4.2. CO_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 (*57*). 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 (*58*).

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. III.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) (*3*, *15*, *59*).

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. III.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. III.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 (*60*). At the AM-forest FACE experiment in Oak Ridge (ORNL), eCO_2 reduced foliar N (due to low N availability), and resulted in a lower 21% stimulation of A_{sat} (*61*) (although with small sample sizes and only occasional measurements rendered this effect non-significant).

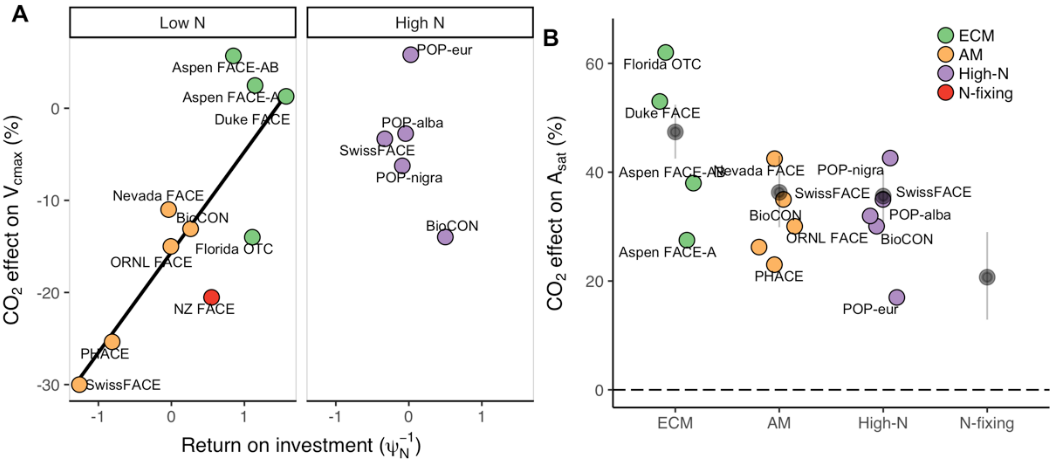


Figure III.2 **A)** Relationship between the effect of elevated CO_2 on V_{cmax} and the N return on investment (Ψ_N^{-1}, Eq. 3) under low (left panel) and high (right panel) N availability. **B)** Summary of eCO_2 effects on light saturated photosynthesis (A_{sat}). Black dots are mean effects ± CI from a meta-analysis by Ainsworth & Long (2005) for trees, grasses, N-fertilised plants and legumes. References in Table III.1.

The effect of eCO_2 on A_{sat} in legumes (*3*, *62*) and N-fertilized plants, however, was not higher than in AM non-fertilized plants (Fig. III.2B), as we hypothesized. 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 (*63*, *64*). 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%) (*64*), 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 (*20*). The lower effect on A_{sat} in legumes than in grasses (Fig. III.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 (*3*). Norby & Zak (*17*) 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 (*65*).

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 (*66*, *67*), but a meta-analysis of eCO_2 experiments did not find a significantly negative effect for an average $[CO_2]$ of 571 ppm (*59*). Furthermore, a meta-analysis found that eCO_2 increased the number of leaves (*3*), an effect that might compensate for any potential reduction on stomatal density at the ecosystem level. The experiments shown in Fig. III.2B did not generally find a decreasing A_{sat} response over time, but the long-term acclimation to eCO_2 requires further investigation (*66*).

Conclusions: although the influence of N on the eCO_2 effect on V_{cmax} is long known, it has commonly been linked to plant functional groups rather than actual N_{acq}-strategies. 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.

3.4.3. CO_2 effects on biomass production

Background: when N availability is low, a positive growth enhancement effect of eCO_2 depends on plants' ability to (i) increase their rate of N_{acq} from the soil (*44*, *68*), 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.* (*69*) 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} (*43*, *44*).

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 significant and positive relationship between Ψ_N^{-1} and the eCO_2 effect on aboveground biomass productivity (ANPP) (Fig. III.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 changes in biomass is part of the Ψ_N^{-1} calculation, increased C investment belowground reduces Ψ_N^{-1}; thus, the positive relationship in Fig. III.3 is not necessarily confounded by this issue (see also ref. *43*).

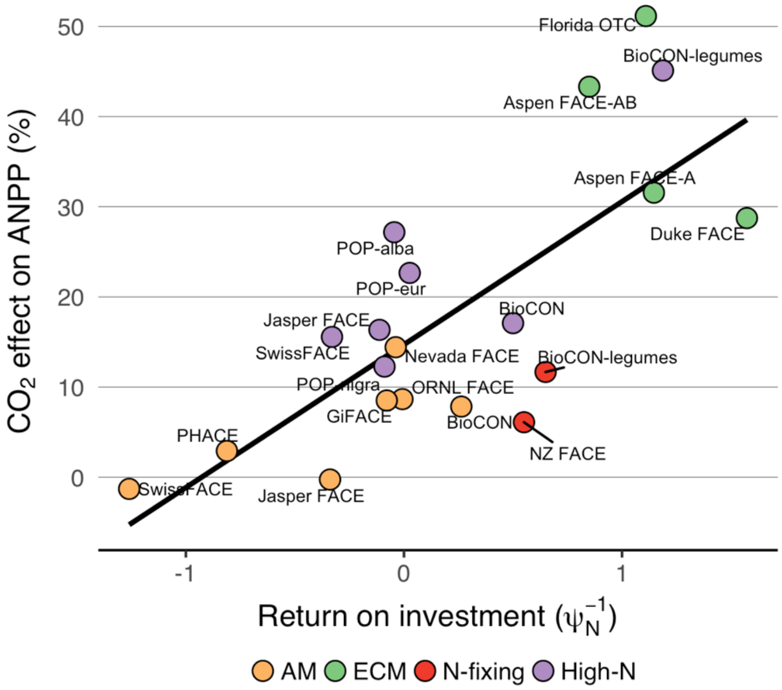


Figure III.3 Relationship between the effect of elevated CO_2 on aboveground biomass production (ANPP) and the N return on investment (Ψ_N^{-1}, Eq. 3). References in Table III.1.

Although N_{acq}-efficiency is a primary factor determining the ANPP response to eCO_2, other factors such as P, water, climate, biodiversity or disturbances are also at play. ECM plants consistently showed the largest increases in ANPP owing to effective investment in N_{acq}. For example, the ECM scrub-oak OTC experiment in Florida showed the largest increase in ANPP (Fig. III.2), possibly linked with a pulse of belowground resource availability associated with disturbance, initially by fire and later by hurricane (*23*). 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 (*24*, *25*). Furthermore, N fertilization in the Duke FACE experiment did not increase productivity further (*25*), consistent with the observation in the US of increased aboveground growth in most AM trees in response to N deposition, but not in ECM trees (*70*). Efficient N_{acq} stimulated trees at the Duke and Aspen FACE experiments to increasingly allocate more C to wood (with low [N]), enhancing NUE (*69*).

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} ratio than other ECM species despite high N availability (Figs. IV.1 and IV.3) due to the lack of an eCO_2-driven N_{acq} enhancement; N_{acq} was already high in both CO_2 treatment plots owing to previous agricultural use and irrigation (*71*). Instead, trees at POP-FACE sustained the eCO_2 fertilization effect by increasing NUE (*44*), which was likely influenced by increased allocation to wood (low [N]).

AM systems, however, showed a wider range of responses, driven by their variable capacity to acquire N efficiently, 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 (*19*), consistent with the lower cost of N_{acq} associated with N-fertilisation (Fig. III.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 (*72*) (data not included in Fig. III.3 because no indication of $C_{transfer}$ was found). Similarly at BioCON, the eCO_2-enhancement in productivity was larger in legumes than in non-legume AM species (Fig. III.3) (see *73*), accompanied by a higher Ψ_N^{-1}. The ANPP response of AM species in the Nevada Desert FACE from 1998 to 2007 (Fig. III.3) showed pronounced interannual variation because growth was limited by water availability, with stronger increases in ANPP under eCO_2 in wet years (*74*, *75*). However, these periodic increases in productivity did not result in increased above or belowground biomass at the end of the experiment (*76*).

AM trees at ORNL FACE showed the opposite pattern than Aspen and Duke FACE trees: as AM fungi have little effect on plant N_{acq}, these trees relied primarily on increased allocation to fine roots (with high [N]) to explore a larger proportion of the soil (*21*, *77*), 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 (*44*), 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. III.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 (*78*).

There are still uncertainties about the role of symbionts as modulators of the magnitude of the eCO_2 fertilization effect. The role of ECM fungi under extremely N-scarce conditions is uncertain. 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 (*22*). Mycorrhizal symbioses are thought to follow a continuum from mutualistic to parasitic (*79*). At the lower limit of N availability, there may be a point below which ECM fungi do not transfer enough N to the plant to elicit and sustain higher rates of eCO_2-growth, as some models suggest (*80*, *81*). Another important uncertainty is about the role of symbiotic types in acquiring nutrients other than N. Unlike organic N, AM-fungi can acquire P and transfer it to the host plant (*82*), opening a potential different landscape of AM and ECM plant responses to eCO_2 when P is the main limitation. 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 (*83*) despite enhanced P and N availability (*84*, *85*). As tropical forests are commonly limited by P, rather than N, more eCO_2 experiments under P limitations are merited (*86*).

Conclusion: the hypothesis that the growth response to eCO_2 is primarily modulated by N_{acq}-efficiency is supported by available data. 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. III.1), so increases in productivity (Fig. III.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 (*87*). 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.

3.4.4. CO_2 effects on priming and soil C content

Background: In previous sections, we have discussed the role of symbiotic types to increase plant N acquisition, and hypothesized that ECM plants and N_2-fixers can bypass N mineralization by free-living microbes to meet plant N-demand. Meta-analyses show that eCO_2 increases belowground C inputs through enhanced fine-root production by 44% (*88*) and rhizodeposition by 37.9% (*89*). 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" (*90*, *91*). Consequently, the balance between C inputs and loses determine whether eCO_2 increases or decreases soil C storage. Meta-analyses have shown that increases in soil C inputs under eCO_2 are offset by loses (*92*, *93*). These studies, however, did not account for potential differential effects among symbiotic types. The quantification of priming effects therefore has important implications on the magnitude of the terrestrial CO_2 sink, but these effects are difficult to measure and model (*94*), introducing an additional uncertainty in attempts to quantify the implications of eCO_2 for the terrestrial C sink.

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 priming – and heterotrophic respiration – in ECM, and soil C storage in AM systems under low N availability. The picture that emerges from Fig. III.4, however, highlights that other factors beyond Ψ_N^{-1} are at play (marginally significant relationship between soil C storage and Ψ_N^{-1}, P=0.0503).

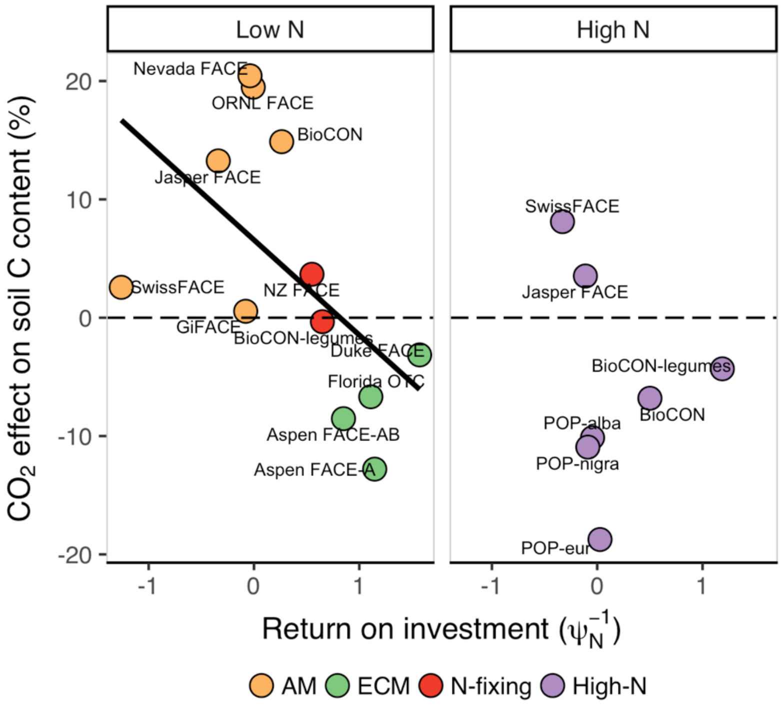


Figure III.4 Relationship between the effect of elevated CO_2 on soil C content (%) and the N return on investment (Ψ_N^{-1}, Eq. 3). References in Table III.1.

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. III.4). For example, the large CO_2 fertilization effect on ANPP in Duke FACE (ECM) (*25*) was likely driven by increased allocation to ECM fungi (*26*) and root exudation (*37*), which stimulated microbial activity and SOM decomposition (priming) increasing N availability to plants (see also *90*). This, however, was accompanied by increased soil respiration (*95*), reducing soil C content (Fig. III.4). In the *Populus tremuloides* (ECM) community from the Aspen FACE experiment, eCO_2 increased litter inputs, but also decreased soil C content (Fig. III.4), suggesting strong stimulation in SOM decomposition (*24*). Similarly in the Florida OTC experiment, eCO_2 increased plant productivity of scrub-oaks (ECM) under low N availability (Fig. III.3) through enhanced N mineralization (*96*), but the stimulation of SOM decomposition yielded no effect on C storage at the ecosystem level (*23*).

In contrast, several AM-ecosystems under low N have shown limited eCO_2-effects on N mineralization and plant productivity, together with significant changes 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) (*76*) was accompanied by a significantly positive effect on soil C content (*97*), with increased fungal activity (*98*), but not fine-root inputs (*99*) – suggesting $C_{transfer}$ as the main driver of this effect (*98*). The same pattern of smaller than average biomass responses but soil C accumulation was observed, for example, in an AM-forest ecosystem at ORNL (*77*), an AM-grassland ecosystem in Australia (*100*), and a shortgrass steppe in the US (*101*), accompanied by a doubling in rhizodeposition (*102*). 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 (*103*), despite a positive effect on photosynthesis (*104*) and a lack of N-mineralization and ANPP response under low N availability (*19*). eCO_2 did not increase soil C content at GiFACE either (*105*), 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 (*106*). A certain degree of CO_2-driven enhancement of N mineralization in grasslands might also follow from increased soil water (e.g. *107*).

An intermediate situation might be found for N_2-fixers (Fig. III.4). eCO_2 generally increases growth in legumes (Fig. III.3; Ainsworth & Long, 2005), and thus likely also enhanced soil C inputs, but whether SOM decomposition offsets additional inputs is uncertain. For example, eCO_2 increased C inputs through biomass and productivity (Fig. III.3) in a grassland FACE experiment with N_2-fixers in New Zealand. But eCO_2 also increased N-mineralization (*108*) and N availability (*109*), yielding a modest increase in soil C storage (*110*) (Fig. III.4). Various factors are probably at play to determine the balance between inputs and outputs, including species, litter quality, climate and nutrient and water availability.

eCO_2 effects on soil C under high N availability do not appear to follow a clear pattern in this dataset (Fig. III.4). Meta-analyses show that N-fertilization may increase the positive effects of eCO_2 on soil respiration further (*111*), but the effect of N has been shown to be negative in trees (*112*), and positive in grasslands and croplands (*113*), which may indicate different effects of N fertilization on soil C cycling between AM and ECM systems.

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 (*92*, *93*, *114*). 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 (*115-117*). On the other hand, the stabilization of labile litter in SOM should protect plant material, constraining the eCO_2-driven priming effect (*118*). 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 (*31*). 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 the sign of the eCO_2 effect on soil C storage. Under low N availability, AM plants show a small eCO_2 stimulation of plant growth (and thus, of litter inputs), and produce litter with low C:N, limiting the priming effect and plant acquisition of additional N in response to eCO_2. In contrast, ECM systems show strong priming effect and N acquisition in response to eCO_2, but this mechanism also enhances SOM decomposition and may thus partially offset the increase in biomass storage and limit CO_2 sequestration at the ecosystem level. It is the balance between the (changes in) C inputs and outputs that eventually determines whether soil C storage increases, decreases or remains unaltered.

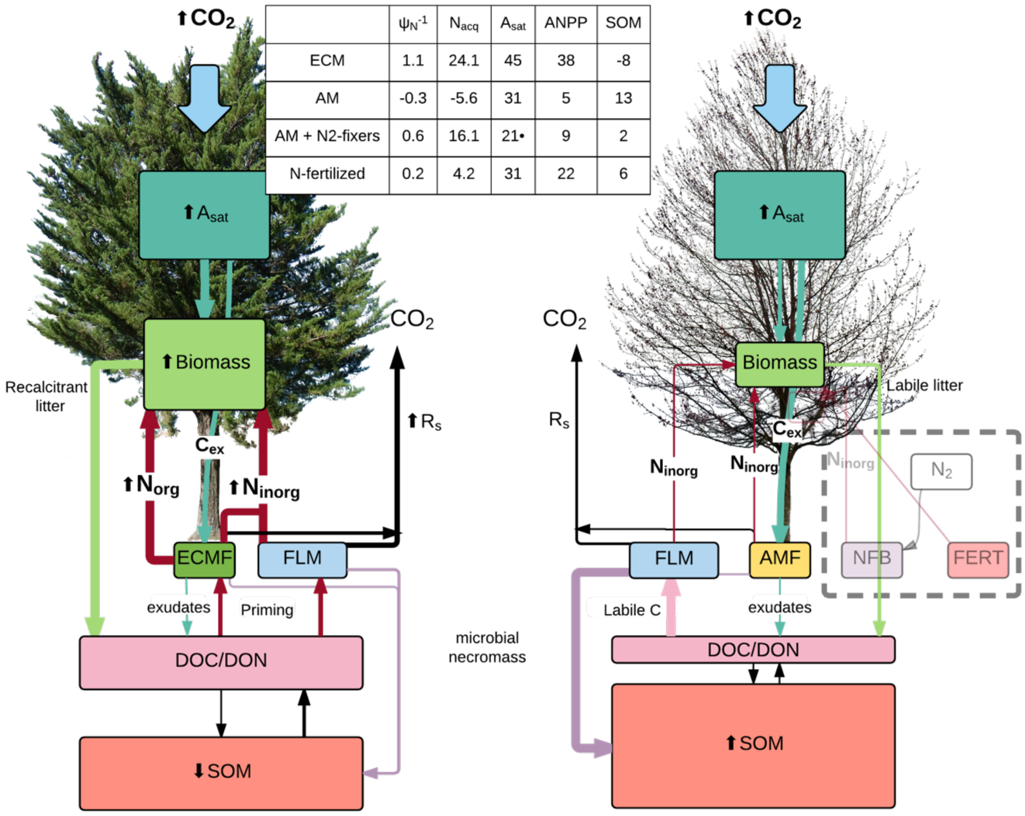
3.5. Discussion

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 (*119*), (ii) allocation to mycorrhizal fungi (*26*), and (iii) allocation to root exudates to increase soil priming (*120*). 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 variables ranging from leaf-level photosynthetic capacity to 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.

As represented in Fig. III.5, we hypothesize that plants that associate with ECM fungi acquire N more efficiently in response to eCO_2 than AM-plants for two reasons: (i) ECM fungi have the enzymes necessary to mine organic N, and (ii) litter produced by ECM plants has a high C:N ratio that promotes slow decomposition and facilitates priming. A similar effect can be achieved by AM plants when N availability is high or in the presence of N_2-fixers. 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. On the other hand, priming increases heterotrophic respiration, limiting C storage at the ecosystem level. As N uptake either through direct root uptake of inorganic N or through SOM-mining have consequences for soil C and N dynamics, it is therefore important that models can identify and distinguish between these two mechanisms to determine the long-term effects of CO_2 on ecosystem C storage.

Plants that associate with AM fungi show a small or non-significant CO_2 fertilization effect on biomass, due to insufficient N uptake. This is because (i) AM fungi do not produce the enzymes required to increase priming in response to eCO_2 (*121*), and (ii) litter produced by AM plants has a lower C:N ratio, promoting greater stabilization of SOM (*28*, *31*). Thereby, AM plants have limited ability to prime the labile SOM that they live on. Consequently, increased $C_{transfer}$ and allocation to roots under eCO_2 does not result in effective N acquisition (high costs), limiting the CO_2-driven growth response. However, we hypothesize that eCO_2 might increase soil C under N-limited conditions in AM systems via increased C allocation belowground. The result is a spectrum of ecosystem responses to eCO_2, primarily driven by the cost of N_{acq}.



	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 + N2-fixers	0.6	16.1	21•	9	2
N-fertilized	0.2	4.2	31	22	6

Figure III.1 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 N2-fixation and external N-fertilization. N inputs through N2-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. 3) 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 N2-fixing capacity and N-fertilized systems derived from Figs. IV.2-IV.4. The CO_2 effect on Asat for AM+N2-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=N2-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.

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 (*22*, *76*, *83*, *122*). 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 (*123*), and there is no evidence that the R_a:GPP ratio consistently increases under eCO_2 (*124*, *125*). 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 (*27*, *35*, *37*, *89*), pointing at $C_{transfer}$ as an important flux of the "missing" C.

A large part of the framework outlined here (see Fig. III.5) is not represented in the current generation of global dynamic vegetation models. Although these models may produce eCO_2-induced increases in growth that are consistent in magnitude with observations (but see *126*), the importance of underlying mechanisms that produce these results is inappropriately represented (*69*). 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 (*69*, *127*). Models do not generally consider $C_{transfer}$ as a separate component of the plant C budget (*128*), 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 (*69*, *129*). 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 III.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 plays a key role. Ecosystems with ECM-associated plants and N_2-fixers can 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. It is still uncertain for how long eCO_2 would sustain enhanced rates of plant growth. Our findings, however, hint at 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.

Table III.1 Current limitations about the interactions between the C and nutrient cycles under elevated CO_2, and recommendations for experiments and methods to fill some of the limitations.

Limitation	**Recommendations**
Quantification of the C cost of N acquisition under eCO_2	Quantification of belowground plant biomass. Priorities: Fine-root production > root production > fine-root biomass > root biomass
	Quantification total N acquisition
	Quantification of C allocated to exudates
	Quantification of C allocated to symbionts
	Quantification N derived from N_2-fixation
	eCO_2 experiments with ericoid mycorrhizal plants
	Mixture of AM and ECM trees within the same eCO_2 experiment
	Test the bottom limit of ECM-fungal N acquisition in boreal forests
	Quantification of changes in mycorrhizal communities with eCO_2 and N (e.g. changes in the proportion of ECM vs AM and proportion of ECM taxa capable of mobilising organic N)
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, ECM and N_2-fixers as above but under P-limitations
Quantification of soil C storage under eCO_2	Quantification of changes in soil C pools
	Quantification 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

3.6. References

1. P. Ciais et al., in Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, T. F. Stocker et al., Eds. (Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 2013), pp. 465–570.
2. P. Smith et al., Biophysical and economic limits to negative CO_2 emissions. Nature Climate change. **6**, 42–50 (2016).
3. E. A. Ainsworth, S. P. Long, 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 Phytol. **165**, 351–372 (2005).
4. Y. Malhi et al., The linkages between photosynthesis, productivity, growth and biomass in lowland Amazonian forests. Global Change Biology. **21**, 2283–2295 (2015).
5. B. A. Hungate, J. S. Dukes, M. R. Shaw, Y. Luo, C. B. Field, Nitrogen and climate change. Science. **302**, 1512–1513 (2003).
6. P. A. Fay et al., Grassland productivity limited by multiple nutrients. Nature Plants, Published online: 6 July 2015; | doi:10.1038/nplants.2015.80. **1**, 15080 (2015).
7. W. Huang, B. Z. Houlton, A. R. Marklein, J. Liu, G. Zhou, Plant stoichiometric responses to elevated CO_2 vary with nitrogen and phosphorus inputs: Evidence from a global-scale meta-analysis. Sci. Rep. **5**, 18225 (2015).
8. P. Vitousek, R. Howarth, Nitrogen limitation on land and in the sea: How can it occur? Biogeochemistry. **13** (1991).
9. D. S. LeBauer, K. K. Treseder, Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. Ecology. **89**, 371–379 (2008).
10. D. N. L. Menge, L. O. Hedin, S. W. Pacala, Nitrogen and Phosphorus Limitation over Long-Term Ecosystem Development in Terrestrial Ecosystems. PLoS ONE. **7**, e42045 (2012).
11. P. Reich et al., Nitrogen limitation constrains sustainability of ecosystem response to CO_2. Nature. **440**, 922–925 (2006).
12. C. Körner, Plant CO_2 responses: an issue of definition, time and resource supply. New Phytol. **172**, 393–411 (2006).
13. C. Terrer, S. Vicca, B. A. Hungate, R. P. Phillips, I. C. Prentice, Mycorrhizal association as a primary control of the CO_2 fertilization effect. Science. **353**, 72–74 (2016).
14. F. Bazzaz, The response of natural ecosystems to the rising global CO_2 levels. Annual Review of Ecology and Systematics. **21**, 167–196 (1990).
15. R. S. Nowak, D. S. Ellsworth, S. D. Smith, 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 (2004).

16. P. B. Reich, B. A. Hungate, Y. Luo, Carbon-Nitrogen Interactions in Terrestrial Ecosystems in Response to Rising Atmospheric Carbon Dioxide. Annu. Rev. Ecol. Evol. Syst. **37**, 611–636 (2006).

17. R. J. Norby, D. R. Zak, Ecological lessons from free-air CO_2 enrichment (FACE) experiments. Annual Review of Ecology. **42**, 181–203 (2011).

18. H. Saxe, D. S. Ellsworth, J. Heath, Tree and forest functioning in an enriched CO_2 atmosphere. New Phytologist. **139**, 395–436 (1998).

19. M. K. Schneider et al., 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 (2004).

20. P. B. Reich, S. E. Hobbie, Decade-long soil nitrogen constraint on the CO_2 fertilization of plant biomass. Nature Climate change. **3**, 278–282 (2013).

21. R. J. Norby, J. M. Warren, C. M. Iversen, B. E. Medlyn, R. E. McMurtrie, CO_2 enhancement of forest productivity constrained by limited nitrogen availability. Proc Natl Acad Sci U S A. **107**, 19368–19373 (2010).

22. B. D. Sigurdsson, J. L. Medhurst, G. Wallin, O. Eggertsson, S. Linder, Growth of mature boreal Norway spruce was not affected by elevated [CO_2] and/or air temperature unless nutrient availability was improved. Tree Physiol. **33**, 1192–1205 (2013).

23. B. A. Hungate et al., Cumulative response of ecosystem carbon and nitrogen stocks to chronic CO_2 exposure in a subtropical oak woodland. New Phytol. **200**, 753–766 (2013).

24. A. F. Talhelm et al., Elevated carbon dioxide and ozone alter productivity and ecosystem carbon content in northern temperate forests. Global Change Biology. **20**, 2492–2504 (2014).

25. H. R. McCarthy et al., 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 Phytol. **185**, 514–528 (2010).

26. J. E. Drake et al., Increases in the flux of carbon belowground stimulate nitrogen uptake and sustain the long-term enhancement of forest productivity under elevated CO_2. Ecol Lett. **14**, 349–357 (2011).

27. O. Alberton, T. W. Kuyper, A. Gorissen, Taking mycocentrism seriously: mycorrhizal fungal and plant responses to elevated CO_2. New Phytol. **167**, 859–868 (2005).

28. R. P. Phillips, E. Brzostek, M. G. Midgley, The mycorrhizal-associated nutrient economy: a new framework for predicting carbon–nutrient couplings in temperate forests. New Phytol. **199**, 41–51 (2013).

29. C. Terrer et al., Response to Comment on "Mycorrhizal association as a primary control of the CO_2 fertilization effect." Science. **355**, 358–358 (2017).

30. R. J. Norby et al., Comment on "Mycorrhizal association as a primary control of the CO_2 fertilization effect." Science. **355**, 358–358 (2017).

31. G. Lin, M. L. McCormack, C. Ma, D. Guo, Similar below-ground carbon cycling dynamics but contrasting modes of nitrogen cycling between arbuscular mycorrhizal and ectomycorrhizal forests. New Phytol. **213**, 1440–1451 (2017).

32. S. G. Pritchard, Soil organisms and global climate change. Plant Pathology. **60**, 82–99 (2011).

33. F. S. Chapin III, P. A. Matson, P. Vitousek, Principles of Terrestrial Ecosystem Ecology (Springer Science & Business Media, 2011).

34. M. Aoki, K. Fujii, K. Kitayama, Environmental Control of Root Exudation of Low-Molecular Weight Organic Acids in Tropical Rainforests. Ecosystems. **15**, 1194–1203 (2012).

35. K. K. Treseder, A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO_2 in field studies. New Phytol. **164**, 347–355 (2004).

36. E. A. Hobbie, Carbon allocation to ectomycorrhizal fungi correlates with belowground allocation in culture studies. Ecology. **87**, 563–569 (2006).

37. R. P. Phillips, A. C. Finzi, E. S. Bernhardt, Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO_2 fumigation. Ecol Lett. **14**, 187–194 (2011).

38. M. N. Högberg et al., 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 Phytol. **187**, 485–493 (2010).

39. E. Nouri, F. Breuillin-Sessoms, U. Feller, D. Reinhardt, Phosphorus and Nitrogen Regulate Arbuscular Mycorrhizal Symbiosis in Petunia hybrida. PLoS ONE. **9**, e90841 (2014).

40. S. Vicca et al., Fertile forests produce biomass more efficiently. Ecol Lett. **15**, 520–526 (2012).

41. M. Fernández-Martínez et al., Nutrient availability as the key regulator of global forest carbon balance. Nature Climate change. **4**, 471–476 (2014).

42. A. L. Gill, A. C. Finzi, Belowground carbon flux links biogeochemical cycles and resource-use efficiency at the global scale. Ecol Lett. **19**, 1419–1428 (2016).

43. Z. Feng et al., Constraints to nitrogen acquisition of terrestrial plants under elevated CO_2. Global Change Biology. **21**, 3152–3168 (2015).

44. A. C. Finzi et al., Increases in nitrogen uptake rather than nitrogen-use efficiency support higher rates of temperate forest productivity under elevated CO_2. Proc Natl Acad Sci U S A. **104**, 14014–14019 (2007).

45. R. Norby et al., Forest response to elevated CO_2 is conserved across a broad range of productivity. Proceedings of the National Academy of Sciences. **102**, 18052–18056 (2005).

46. P. C. D. Newton et al., Selective grazing modifies previously anticipated responses of plant community composition to elevated CO_2 in a temperate grassland. Global Change Biology. **20**, 158–169 (2014).

47. C. M. Litton, J. W. Raich, M. G. Ryan, Carbon allocation in forest ecosystems. Global Change Biology. **13**, 2089–2109 (2007).

48. E. C. Adair, P. B. Reich, S. E. Hobbie, J. M. H. Knops, 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 (2009).

49. N. Wurzburger, E. N. J. Brookshire, M. L. McCormack, R. A. Lankau, Mycorrhizal fungi as drivers and modulators of terrestrial ecosystem processes. New Phytol. **213**, 996–999 (2017).

50. J. A. Bennett et al., Plant-soil feedbacks and mycorrhizal type influence temperate forest population dynamics. Science. **355**, 181–184 (2017).

51. F. P. Teste et al., Plant-soil feedback and the maintenance of diversity in Mediterranean-climate shrublands. Science. **355**, 173–176 (2017).

52. N. C. Johnson, G. W. T. Wilson, J. A. Wilson, R. M. Miller, M. A. Bowker, Mycorrhizal phenotypes and the Law of the Minimum. New Phytol. **205**, 1473–1484 (2015).

53. H. L. Reynolds, A. E. Hartley, K. M. Vogelsang, J. D. Bever, P. A. Schultz, Arbuscular mycorrhizal fungi do not enhance nitrogen acquisition and growth of old-field perennials under low nitrogen supply in glasshouse culture. New Phytol. **167**, 869–880 (2005).

54. T. J. Thirkell, D. D. Cameron, A. Hodge, Resolving the “nitrogen paradox” of arbuscular mycorrhizas: fertilization with organic matter brings considerable benefits for plant nutrition and growth. Plant Cell Environ. **39**, 1683–1690 (2016).

55. F. Shah et al., Ectomycorrhizal fungi decompose soil organic matter using oxidative mechanisms adapted from saprotrophic ancestors. New Phytol. **209**, 1705–1719 (2015).

56. B. D. Lindahl, A. Tunlid, Ectomycorrhizal fungi - potential organic matter decomposers, yet not saprotrophs. New Phytol. **205**, 1443–1447 (2015).

57. H. Wang et al., Photosynthetic responses to altitude: an explanation based on optimality principles. New Phytol. **213**, 976–982 (2017).

58. R. J. Spreitzer, M. E. Salvucci, RUBISCO: Structure, Regulatory Interactions, and Possibilities for a Better Enzyme. Annual Review of Plant Biology. **53**, 449–475 (2002).

59. E. A. Ainsworth, A. Rogers, The response of photosynthesis and stomatal conductance to rising [CO_2]: mechanisms and environmental interactions. Plant Cell Environ. **30**, 258–270 (2007).

60. D. S. Ellsworth et al., 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 (2012).

61. J. M. Warren, A. M. Jensen, B. E. Medlyn, R. J. Norby, D. T. Tissue, Carbon dioxide stimulation of photosynthesis in Liquidambar styraciflua is not sustained during a 12-year field experiment. AoB PLANTS. **7**, plu074–plu074 (2015).

62. D. Wang, S. A. Heckathorn, X. Wang, S. M. Philpott, A meta-analysis of plant physiological and growth responses to temperature and elevated CO_2. Oecologia. **169**, 1–13 (2012).

63. A. Rogers et al., 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 Physiol. **118**, 683–689 (1998).

64. T. D. Lee, S. H. Barrott, P. B. Reich, 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 (2011).

65. R. A. Duursma et al., 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 (2016).

66. P. J. Franks et al., Sensitivity of plants to changing atmospheric CO_2 concentration: from the geological past to the next century. New Phytologist. **197**, 1077–1094 (2013).

67. J. Peñuelas, R. Matamala, Changes in N and S Leaf Content, Stomatal Density and Specific Leaf Area of 14 Plant Species during the Last Three Centuries of CO Increase. J. Exp. Bot. **41**, 1119–1124 (1990).

68. R. Oren et al., Soil fertility limits carbon sequestration by forest ecosystems in a CO_2-enriched atmosphere. Nature. **411**, 469–472 (2001).

69. S. Zaehle et al., Evaluation of 11 terrestrial carbon–nitrogen cycle models against observations from two temperate Free-Air CO_2 Enrichment studies. New Phytol. **202**, 803–822 (2014).

70. R. Q. Thomas, C. D. Canham, K. C. Weathers, C. L. Goodale, Increased tree carbon storage in response to nitrogen deposition in the US. Nature Geosci. **3**, 13–17 (2010).

71. M. Liberloo et al., 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 (2006).

72. A. Lüscher, U. A. Hartwig, D. Suter, J. Nösberger, Direct evidence that symbiotic N2 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 (2000).

73. K. E. Mueller, S. E. Hobbie, D. Tilman, P. B. Reich, 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 (2013).

74. S. D. Smith et al., Long-term response of a Mojave Desert winter annual plant community to a whole-ecosystem atmospheric CO 2manipulation (FACE). Global Change Biology. **20**, 879–892 (2014).

75. D. C. Housman et al., Increases in Desert Shrub Productivity under Elevated Carbon Dioxide Vary with Water Availability. Ecosystems. **9**, 374–385 (2006).

76. B. A. Newingham et al., 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 (2013).

77. C. M. Iversen, J. K. Keller, C. T. Garten Jr, R. J. Norby, 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 (2012).

78. A. P. Walker et al., Predicting long-term carbon sequestration in response to CO_2 enrichment: How and why do current ecosystem models differ? Global Biogeochem. Cycles. **29**, 476–495 (2015).

79. N. C. Johnson, J. H. Graham, F. A. Smith, Functioning of mycorrhizal associations along the mutualism–parasitism continuum*. New Phytol (1997), doi:10.1046/j.1469-8137.1997.00729.x/epdf.

80. P. Baskaran et al., Modelling the influence of ectomycorrhizal decomposition on plant nutrition and soil carbon sequestration in boreal forest ecosystems. New Phytol. **213**, 1452–1465 (2017).

81. O. Franklin, T. Näsholm, P. Högberg, M. N. Högberg, Forests trapped in nitrogen limitation - an ecological market perspective on ectomycorrhizal symbiosis. New Phytol. **203**, 657–666 (2014).

82. S. E. Smith, F. A. Smith, Roles of Arbuscular Mycorrhizas in Plant Nutrition and Growth: New Paradigms from Cellular to Ecosystem Scales. Annual Review of Plant Biology. **62**, 227–250 (2011).

83. D. S. Ellsworth et al., Elevated CO_2 does not increase eucalypt forest productivity on a low-phosphorus soil. Nature Climate change. **320**, 1444 (2017).

84. R. Ochoa-Hueso et al., Rhizosphere-driven increase in nitrogen and phosphorus availability under elevated atmospheric CO_2 in a mature Eucalyptus woodland. Plant Soil. **1**, 1 (2017).

85. S. Hasegawa, C. A. Macdonald, S. A. Power, Elevated carbon dioxide increases soil nitrogen and phosphorus availability in a phosphorus-limitedEucalyptuswoodland. Global Change Biology. **22**, 1628–1643 (2016).

86. R. J. Norby et al., Model-data synthesis for the next generation of forest free-air CO_2 enrichment (FACE) experiments. New Phytol. **209**, 17–28 (2015).

87. H. L. Gholz, R. F. Fisher, W. L. Prichett, Nutrient Dynamics in Slash Pine Plantation Ecosystems. Ecology. **66**, 647–659 (1985).

88. W. I. J. Dieleman et al., Soil [N] modulates soil C cycling in CO_2-fumigated tree stands: a meta-analysis. Plant Cell Environ. **33**, 2001–2011 (2010).

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

90. W. Cheng et al., Synthesis and modeling perspectives of rhizosphere priming. New Phytol. **201**, 31–44 (2014).

91. A. C. Finzi et al., Rhizosphere processes are quantitatively important components of terrestrial carbon and nutrient cycles. Global Change Biology. **21**, 2082–2094 (2015).

92. K. J. van Groenigen, X. Qi, C. W. Osenberg, Y. Luo, B. A. Hungate, Faster Decomposition Under Increased Atmospheric CO_2 Limits Soil Carbon Storage. Science. **344**, 508–509 (2014).

93. B. A. Hungate et al., Assessing the effect of elevated carbon dioxide on soil carbon: a comparison of four meta-analyses. Global Change Biology. **15**, 2020–2034 (2009).

94. K. Georgiou, C. D. Koven, W. J. Riley, M. S. Torn, Toward improved model structures for analyzing priming: potential pitfalls of using bulk turnover time. Global Change Biology. **21**, 4298–4302 (2015).

95. A. C. Oishi, S. Palmroth, K. H. Johnsen, H. R. McCarthy, R. Oren, Sustained effects of atmospheric [CO_2] and nitrogen availability on forest soil CO_2 efflux. Global Change Biology. **20**, 1146–1160 (2014).

96. J. A. Langley et al., Priming depletes soil carbon and releases nitrogen in a scrub-oak ecosystem exposed to elevated CO_2. Soil Biology and Biochemistry. **41**, 54–60 (2009).

97. R. D. Evans et al., Greater ecosystem carbon in the Mojave Desert after ten years exposure to elevated CO_2. Nature Climate change. **4**, 394–397 (2014).

98. V. L. Jin, R. D. Evans, Microbial 13C 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 (2010).

99. S. D. Ferguson, R. S. Nowak, 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 Phytol. **190**, 953–967 (2011).

100. E. Pendall, Y. Osanai, A. L. Williams, M. J. Hovenden, Soil carbon storage under simulated climate change is mediated by plant functional type. Global Change Biology. **17**, 505–514 (2011).

101. E. Pendall, J. Y. King, 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 (2007).

102. E. Pendall, A. R. Mosier, J. A. Morgan, Rhizodeposition stimulated by elevated CO_2 in a semiarid grassland. New Phytol. **162**, 447–458 (2004).

103. C. van Kessel et al., Total soil C and N sequestration in a grassland following 10 years of free air CO_2 enrichment. Global Change Biology. **12**, 2187–2199 (2006).

104. E. A. Ainsworth et al., 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_2Enrichment (FACE). Plant Cell Environ. **26**, 705–714 (2003).

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

106. L. C. Andresen et al., Global Change Biology, in press, doi:10.1111/gcb.13705.

107. E. Pendall et al., Global Biogeochem. Cycles, in press, doi:10.1029/2001GB001821.

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

109. P. Newton, M. Lieffering, W. Bowatte, S. C. Brock, 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 Soil. **336**, 433–441 (2010).

110. D. J. Ross, P. C. D. Newton, K. R. Tate, D. Luo, 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 (2013).

111. L. Zhou et al., Interactive effects of global change factors on soil respiration and its components: a meta-analysis. Global Change Biology. **22**, 3157–3169 (2016).

112. I. A. Janssens et al., Reduction of forest soil respiration in response to nitrogen deposition. Nature Geosci. **3**, 315–322 (2010).

113. L. Zhou et al., Different responses of soil respiration and its components to nitrogen addition among biomes: a meta-analysis. Global Change Biology. **20**, 2332–2343 (2014).

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

115. M. F. Cotrufo et al., Formation of soil organic matter via biochemical and physical pathways of litter mass loss. Nature Geosci. **8**, 776–779 (2015).

116. M. J. Castellano, K. E. Mueller, D. C. Olk, J. E. Sawyer, J. Six, Integrating plant litter quality, soil organic matter stabilization, and the carbon saturation concept. Global Change Biology. **21**, 3200–3209 (2015).

117. H. Knicker, Soil organic N - An under-rated player for C sequestration in soils? Soil Biology and Biochemistry. **43**, 1118–1129 (2011).

118. B. N. Sulman, R. P. Phillips, A. C. Oishi, E. Shevliakova, S. W. Pacala, Microbe-driven turnover offsets mineral-mediated storage of soil carbon under elevated CO_2. Nature Climate change. **4**, 1099–1102 (2014).

119. C. M. Iversen, Digging deeper: fine-root responses to rising atmospheric CO_2 concentration in forested ecosystems. New Phytol. **186**, 346–357 (2010).

120. R. P. Phillips et al., Roots and fungi accelerate carbon and nitrogen cycling in forests exposed to elevated CO_2. Ecol Lett. **15**, 1042–1049 (2012).

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

122. M. K. F. Bader et al., 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 (2013).

123. N. G. Smith, in Contribution to Advances in Photosynthesis and Respiration special volume on plant respiration, Govindjee, T. D. Sharkey, G. Tcherkez, J. Ghashghaie, Eds. (New York: Springer-Verlag New York, Inc, in press., 2017).

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

125. N. G. Smith, J. S. Dukes, Plant respiration and photosynthesis in global-scale models: incorporating acclimation to temperature and CO_2. Global Change Biology. **19**, 45–63 (2013).

126. M. G. De Kauwe et al., Challenging terrestrial biosphere models with data from the long-term multifactor Prairie Heating and CO_2 Enrichment experiment. Global Change Biology. **348**, 895 (2017).

127. R. Q. Thomas, E. N. J. Brookshire, S. Gerber, Nitrogen limitation on land: how can it occur in Earth system models? Global Change Biology. **21**, 1777–1793 (2015).

128. B. E. Medlyn et al., Using ecosystem experiments to improve vegetation models. Nature Climate change. **5**, 528–534 (2015).

129. M. G. De Kauwe et al., 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 Phytol. **203**, 883–899 (2014).

130. D. S. Ellsworth et al., 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 (2004).

131. J. N. T. Darbah, T. D. Sharkey, C. Calfapietra, D. F. Karnosky, Differential response of aspen and birch trees to heat stress under elevated carbon dioxide. Environ. Pollut. **158**, 1008–1014 (2010).

132. J. Lichter et al., Soil carbon sequestration in a pine forest after 9 years of atmospheric CO_2 enrichment. Global Change Biology. **14**, 2910–2922 (2008).

133. J. H. Li, P. Dijkstra, C. R. Hinkle, R. M. Wheeler, B. G. Drake, 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 Physiol. **19**, 229–234 (1999).

134. D. C. Housman, K. T. Killingbeck, R D Evans, T. N. Charlet, S. D. Smith, Foliar nutrient resorption in two Mojave Desert shrubs exposed to Free-Air CO_2 Enrichment (FACE). Journal of Arid Environments. **78**, 26–32 (2012).

135. K. E. Mueller et al., Impacts of warming and elevated CO_2 on a semi-arid grassland are non-additive, shift with precipitation, and reverse over time. Ecol Lett. **19**, 956–966 (2016).

136. D. M. Blumenthal et al., Invasive forb benefits from water savings by native plants and carbon fertilization under elevated CO_2 and warming. New Phytol. **200**, 1156–1165 (2013).

137. K. Y. Crous, P. B. Reich, M. D. Hunter, D. S. Ellsworth, 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 (2010).

138. V. Allard et al., 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 Soil. **276**, 49–60 (2005).

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

140. S. Bazot, L. Ulff, H. Blum, C. Nguyen, C. Robin, 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 (2006).

141. M. J. Hovenden, 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 (2003).

142. M. R. Hoosbeek, G. E. Scarascia-Mugnozza, 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 (2009).

143. K. Zhu, N. R. Chiariello, T. Tobeck, T. Fukami, C. B. Field, Nonlinear, interacting responses to climate limit grassland production under global change. Proceedings of the National Academy of Sciences. **113**, 10589–10594 (2016).

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

Chapter IV: Quantification and distribution of the CO_2 fertilization effect on plant biomass

Quantification and distribution of the CO_2 fertilization effect on plant biomass

4.1. Overview

Carbon dioxide (CO_2) stimulates photosynthesis, and this effect can potentially increase plant growth. Elevated CO_2 experiments attempt to simulate the atmosphere of the future to study the extent to which plants are likely to continue absorbing increasing quantities of CO_2, partially buffering CO_2 emissions. These experiments, however, show apparently contradictory results, ranging from no effect of CO_2 on plant biomass in some ecosystems, to a large effect in others, complicating projections of climate change. Here, we synthesized a dataset of 91 CO_2 experiments through meta-analysis, and found that the magnitude of the CO_2 effect on aboveground biomass is primarily driven by temperature, precipitation and the C:N ratio of the soil, and modulated by the type of mycorrhizal fungi associated with the plants. We have created a data-driven model based on observational data, upscaling the CO_2 fertilization effect from elevated CO_2 experiments to the globe. Overall, for an increase in atmospheric CO_2 from 400 to 700 ppm, we found an ~8% increase in aboveground biomass. Boreal forests appear to have limited capacity to accumulate carbon in biomass in response to elevated CO_2 due to low temperatures; temperate forests show larger enhancements. We found the largest increases in specific areas of Asia and Africa, although the response in tropical forests is far more uncertain. In grasslands, arid regions, and some temperate forests the CO_2 fertilization effect may be strongly constrained by N or water availability.

4.2. Introduction

Forest inventories, models and satellite observations indicate that the land carbon (C) sink has been increasing during the last decades (*1-5*), and vegetation has been “greening”, apparently primarily driven by the increase in atmospheric CO_2 (*6-8*). However, the magnitude of terrestrial ecosystems’ future capacity to continue absorbing CO_2 is one of the most uncertain effects in Earth system models (*9-13*). Nitrogen (N) availability has been proposed to limit the CO_2 fertilization effect with rising CO_2 (*14, 15*). Experiments in which plants are fumigated with elevated CO_2 (eCO_2) show contrasting results concerning the N limitation on CO_2 fertilization (*16*). Chapters II and III presented evidence that mycorrhizal association can explain these results. Under low N availability, only plants that associate with ectomycorrhizal (ECM) fungi, and not arbuscular mycorrhizal (AM) fungi, show a positive CO_2 fertilization effect on biomass (*17*). These differences are likely driven by the capacity of ECM plants to acquire N at a lower C cost than AM plants in response to eCO_2 (Chapter III). This difference can be explained by the capacity of ECM fungi to produce extracellular enzymes that can decompose organic forms of N (*18*) that the plant roots cannot normally access, a feature that is not found in AM fungi (*19*).

Biomass responses to eCO_2 can therefore be predicted based on the interaction between N availability and mycorrhizal association. But two challenges limit our ability to upscale these effects from eCO_2 experiments to the globe: first, the global distribution of mycorrhizal plants had not been adequately mapped, and second, the lack of an accepted metric to quantitatively assess plant N availability. Here, we compiled a large dataset of eCO_2 experiments, and statistically determined the most important drivers that explain the variability of the aboveground biomass response to CO_2 in the dataset. Finally, we combined global spatial datasets of these drivers with a recently developed global map of mycorrhizal distribution (Soudzilovskaia, *et al.*, in review) to develop the first data-driven statistical model to quantify the magnitude and distribution of the aboveground biomass response to eCO_2.

4.3. Methods

The goal of this Chapter is to upscale the effects of CO_2 on biomass. This requires a quantification of “current” plant biomass and its distribution worldwide. As satellites can only measure aboveground biomass, we collected data on aboveground biomass from 91 eCO_2 experiments (Fig. IV.1, Fig. S-IV.1, Table S-IV.1). These studies represented, on average, an increase in atmospheric CO_2 from 400 to 650 ppm. Previous work (e.g. *17*) studied the role of N availability on the CO_2 fertilization effect using a qualitative assessment (i.e. either high or low N availability) on a per-experiment basis, taking into account N fertilization, soil characteristics and the assessments made by the authors of the experiments. But this categorical approach to N limitation cannot readily be applied to scale up CO_2 effects from experiments to the globe. As potential indicators of the role of N availability on CO_2

fertilization effect we considered pH and soil C:N ratio, both of which have been traditionally linked with plant fertility and can be applied at a global scale in a continuous fashion. We also considered other quantities that could potentially explain part of the variability of the biomass responses to CO_2 in our dataset ("predictors"). Several hypotheses have been suggested to explain the differences in the CO_2 effects among experiments, including a larger response of trees compared to grasslands (*20*) or differences between seedlings and older plants (*21*). We considered the following potential predictors: plant type (trees, grasslands, crops), vegetation age (years), length of the experiment (years), mean annual precipitation (MAP), mean annual temperature (MAT), the increase in atmospheric CO_2 concentration ($[CO_2]$), the type of mycorrhizal association (AM or ECM), and soil C:N and pH as potential indicators of N availability. We considered the relative support for all models containing the predictors, and calculated the relative importance for a particular predictor as the sum of the weights (i.e. probabilities) for the models in which the variable appears. A cut-off value of the sum of Akaike weights of 0.7 was adopted here to differentiate between important and non-essential predictors.

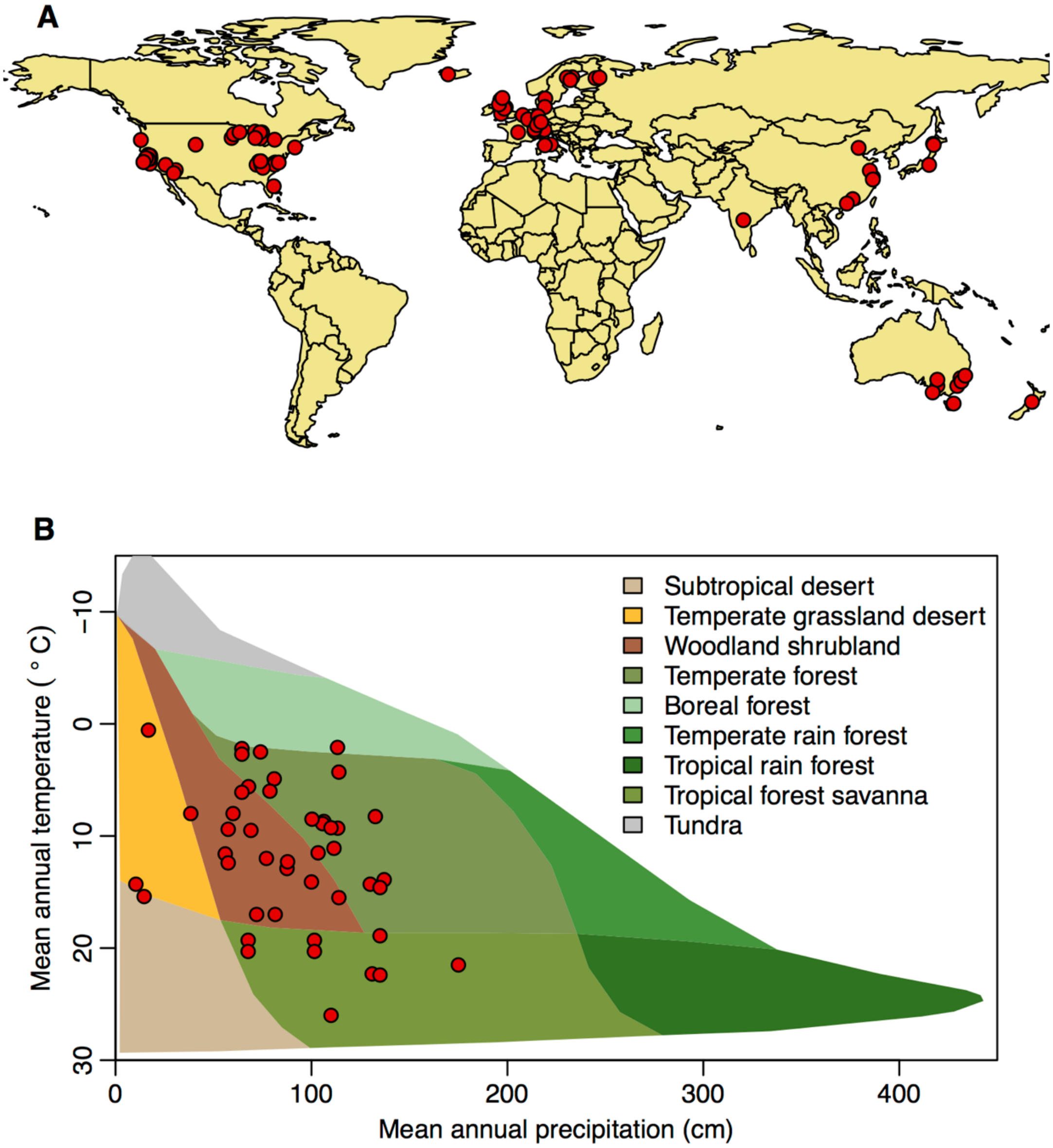


Figure IIV.1 Geographical and climatic distribution of the elevated CO_2 experiments included in the dataset. (A) Red dots indicate individual study locations. Experiments from the same site are spaced to avoid overlapping. (B) Individual studies overlaid on Whittaker's terrestrial biomes, defined as a function of mean annual temperature and precipitation. Croplands and irrigated studies are not included in (B).

Data collection

We collected published and unpublished data on aboveground biomass from eCO_2 experiments, together with data on the climate of the sites, soil fertility (C:N ratio, pH) and the age of the vegetation. We used the dataset of aboveground biomass of Chapter II, Fig. S-II.2 (*17*), and searched for soil C:N and pH data using the Web of Science and contacting the principal investigators (PIs) of the studies, yielding a total of 40 studies with available data for all predictors considered here (Fig. IV.2). Soil C:N ratio, in particular, was the type of data that more clearly limited the amount of studies that could be included in the dataset, as this parameter was not measured in many studies. We then searched for additional studies not included in Chapter II (*17*).

Criteria for exclusion from the analysis were: i) species did not form associations with either AM or ECM; ii) the duration of the experiment was less than 3 months. Additionally, soil C:N data were only considered valid under certain conditions: i) collected preferentially from elevated-CO_2 plots; ii) collected in the later years of the experiments (to reflect more accurately the conditions that future plants may experience under eCO_2); iii) plots with a N fertilization treatment were only included when the soil C:N data available were specifically measured in those plots, and not in unfertilized plots; and iv) in N-fertilized plots, C:N measurements were only considered valid when measured at least one year, and preferentially more than three years, after the start of the N-fertilization treatment.

We considered the inclusion of factorial CO_2 x warming or CO_2 x irrigation studies when specific soil C:N data for those additional treatments were measured and reported. These treatments were treated as independent and were included in the dataset using the specific MAT and MAP for the warming and irrigation treatments, respectively. When plants in the studies were irrigated and the total amount of water was not indicated, we did not use the corresponding MAP data of the site in the analysis, but instead we assigned the maximum value of MAP in the dataset (1750 mm y^{-1}). When the age of the vegetation at the start of the experiment was not specified in the study, we assigned a value of 1 for seedlings, annuals, frequently grazed vegetation, or experiments under controlled burning, and the maximum value in the dataset (50 years) when the site was classified as “intact” or similar.

Where possible, data were collected at the species level, and different species within experiments were considered independent when grown in monoculture. When available data were pooled across several species, these were only included in the analysis if the dominant species were associated with the same type of mycorrhizal fungus. Experiments in which the most abundant species were C_4 species were excluded from the analysis because they are less responsive to CO_2 than C_3 species. As N_2-fixing species can potentially benefit from increased N availability through the fixation of atmospheric N, we excluded these species from the analysis if the data were available at the species level, or removed the experiment from the dataset when biomass data were pooled across species with a dominant role of N_2-fixing species.

Using these criteria, we found a total of 154 studies with data on aboveground biomass, with 91 of them including data for all the predictors considered. For the final selection of studies, we took into consideration the suggestions by Norby *et al.* (*22*) about studies that were not methodologically adequate for our analysis (e.g. pot studies), as well as their recommendations for additional studies if they met the criteria of inclusion. Overview of the experiments included in the dataset is in Table S-IV.1, data included in the meta-analysis in Fig. S-IV.1, and location of the studies in Fig. IV.1.

Meta-Analysis

We used the response ratio (mean response in elevated to ambient CO_2 plots) to measure effect sizes (*23*). We calculated the natural logarithm of the response ratio (logR) and its variance for each experimental unit to obtain a single response metric in a weighted, mixed-effects model using the R package *metafor* (*24*).

Effect size measurements from individual studies were weighted by the inverse of the variance (*25*).

Measurements across different time-points (i.e. over several years or harvests) were considered non-independent, and we computed a combined effect across time-points so that only one effect size was analysed per study. The combined variance that takes account of the correlation among the different time-point measurements was calculated following the method described in Borenstein *et al* (*26*), with a conservative approach and non independence (r=1) and performed using the *MAd* package in *R* (*27*).

The mixed-effects meta-regression model was fitted using maximum likelihood for the amount of residual heterogeneity. The Knapp and Hartung method (*28*) was included as an adjustment to the standard errors of the estimated coefficients to control the Type I error rate (*29*). This method leads to an F-test for sets of model predictors (test of moderators) to test their significance to influence the average effect of CO_2. For individual model coefficients, the method leads to t-tests. We inferred CO_2 effects if the calculated 95 % CI did not overlap with zero. The log response ratio was back-transformed and expressed as percentage CO_2 effect ($[\log R\text{-}1] \times 100$) to ease interpretation in figures and text.

Model selection and relative importance

We analysed the plausibility of models containing all potential combinations of the studied predictors in a mixed-effects meta-regression model using maximum likelihood estimation. For this purpose, we used the R packages *gmulti* (*30*) and *metafor* (*24*). Model selection was based on AICc. The relative importance value for a particular predictor was equal to the sum of the Akaike weights (probability that a model is the most plausible model) for the models in which the predictor appears. Hence, a predictor that is included in models with large Akaike weights will receive a high importance value. These values can be regarded as the overall support for each variable across all models. A cut-off of 0.7 was set to differentiate between important and non-essential predictors.

Global estimates of soil C:N ratio

Initially, we used soil C:N ratio for the individual studies reported in the papers as an indicator of soil N availability, with decreasing N availability with increasing soil C:N. For example, N-fertilized croplands are generally characterised by soil C:N around 9, sometimes slightly lower, whereas some soils in Northern latitudes have values of 30. As soil C:N ratio was an important predictor of the CO_2-driven increase in biomass of AM plants in our dataset (Fig. IV.2), we used a global dataset on soil C:N ratio from ISRIC-WISE on a 30 by 30 arcsec grid (*31*) to upscale this effect. Soils with low soil C:N are characteristic from arid regions, and are the result of a small organic C pool, but also low N content (*32*, *33*). Therefore, soil C:N is not a good indicator of N availability in arid soils, and the model would overestimate the CO_2 effect in these areas, as it would assume high N availability. To avoid the overestimation of the CO_2 effect in arid areas with low C:N, yet low N availability, we followed the approach of Wang *et al.* (*36*), which found a threshold of 0.32 in aridity index (ratio of precipitation to mean temperature) below which plant N uptake is limited by water availability, and characterised by low soil C:N despite extremely low soil N content. We converted areas with aridity index < 0.32 to null values in the map of soil C:N, thereby assuming the increase in plant biomass by elevated CO_2 in these areas is zero. We used the aridity data from the CGIAR-CSI Global-Aridity Database (*37*). In our dataset of CO_2 experiments, the Nevada Desert FACE fell within this category, with low soil C:N = 0.87, but low total N = 0.49 g/kg (*34*), and no CO_2 effect on biomass (*35*), supporting this assumption. Running the model strictly in areas with aridity index > 0.32 resulted in 0.4 Pg C less than by running the model globally. This small difference was the result of the general lack of aboveground biomass in arid regions (Fig. S-IV.4), rendering small absolute increases in biomass when incorporated in the analysis. Nevertheless, we support the exclusion of these areas as it is not likely they could increase their biomass under elevated CO_2 due to extremely low water and N availability.

Current aboveground biomass

As global estimates of current aboveground biomass we used passive microwave-based global aboveground biomass carbon in 2012 from Liu *et al.* (*38*) (Version 1.0) at 0.25º resolution and available online for the period 1993-2012 (http://www.wenfo.org/wald/global-biomass/).

Climate data

For the model selection analysis (Fig. IV.2) we used MAT and MAP data for the individual studies reported in the papers. As MAT and MAP were among the important predictors of the biomass response to elevated CO_2 in ECM plants in our dataset, we upscaled the CO_2 effects globally using MAT and MAP calculated from CRU TS v. 4.00 (*39*) for the period 2010-2015 at 0.5º resolution.

In addition to MAT, we tested growing season temperature (GST) and growing degree days (GDD, day degrees ≥5 °C) as alternative metrics to account for the effect of temperature on eCO_2 fertilization in the model selection analysis. As these data are not commonly reported in the papers, we calculated these values globally using monthly temperatures from CRU, and extracted the corresponding values for each study using the coordinates of the sites. Replacing MAT for the extracted measurements of GST or GDD rendered models with less amount of heterogeneity accounted for than using MAT, and were not considered in the analyses. Due to the nature of the regression of ECM species (Fig. IV.2D, Table IV.1), the model would predict a decrease in biomass by eCO_2 under extremely low MAT, and very large relative increases in biomass under very high temperatures, effects not supported by experimental evidence. We normalised the map of MAT to adjust its range of values to the max and min values in the dataset of studies (Table S-IV.1, Fig. S-IV.1).

As an alternative predictor to MAP, we considered the moisture index, defined as the ratio of MAP and mean annual potential evapotranspiration (PET) from SPLASH (*40*). We extracted values of moisture index for the individual studies, including it as a potential predictor of the CO_2 effect. Aridity index extracted from SPLASH accounted for a lower amount of heterogeneity than MAP, and so it was not used to upscale CO_2 effects.

Global Ecological Zones

Calculations of the enhancement in biomass in response to CO_2 within each habitat type or biome (Table IV.2) were performed through zonal statistics with the map of Terrestrial Ecoregions from The Nature Conservancy (http://maps.tnc.org/gis_data.html), and based on the ecoregions defined by World Wildlife Fund (*41*).

4.4. Results

When considering all the predictors together, we found that mycorrhizal type was the only predictor with a sum of Akaike weights higher than 0.7, whereas the rest of the predictors that have been referenced to explain individual responses to CO_2 did not have enough power to explain overall responses (Fig. S-IV.2). We then studied the relative importance of the predictors for the subset of AM and ECM plants separately. In AM plants, soil C:N was by far the most important predictor (Fig V.2A). Therefore, the range of AM responses to CO_2 in the dataset was primarily explained by soil C:N, with a decreasing eCO_2 effect on aboveground biomass with increasing soil C:N (Fig. IV.2B, pseudo R^2=89%, $P<0.001$). If we assume soil C:N is an indicator of N availability, these results confirm that the effects of eCO_2 decrease with decreasing N availability in AM plants. For ECM species, however, soil C:N was not a good predictor of the biomass response to eCO_2 (Fig. IV.2C), suggesting that ECM responses to eCO_2 in our dataset do not significantly decrease with decreasing N availability (Fig. S-IV.3). Including interactions between predictors in model selection, we found that the most parsimonious model explaining the variety of ECM responses to eCO_2 was ~ MAT + MAT * $[CO_2]$ (Fig. IV.2C, pseudo R^2=46%, $P=0.002$). The relationship between MAP and the CO_2 effect was positive (Fig. IV.2D, Table IV.1), as well as the interaction between MAT and $[CO_2]$, indicating a stronger and more positive relationship between MAT and the eCO_2 effect with higher levels of $[CO_2]$ (Fig. IV.2E, Table IV.1). This relationship indicated that experiments with $[CO_2] > +300$ppm generally showed relatively small eCO_2-driven effects on biomass under low temperatures, especially in areas with low precipitation

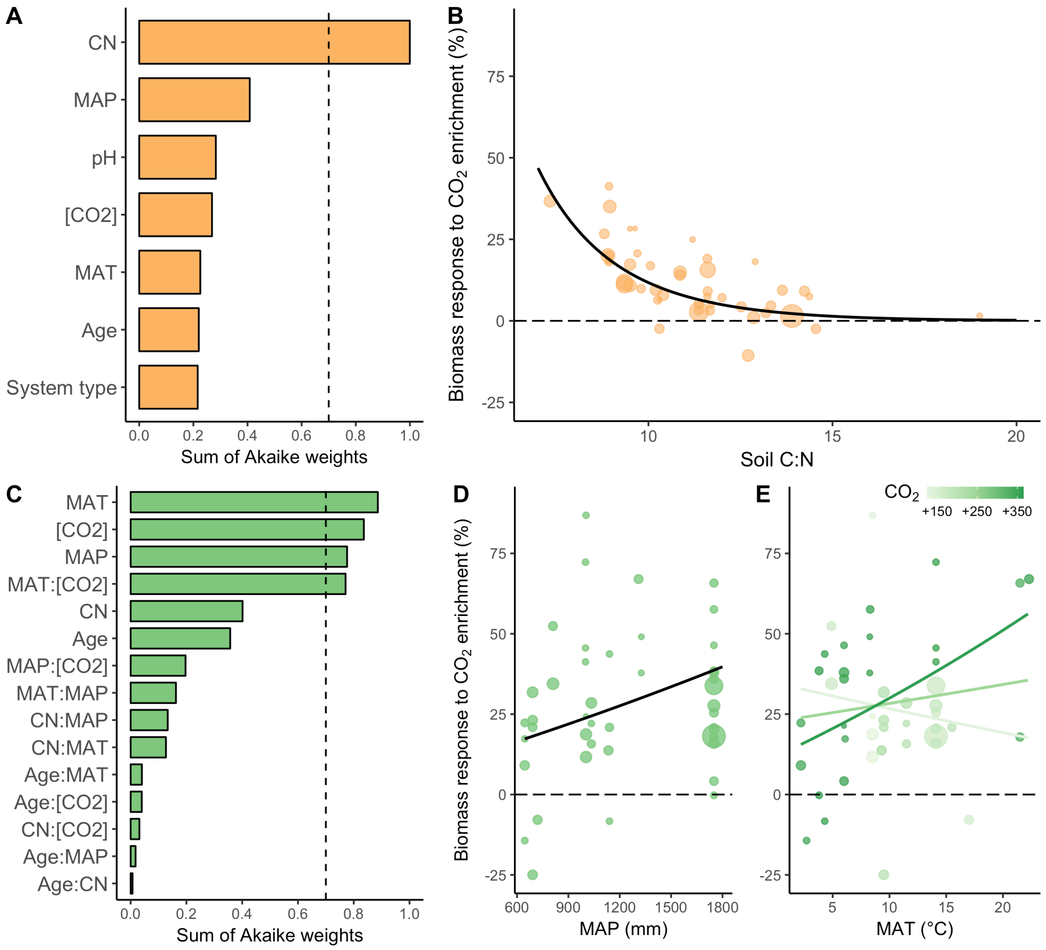


Figure IIV.2 Model selection of the CO_2 fertilization effect on aboveground biomass in AM (A and B) and ECM plants (C, D, E). (A and C) Model-averaged importance of the predictors. (B, D, E) Meta-analytic scatterplot showing the observed outcomes (percentage CO_2 effect) of the individual studies against the most important predictors. Line are based on a mixed-effects meta-regression model. Dots represent the individual studies in the dataset, with the size of the dots drawn proportional to the weights in the model. The lines in (E) represent the interaction between MAT and $[CO_2]$, showing the relationship between the eCO_2 effect on biomass and MAT under three different levels of $[CO_2]$.

The C:N ratio of soil organic matter (SOM) is associated with stoichiometric limitations of microbial processes in the soil (*42*, *43*). If the C:N ratio in SOM is high compared to microbial demand (i.e. above the "threshold element ratio" often considered to be at around 20 (*44*)), N will be mainly used by microbial decomposers themselves, and only a minor fraction will be released to the soil solution. If the C:N ratio is low, on the other hand, microbes will mineralise excess N, increasing its availability for plants (*45-47*). The identification of soil C:N as an indicator on the N limitation on the CO_2 fertilization effect in AM plants (Fig. IV.2B, Table IV.1) solves the problem of finding a metric to compare CO_2 effects across sites and levels of N availability, avoiding the somewhat arbitrary assessment of high versus low N availability (*17*, *48-50*).

A positive interaction between [CO_2] and temperature on photosynthesis is predicted by theory (*51*), and many models predict a larger enhancement in plant productivity by eCO_2 with increasing MAT (e.g. *52*). A previous meta-analysis, however, did not find a significant relationship between MAT and the eCO_2 on biomass (*53*), perhaps because the effects of mycorrhizal type, MAP and [CO_2] were not taken into account. We found clear evidence from observational studies supporting this important interaction (Fig. IV.2E, Table IV.1). MAT was a better predictor of the response to CO_2 in ECM than GDD or GST (see Methods section), so these alternatives were not considered further.

In addition to the direct effect of eCO_2 on photosynthesis (*20*), eCO_2 lowers stomatal conductance (*54*), potentially reducing plant water use (*55*, *56*). Therefore it has been hypothesized that this indirect effect of eCO_2 might result in greater growth responses to eCO_2 in drier than wetter environments (*57*). This hypothesis is supported by some grassland experiments from dry regions that have shown greater biomass responses to eCO_2 in dry than wet years (*58*, *59*), but a recent study suggest these results might have been driven by the effects of seasonal precipitation on N availability (*60*). A meta-analysis of widely distributed grasslands found no relationship between MAP and eCO_2-driven biomass increase (*61*), and many studies show low growth enhancements by eCO_2 when water supply is reduced (*62*, *63*). Thus, no general support for this hypothesis has been found. Here, we show a significant and positive relationship between MAP and the eCO_2 effect on biomass in ECM species (Fig. IV.2D, Table IV.1), suggesting that the indirect effects of eCO_2 (reduced water use) do not mitigate the inhibitory effects of low levels of soil moisture on plant biomass. The hypothesis that the eCO_2 response will be greater in drier environments, incorporated in some models (*64*), is not supported. We ran the model with moisture index (MI, see Methods section) instead of MAP, and found a significantly positive effect of MI (P_{MI}= 0.0489) in the overall model (~ MI + MAT * [CO_2], R^2=35%, P=0.0076). As the original model with MAP (Table IV.1) was better in explaining the effect of eCO_2 on ECM-biomass, we did not consider MI further.

Table IV.1 Coefficients of retained terms in best mixed-effects meta-regression models for AM and ECM species. SE: standard error; LCI: lower limit 95% Confidence Interval; UCI: upper limit 95% Confidence Interval; *P*: individual moderator *P*-value; F: model support based on omnibus test of moderators using an F-distribution with m and $k - p$ degrees of freedom (m being the number of coefficients tested, p being the total number of model coefficients; PF: *P*-value of the model based on F. R^2: McFadden's pseudo R^2; C:N: soil C:N ratio; MAT: mean annual temperature; MAP: mean annual precipitation; $[CO_2]_{dif}$: difference in CO_2 concentration between elevated and ambient CO_2 treatments. Model for AM is based on a mixed-effects non-linear model of the form: $AM \sim P_1 * e^{P_2}$

Moderators	**Estimate**	**SE**	**LCI**	**UCI**	***P***	**F**	P_F	R^2
AM								
P_1	11.7384	9.3075	-6.5043	29.9811	0.2072	54.6128	0.0000	89%
C:N (P_2)	-0.4772	0.0884	0.3039	0.6504	0.0000			
ECM								
Intercept	0.5754	0.1828	0.2057	0.9451	0.0031	4.9266	0.0026	46%
MAT	-0.0589	0.0179	-0.0952	-0.0226	0.0022			
MAP	0.0002	0.0001	0.0000	0.0003	0.0111			
$[CO_2]_{dif}$	-0.0019	0.0007	-0.0032	-0.0005	0.0093			
MAT: $[CO_2]_{dif}$	0.0002	0.0001	0.0001	0.0003	0.0008			

We have developed the first model that accounts for the observations in CO_2 experiments across a wide range of plant species and ecosystems. Using the parameters in Table IV.1, we have calculated the total change in aboveground biomass to be expected if CO_2 is stabilized at the level of CO_2 used in the experiments. at a global scale. In summary, we calculated the percentage and distribution of the CO_2 effect on aboveground biomass at a resolution of 0.25° combining the meta-analysis-derived equations in Table IV.1 with global maps of: i) mycorrhizal distribution (Soudzilovskaia, et al., *in review*), ii) soil C:N ratio from ISRIC-WISE (*31*), and iii) MAT and MAP 2010-2015 from CRU TS v.4.00 (*39*). The results in Fig. IV.3A correspond to the percentage effect for a standardized magnitude of $[CO_2]$ at 700 ppm compared to 400 ppm, an increase in atmospheric $[CO_2]$ that may be experienced by the end of this century (*65*). In other words, we have estimated the outcome of a "global elevated CO_2 experiment". Despite the lack of data from eCO_2 experiments in tropical regions of South America and Africa, and only few in boreal forests, this approach allows us to estimate the potential increase in biomass in these ecosystems. To convert from percentage change to absolute terms (Mg C ha^{-1}) we used the global estimates of aboveground biomass by Liu et al. (*38*) from satellite passive microwave observations in the year 2012 as a basis ("current biomass").

Using this approach, we estimated the potential enhancement in aboveground biomass at 700 ppm in Mg C h^{-1} (Fig. IV.3B), with a global increase of 29.8 Pg (7.9%) from 2012 (~400ppm). But this increase is not geographically uniform. Tropical, Subtropical and

Temperate coniferous forests, and Temperate Broadleaf and Mixed forests show the largest relative increases in biomass (Table IV.2, Fig. IV.3A). The ECM association appears to be more common in Asia and Africa than South America (*66*), leading to a corresponding higher relative predicted biomass increase in some regions of Southeast Asia and Central Africa than in the Amazon (Fig. IV.3A). Rainforests show the largest absolute enhancement in aboveground biomass (Table IV.2). Boreal regions, despite their association with ECM fungi, show only a 7% increase (Table IV.2), a lower response than other biomes, driven by low temperatures. Grasslands and some tree species in temperate regions generally show low relative increases in biomass (Table IV.2), as these plants commonly occur in soils with low N availability and associate with AM fungi, which are not able to alleviate the N limitation of the CO_2 fertilization effect.

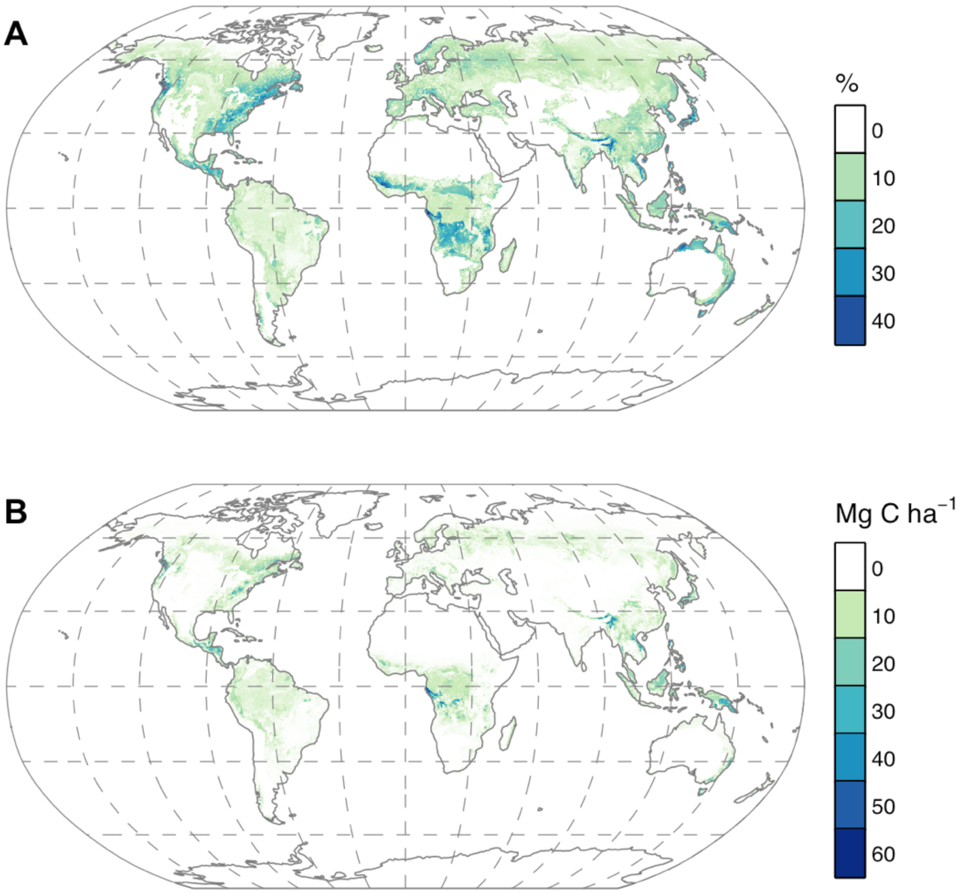


Figure IIV.3 Potential aboveground biomass enhancement in terrestrial ecosystems for an increase in CO_2 concentration from 400 to 700 ppm, in (A) relative (%) and (B) absolute (Mg C ha^{-1}) terms.

4.5. Discussion

Put into perspective, global anthropogenic C emissions including land-use changes are currently around 10 Pg annually (*67*). The estimated value of an additional C storage in aboveground biomass of around 30 Pg for an increase in CO_2 would help continue to slow climate change, as otherwise CO_2 would accumulate faster in the atmosphere. In addition, the distribution of this eCO_2-effect might be similar to the distribution of the current aboveground C sink.

A previous analysis based on four sites estimated that the effect of eCO_2 on forest productivity was 23% across different ranges of productivity (*68*). Although Norby *et al.*'s study focused on total biomass productivity while we focus on aboveground biomass, the relative effects of eCO_2 on total, aboveground and belowground biomass, as well as aboveground productivity, follow the same patterns and are of similar magnitude across mycorrhizal types and levels of N-availability (*17*, *69*). As the biomass enhancement in grasslands is generally low, and many grid cells are not entirely covered by vegetation, a 23% increase is only to be expected in specific hotspots, representing maximum values (Table IV.2) rather than a generalizable effect (Fig. IV.3A). Our analysis suggests that in most areas of the planet the CO_2 fertilization effect is constrained by N availability and/or climate, with an overall ~8% effect.

A component of the CO_2 fertilization effect is the increase in leaf area ("greening"), which is more directly detectable by satellites than other components of plant biomass. Although changes in greenness and total plant productivity are not perfectly correlated, changes in observed greening in the recent past provide an indication of the direction and geographical distribution of ongoing CO_2 fertilization effects. Satellite observations reveal that leaf area index (LAI) has increased during the last 30 years, with rising CO_2 postulated to be the main driver (*6*). The geographical distribution of the magnitude of this CO_2-driven effect is very similar to our projections of an absolute increase in biomass under eCO_2 (Fig. IV.3B), with large increases in Southeast Asia, Central Africa and the East coast of the US, which may suggest that the same areas that are currently responsible for the increase in the land C sink (*67*) might continue absorbing CO_2 in the future. Zhu *et al.* (2016), however, also found a large increase in LAI in the Amazon forest, whereas we found a somewhat lower increase in this region compared to tropical regions in Africa and Asia. Several hypotheses could explain this result: i) changes in allocation among biomass pools under eCO_2 might hinder a direct comparison of CO_2 effects on LAI and aboveground biomass; ii) our statistical model predicts a stronger degree of N limitation in the Amazon than tropical regions of Africa and Asia as a consequence of the dominance of the AM association (*66*). In the Amazon, phosphorus (P), rather than N, is considered the most limiting factor (*70*, *71*). As AM fungi can transfer large quantities of P to their host plant (*72*, *73*), it is possible that under higher levels of CO_2 these plants can overcome P-limitations through the exchange of P for C with AM fungi, increasingly becoming more N-limited as we show. The capacity of tropical plants to acquire additional N through N_2-fixation under eCO_2 is highly uncertain.

Our results are based on the most comprehensive dataset of CO_2 fertilization effects currently available. However, most eCO_2 experiments have been carried out in the Northern Hemisphere (Fig. IV.1A), and tropical ecosystems are strongly underrepresented (Fig. IV.1B). Also, relatively few eCO_2 experiment have been carried out in cold climates (Fig. IV.1B), where N mineralization rates are generally extremely low, and ECM fungi may have limited capacity to transfer sufficient N to their host plant to allow a significant CO_2 fertilization effect (*74*). The relatively small effect in boreal ecosystems shown here (Table IV.2), however, is consistent with the response shown in observational studies (*75*). The role of nutrients in the CO_2 fertilization effect in P-limited, and extremely N-limited, forests is greatly in need of further exploration (*76*).

We found that CO_2 is not a universal fertilizer, and extensive areas may show limited capacity to sustain higher rates of growth under eCO_2. On the other hand, N limitation is not a universal constraint on the CO_2 response, and some plants may be able to overcome N limitations and accumulate more biomass under eCO_2 when temperature and precipitation are adequate. Soil C:N can be used as a quantitative metric of the N limitation of CO_2 fertilization in AM systems, thus, combining global data on soil C:N, mycorrhizal distribution, and climate, we could scale up CO_2 effects on biomass from experiments to a global scale. This approach, combining observational data synthesized through meta-analysis with global predictors, complements the representation of the CO_2 fertilization effect in Dynamic Global Vegetation Models and Earth System models, which incorporating other drivers of global change (e.g. N deposition, warming, droughts, land use, biodiversity) should lead to better predictions of the magnitude of the land C sink. The body of observations from eCO_2 experiments suggest an ~8% increase in aboveground biomass as a result of an increase in $[CO_2]$ from 400 to 700 ppm, which is considerably lower than the magnitude of increase found in some individual forest studies (*53*). To fully elucidate the magnitude and distribution of the land C sink, future synthesis work should account for the effects of eCO_2 on soil C storage.

Table IV.2 Summary of changes in plant aboveground biomass to elevated CO_2 across habitat types. Mean, maximum, and minimum relative changes in percentage effect, and absolute effect in Pg C as the difference in aboveground biomass at 700 ppm versus 400 ppm.

Habitat type	**Relative change (%)**			**Abs change (Pg C)**
	Mean	**Max**	**Min**	
Boreal Forests/Taiga	7	12	3	4.44
Deserts and Xeric Shrublands	1	17	0	0.19
Flooded Grasslands and Savannas*	5	9	0	0.11
Mangroves*	5	15	0	0.1
Mediterranean Forests, Woodlands and Scrub	5	16	0	0.24
Montane Grasslands and Shrublands	4	23	0	0.33
Temperate Broadleaf and Mixed Forests	10	27	2	4.45
Temperate Conifer Forests	10	32	1	1.74
Temperate Grasslands, Savannas and Shrublands	4	7	0	0.29
Tropical and Subtropical Coniferous Forests*	12	31	4	0.42
Tropical and Subtropical Dry Broadleaf Forests*	6	17	1	0.87
Tropical and Subtropical Grasslands, Savannas and Shrublands*	7	19	1	4.1
Tropical and Subtropical Moist Broadleaf Forests*	7	30	1	12.33
Tundra*	2	8	0	0.14
Global	7.9			29.81

* Ecosystems not represented in the dataset of available CO_2 experiments (Table S-IV.1).

4.6. Supplementary Material

Table S-IV.1 Overview of CO2 enrichment experiments included in our analysis. Abbreviations: Myc: mycorrhizal type (AM: arbuscular mycorrhizae, ECM: ectomycorrhizae), F: fertilization treatment, P: irrigation treatment, W: warming treatment, FACE: Free Air Carbon Dioxide Enrichment, G: Greenhouse/Growth chamber, OTC = Open Top Chamber.

Study name	**Species**	**System**	**Country**	**Myc**	**Facility**	**Ref.**
AG FACE	Yitpi	Agricultural	Australia	AM	FACE	(*77*)
BangorFACE	*Alnus glutinosa*	Tree Stand	UK	ECM	FACE	(*78*)
BangorFACE	*Betula pendula*	Tree Stand	UK	ECM	FACE	(*78*)
BangorFACE	*Fagus sylvatica*	Tree Stand	UK	ECM	FACE	(*79*)
BioCON	perennial grassland	Grassland	USA	AM	FACE	(*80*)
BioCON F	perennial grassland	Grassland	USA	AM	FACE	(*80*)
Birmensdorf acidic	*Fagus sylvatica*	Tree Stand	Switzerland	ECM	OTC	(*81*)
Birmensdorf acidic	*Picea abies*	Tree Stand	Switzerland	ECM	OTC	(*81*)
Birmensdorf calcareous	*Fagus sylvatica*	Tree Stand	Switzerland	ECM	OTC	(*81*)
Birmensdorf calcareous	*Picea abies*	Tree Stand	Switzerland	ECM	OTC	(*81*)
Brandbjerg	temperate heath	Scrubland	Denmark	AM	FACE	(*82*)
China FACE F	*Triticum aestivum*	Agricultural	China	AM	FACE	(*83*)
China FACE FF	*Triticum aestivum*	Agricultural	China	AM	FACE	(*83*)
China mini-FACE	*Glycine max*	Agricultural	China	AM	FACE	(*84*)
China mini-FACE	*Triticum aestivum*	Agricultural	China	AM	FACE	(*85*)
DUKE FACE	*Pinus taeda*	Tree Stand	USA	ECM	FACE	(*86*)
ETH FACE	*Betula pendula*	Tree Stand	Switzerland	ECM	FACE	(*87*)
EucFACE	*Eucalyptus tereticornis*	Tree Stand	Australia	ECM	FACE	(*88*)
EUROFACE	*Populus alba*	Tree Stand	Italy	ECM	FACE	(*89*)
EUROFACE	*Populus euramericana*	Tree Stand	Italy	ECM	FACE	(*89*)
EUROFACE	*Populus nigra*	Tree Stand	Italy	ECM	FACE	(*89*)
FACTS II FACE	*Populus tremuloides*	Tree Stand	USA	ECM	FACE	(*90*)
FACTS II FACE	*Populus tremuloides-Betula papyrifera*	Tree Stand	USA	ECM	FACE	(*90*)
Flakaliden F	*Picea abies*	Tree Stand	Sweden	ECM	OTC	(*75*)
Flakaliden	*Picea abies*	Tree Stand	Sweden	ECM	OTC	(*75*)
Flakaliden II	*Picea abies*	Tree Stand	Sweden	ECM	OTC	(*75*)
Flakaliden II W	*Picea abies*	Tree Stand	Sweden	ECM	OTC	(*75*)
FMC (IMAGINE)	Mix grassland	Grassland	France	AM	FACE	(*91*)
GiFACE	Mix grassland	Grassland	Germany	AM	FACE	(*92*)
Glencorse	*Betula pendula*	Tree Stand	UK	ECM	OTC	(*93*)
Glendevon F	*Alnus glutinosa*	Tree Stand	UK	ECM	OTC	(*94*)
Glendevon	*Alnus glutinosa*	Tree Stand	UK	ECM	OTC	(*94*)
Gunnarsholt F	*Populus trichocarpa*	Tree Stand	Iceland	ECM	G	(*95*)
Gunnarsholt	*Populus trichocarpa*	Tree Stand	Iceland	ECM	G	(*95*)
Heidfeldhof F	oilseed rape	Agricultural	Germany	AM	FACE	(*96*)
Heidfeldhof	wheat, weet, oilseed rape	Agricultural	Germany	AM	FACE	(*97*)
Horsham	Janz	Agricultural	Australia	AM	FACE	(*98*)
Horsham	Yitpi	Agricultural	Australia	AM	FACE	(*98*)
Hyderabad	*Gmelina arborea*	Tree Stand	India	AM	OTC	(*99*)
Jasper Ridge FACE	annual grassland	Grassland	USA	AM	FACE	(*100*)
Jasper Ridge FACE F	annual grassland	Grassland	USA	AM	FACE	(*100*)
Jasper Ridge FACE FP	annual grassland	Grassland	USA	AM	FACE	(*100*)
Jasper Ridge FACE P	annual grassland	Grassland	USA	AM	FACE	(*100*)
Jasper Ridge FACE FW	annual grassland	Grassland	USA	AM	FACE	(*100*)
Jasper Ridge FACE W	annual grassland	Grassland	USA	AM	FACE	(*100*)

Jasper Ridge FACE FWP	annual grassland	Grassland	USA	AM	FACE	(*100*)
Jasper Ridge FACE WP	annual grassland	Grassland	USA	AM	FACE	(*100*)
Jasper Ridge OTC	sandstone grassland	Grassland	USA	AM	OTC	(*101*, *102*)
Jasper Ridge OTC	serpentine grassland	Grassland	USA	AM	OTC	(*101*)
Kangbo FACE	*Oryza sativa*	Agricultural	China	AM	FACE	(*103*)
Kangbo FACE	*Triticum aestivum*	Agricultural	China	AM	FACE	(*103*)
Maricopa FACE	*Gossypium hirsutum*	Agricultural	USA	AM	FACE	(*104*)
Maricopa FACE	*Sorghum bicolor*	Agricultural	USA	AM	FACE	(*105*)
Maricopa FACE	*Triticum aestivum*	Agricultural	USA	AM	FACE	(*106*)
Maricopa FACE P	*Sorghum bicolor*	Agricultural	USA	AM	FACE	(*105*)
Maricopa FACE P	*Triticum aestivum*	Agricultural	USA	AM	FACE	(*106*)
Maricopa FACE P	*Triticum aestivum*	Agricultural	USA	AM	FACE	(*106*)
Merrit Island	Shrub-*Quercus* system	Tree Stand	USA	ECM	OTC	(*107*)
New Zealand FACE	temperate pasture	Grassland	New Zealand	AM	FACE	pers com.
Oak Ridge OTC III	model grassland	Grassland	USA	AM	OTC	(*108*)
ORNL FACE	*Liquidambar styraciflua*	Tree Stand	USA	AM	FACE	(*109*)
PHACE	Mixed-grass prairie	Grassland	USA	AM	FACE	(*110*)
POPFACE	*Populus alba*	Tree Stand	Italy	ECM	FACE	(*111*)
POPFACE	*Populus euramericana*	Tree Stand	Italy	ECM	FACE	(*111*)
POPFACE	*Populus nigra*	Tree Stand	Italy	ECM	FACE	(*111*)
Riso	*Pisum sativum*	Agricultural	Denmark	AM	G	(*112*)
Sapporo - brown soil	*Betula platyphylla*	Tree Stand	Japan	ECM	FACE	(*113*)
Sapporo - brown soil	*Kalopanax semptemlobus*	Tree Stand	Japan	AM	FACE	(*113*)
Sapporo - brown soil	*Larix gmelinii*	Tree Stand	Japan	ECM	FACE	(*113*)
Sapporo - brown soil	*Larix kaempferi*	Tree Stand	Japan	ECM	FACE	(*113*)
Sapporo - volcanic ash	*Larix gmelinii*	Tree Stand	Japan	ECM	FACE	(*114*)
SCBG	Subtrop forest	Tree Stand	China	ECM	OTC	(*115*)
SCBG F	Subtrop forest	Tree Stand	China	ECM	OTC	(*115*)
Suonenjoki	*Betula pendula* clone 4	Tree Stand	Finland	ECM	OTC	(*116*)
Suonenjoki	*Betula pendula* clone 80	Tree Stand	Finland	ECM	OTC	(*116*)
Swiss FACE F	*Lolium perenne*	Grassland	Switzerland	AM	FACE	(*117*)
Swiss FACE F	*Medicago sativa*	Grassland	Switzerland	AM	FACE	(*118*)
Swiss FACE FF	*Lolium perenne*	Grassland	Switzerland	AM	FACE	(*117*)
Swiss FACE FF	*Medicago sativa*	Grassland	Switzerland	AM	FACE	(*118*)
Tas FACE	Temperate grassland	Grassland	Australia	AM	FACE	(*60*)
Tsukuba FACE	*Oryza sativa*	Agricultural	Japan	AM	FACE	(*119*)
Tsukuba FACE F	*Oryza sativa*	Agricultural	Japan	AM	FACE	(*120*)
UMBS	*Populus euramericana*	Tree Stand	USA	ECM	OTC	(*121*)
UMBS F	*Populus euramericana*	Tree Stand	USA	ECM	OTC	(*121*)
UMBS II	*Populus grandidentata*	Tree Stand	USA	ECM	OTC	(*122*)
UMBS III	*Populus tremuloides*	Tree Stand	USA	ECM	OTC	(*123*)
UMBS III F	*Populus tremuloides*	Tree Stand	USA	ECM	OTC	(*123*)
USDA Placerville	*Pinus ponderosa*	Tree Stand	USA	ECM	OTC	(*124*)
USDA Placerville F	*Pinus ponderosa*	Tree Stand	USA	ECM	OTC	(*124*)
USDA Placerville FF	*Pinus ponderosa*	Tree Stand	USA	ECM	OTC	(*124*)
Walpeup	Yitpi	Agricultural	Australia	AM	FACE	(*98*)

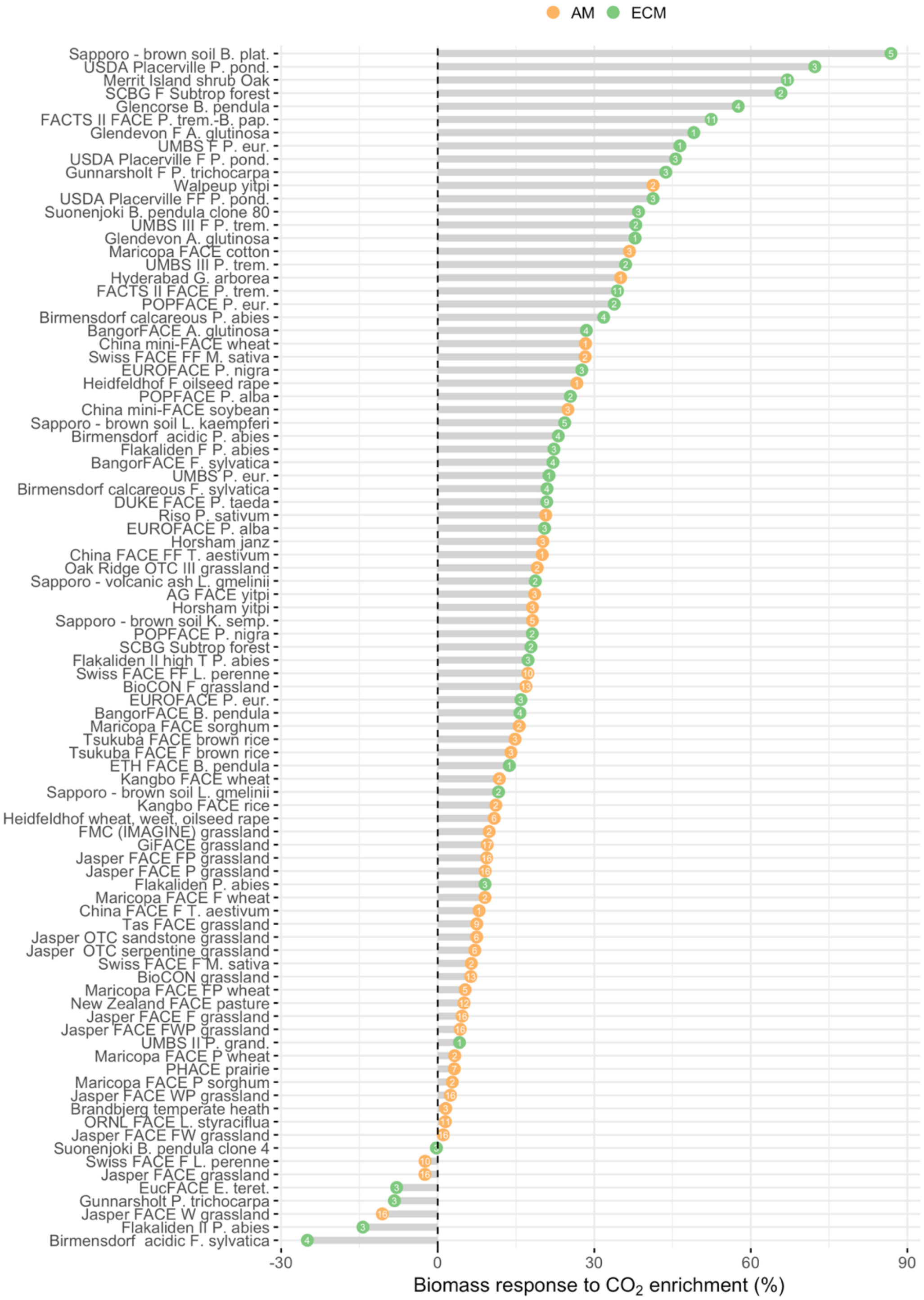


Figure S-IIV.1 Data on CO_2 effects (%) on aboveground biomass included in our meta-analysis. More information in Table S-IV.1. Numbers inside the dots indicate the number of years included in each study.

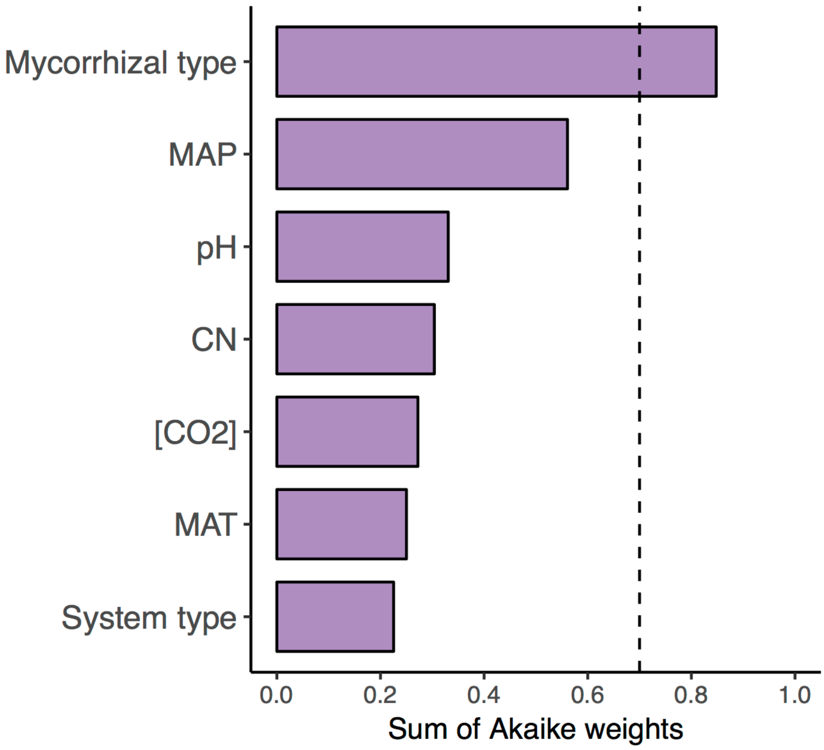


Figure S-IIV.2 Model-averaged importance of the predictors of the CO_2 fertilization effect on aboveground biomass for the entire dataset of eCO_2 experiments. The importance is based on the sum of Akaike weights derived from model selection using AICc (Akaike's Information Criteria corrected for small samples). Cutoff is set at 0.7 (dashed line) to differentiate among the most important predictors.

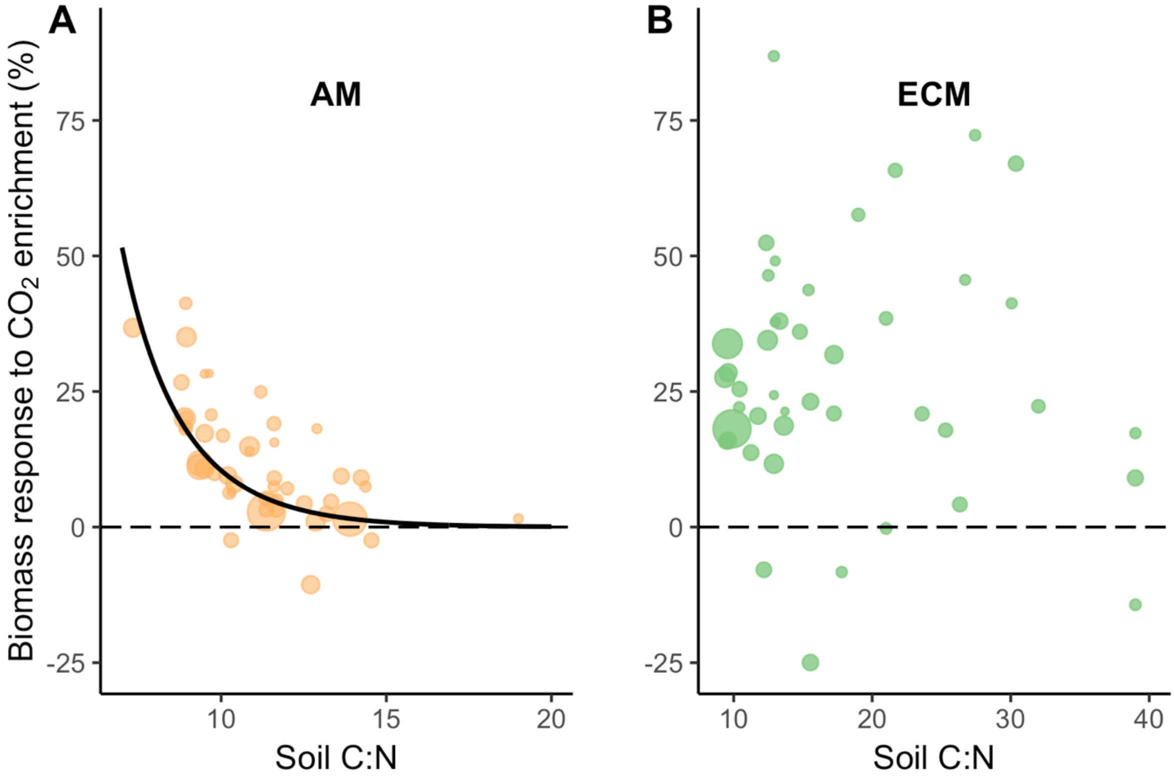


Figure S-IIV.3 Relationship between soil C:N ratio and the aboveground biomass response to elevated CO_2 in AM (A) and ECM (B) plants. Line in (A) is based on a non-linear mixed-effects meta-regression model. Dots represent the individual studies in the dataset, with the size of the dots drawn proportional to the weights in the model. No line was drawn in (B) because the relationship was not significant.

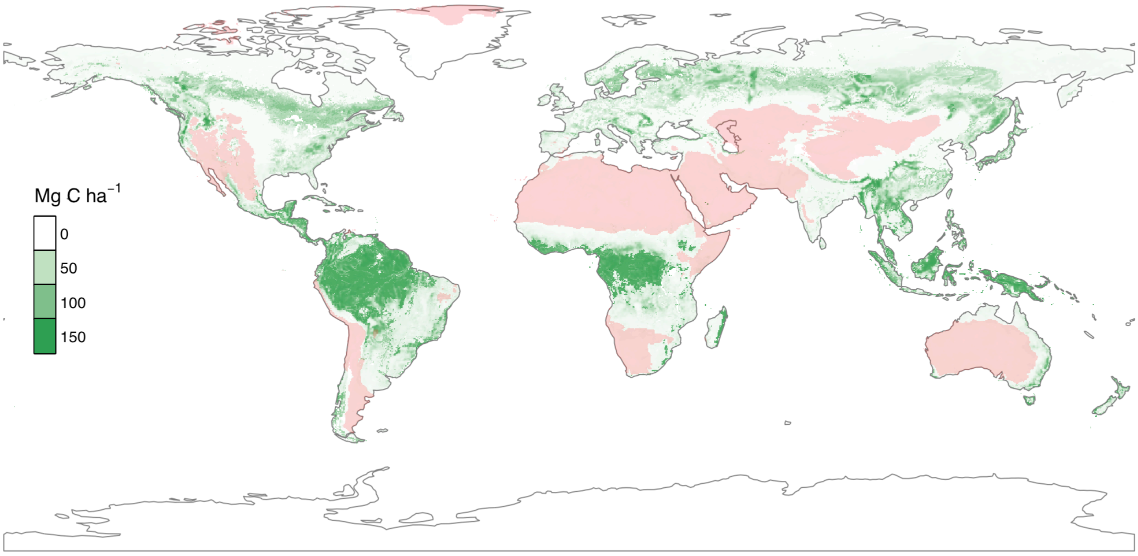


Figure S-IIV.4 Aboveground biomass in 2012 from Liu *et al*. (*38*). Areas shaded in red have aridity index < 0.32.

4.7. References

1. J. Mao *et al.*, Global Latitudinal-Asymmetric Vegetation Growth Trends and Their Driving Mechanisms: 1982–2009. *Remote Sensing*. **5**, 1484–1497 (2013).
2. S. O. Los, Analysis of trends in fused AVHRR and MODIS NDVI data for 1982-2006: Indication for a CO 2fertilization effect in global vegetation. *Global Biogeochem. Cycles*. **27**, 318–330 (2013).
3. Y. Pan *et al.*, A Large and Persistent Carbon Sink in the World's Forests. *Science*. **333**, 988–993 (2011).
4. A. P. A. Ballantyne, C. B. C. Alden, J. B. J. Miller, P. P. P. Tans, J. W. C. J. White, Increase in observed net carbon dioxide uptake by land and oceans during the past 50 years. *Nature*. **488**, 70–72 (2012).
5. T. Keenan *et al.*, Recent pause in the growth rate of atmospheric CO2 due to enhanced terrestrial carbon uptake. *Nature Communications*. **7**, 13428 (2016).
6. Z. Zhu *et al.*, Greening of the Earth and its drivers. *Nature Climate change*. **6**, 791–795 (2016).
7. S. Piao *et al.*, Detection and attribution of vegetation greening trend in China over the last 30 years. *Global Change Biology*. **21**, 1601–1609 (2015).
8. D. Schimel, B. B. Stephens, J. B. Fisher, Effect of increasing CO 2on the terrestrial carbon cycle. *Proc Natl Acad Sci U S A*. **112**, 436–441 (2015).
9. V. K. Arora *et al.*, Carbon–Concentration and Carbon–Climate Feedbacks in CMIP5 Earth System Models. *J. Climate*. **26**, 5289–5314 (2013).
10. V. K. Arora, J. F. Scinocca, On constraining the strength of the terrestrial CO2 fertilization effect in an Earth system model. *GMDD*. **2016**, 1–46 (2016).
11. P. Friedlingstein *et al.*, Climate–Carbon Cycle Feedback Analysis: Results from the C 4MIP Model Intercomparison. *J. Climate*. **19**, 3337–3353 (2006).
12. M. R. Raupach, J. G. Canadell, C. Le Quéré, Anthropogenic and biophysical contributions to increasing atmospheric CO₂ growth rate and airborne fraction. *Biogeosciences*. **5**, 1601–1613 (2008).
13. P. Bousquet, Regional Changes in Carbon Dioxide Fluxes of Land and Oceans Since 1980. *Science*. **290**, 1342–1346 (2000).
14. B. A. Hungate, J. S. Dukes, M. R. Shaw, Y. Luo, C. B. Field, Nitrogen and climate change. *Science*. **302**, 1512–1513 (2003).
15. W. R. Wieder, C. C. Cleveland, W. K. Smith, K. Todd-Brown, Future productivity and carbon storage limited by terrestrial nutrient availability. *Nature Geosci*. **8**, 441–444 (2015).
16. R. J. Norby, D. R. Zak, Ecological lessons from free-air CO2 enrichment (FACE) experiments. *Annual Review of Ecology*. **42**, 181–203 (2011).
17. C. Terrer, S. Vicca, B. A. Hungate, R. P. Phillips, I. C. Prentice, Mycorrhizal association as a primary control of the CO_2 fertilization effect. *Science*. **353**, 72–74 (2016).

18. F. Shah *et al.*, Ectomycorrhizal fungi decompose soil organic matter using oxidative mechanisms adapted from saprotrophic ancestors. *New Phytol.* **209**, 1705–1719 (2015).

19. T. J. Thirkell, D. D. Cameron, A. Hodge, Resolving the “nitrogen paradox” of arbuscular mycorrhizas: fertilization with organic matter brings considerable benefits for plant nutrition and growth. *Plant Cell Environ.* **39**, 1683–1690 (2016).

20. E. A. Ainsworth, S. P. Long, What have we learned from 15 years of free-air CO2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO2. *New Phytol.* **165**, 351–372 (2005).

21. C. Körner *et al.*, Carbon flux and growth in mature deciduous forest trees exposed to elevated CO2. *Science*. **309**, 1360–1362 (2005).

22. R. J. Norby *et al.*, Comment on “Mycorrhizal association as a primary control of the CO2 fertilization effect.” *Science*. **355**, 358–358 (2017).

23. L. V. Hedges, J. Gurevitch, P. S. Curtis, The meta-analysis of response ratios in experimental ecology. *Ecology*. **80**, 1150 (1999).

24. W. Viechtbauer, Conducting Meta-Analyses in R with the metafor Package. *Journal of Statistical Software*. **36** (2010), doi:10.18637/jss.v036.i03.

25. C. W. Osenberg, O. Sarnelle, S. D. Cooper, R. D. Holt, Resolving ecological questions through meta-analysis: goals, metrics, and models. *Ecology*. **80**, 1105–1117 (1999).

26. M. Borenstein, L. V. Hedges, J. P. T. Higgins, H. R. Rothstein, in *Introduction to Meta-Analysis* (John Wiley & Sons, Ltd, Chichester, UK, 2009), pp. 225–238.

27. A. C. Del Re, W. T. Hoyt, MAd: Meta-Analysis with Mean Differences. *R package version 0.8-2* (2014) (available at http://cran.r-project.org/web/packages/MAd).

28. G. Knapp, J. Hartung, Improved tests for a random effects meta-regression with a single covariate. *Statistics in Medicine*. **22**, 2693–2710 (2003).

29. W. Viechtbauer, J. A. López-López, J. Sánchez-Meca, F. Marín-Martínez, A comparison of procedures to test for moderators in mixed-effects meta-regression models. *Psychological Methods*. **20**, 360–374 (2015).

30. V. Calcagno, C. de Mazancourt, glmulti: an R package for easy automated model selection with (generalized) linear models. *Journal of Statistical Software*. **34** (2010), doi:10.18637/jss.v034.i12.

31. N. H. Batjes, Harmonized soil property values for broad-scale modelling (WISE30sec) with estimates of global soil carbon stocks. *Geoderma*. **269**, 61–68 (2016).

32. W. M. Post, J. Pastor, P. J. Zinke, A. G. Stangenberger, Global patterns of soil nitrogen storage. *Nature*. **317**, 613–616 (1985).

33. F. Jiao, X.-R. Shi, F.-P. Han, Z.-Y. Yuan, Increasing aridity, temperature and soil pH induce soil C-N-P imbalance in grasslands. *Sci. Rep.* **6**, 19601 (2016).

34. S. A. Billings, S. M. Schaeffer, R. D. Evans, Trace N gas losses and N mineralization in Mojave desert soils exposed to elevated CO2. *Soil Biology and Biochemistry*. **34**, 1777–1784 (2002).

35. R. D. Evans *et al.*, Greater ecosystem carbon in the Mojave Desert after ten years exposure to elevated CO2. *Nature Climate change*. **4**, 394–397 (2014).

36. C. Wang *et al.*, Aridity threshold in controlling ecosystem nitrogen cycling in arid and semi-arid grasslands. *Nature Communications*. **5**, 4799–4799 (2013).

37. R. J. Zomer, A. Trabucco, D. A. Bossio, L. V. Verchot, Climate change mitigation: A spatial analysis of global land suitability for clean development mechanism afforestation and reforestation. *Agriculture, Ecosystems & Environment*. **126**, 67–80 (2008).

38. Y. Y. Liu *et al.*, Recent reversal in loss of global terrestrial biomass. *Nature Climate change*. **5**, 470–474 (2015).

39. I. Harris, P. D. Jones, T. J. Osborn, D. H. Lister, Updated high-resolution grids of monthly climatic observations – the CRU TS3.10 Dataset. *Int. J. Climatol.* **34**, 623–642 (2014).

40. T. W. Davis *et al.*, Simple process-led algorithms for simulating habitats (SPLASH v.1.0): robust indices of radiation, evapotranspiration and plant-available moisture. *Geoscientific Model Development*. **10**, 689–708 (2017).

41. D. M. Olson, E. Dinerstein, The Global 200: Priority ecoregions for global conservation. *Annals of the Missouri Botanical garden*, 199–224 (2002).

42. S. Manzoni, R. B. Jackson, J. A. Trofymow, A. Porporato, The Global Stoichiometry of Litter Nitrogen Mineralization. *Science*. **321**, 684–686 (2008).

43. B. Berg, C. McClaugherty, *Plant Litter* (Springer Science & Business Media, 2013).

44. M. Mooshammer *et al.*, Adjustment of microbial nitrogen use efficiency to carbon:nitrogen imbalances regulates soil nitrogen cycling. *Nature Communications*. **5**, 3694–3694 (2014).

45. C. Kaiser, O. Franklin, U. Dieckmann, A. Richter, Microbial community dynamics alleviate stoichiometric constraints during litter decay. *Ecol Lett*. **17**, 680–690 (2014).

46. S. Manzoni, A. Porporato, Soil carbon and nitrogen mineralization: Theory and models across scales. *Soil Biology and Biochemistry*. **41**, 1355–1379 (2009).

47. R. L. Sinsabaugh, S. Manzoni, D. L. Moorhead, A. Richter, Carbon use efficiency of microbial communities: stoichiometry, methodology and modelling. *Ecol Lett*. **16**, 930–939 (2013).

48. R. S. Nowak, D. S. Ellsworth, S. D. Smith, Functional responses of plants to elevated atmospheric CO2- do photosynthetic and productivity data from FACE experiments support early predictions? *New Phytologist*. **162**, 253–280 (2004).

49. M.-A. de Graaff, K. J. van Groenigen, J. Six, B. Hungate, C. van Kessel, Interactions between plant growth and soil nutrient cycling under elevated CO2: a meta-analysis. *Global Change Biology*. **12**, 2077–2091 (2006).

50. B. A. Hungate *et al.*, Assessing the effect of elevated carbon dioxide on soil carbon: a comparison of four meta-analyses. *Global Change Biology*. **15**, 2020–2034 (2009).

51. S. P. Long, Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO2 concentrations: Has its importance been underestimated? *Plant, Cell & Environment*. **14**, 729–739 (1991).

52. T. Hickler *et al.*, CO2 fertilization in temperate FACE experiments not representative of boreal and tropical forests. *Global Change Biology*. **14**, 1531–1542 (2008).

53. S. Baig, B. E. Medlyn, L. M. Mercado, S. Zaehle, Does the growth response of woody plants to elevated CO2 increase with temperature? A model-oriented meta-analysis. *Global Change Biology*. **21**, 4303–4319 (2015).

54. E. A. Ainsworth, A. Rogers, The response of photosynthesis and stomatal conductance to rising [CO2]: mechanisms and environmental interactions. *Plant Cell Environ*. **30**, 258–270 (2007).

55. T. Keenan *et al.*, Increase in forest water-use efficiency as atmospheric carbon dioxide concentrations rise. *Nature*. **499**, 324–7 (2013).

56. G. Battipaglia *et al.*, Elevated CO2increases tree-level intrinsic water use efficiency: insights from carbon and oxygen isotope analyses in tree rings across three forest FACE sites. *New Phytol*. **197**, 544–554 (2012).

57. C. Körner, Biosphere Responses to CO 2 Enrichment. *Ecological Applications*. **10**, 1590 (2000).

58. J. A. Morgan *et al.*, Water relations in grassland and desert ecosystems exposed to elevated atmospheric CO2. *Oecologia*. **140**, 11–25 (2004).

59. J. A. Morgan *et al.*, C4 grasses prosper as carbon dioxide eliminates desiccation in warmed semi-arid grassland. *Nature*. **476**, 202–205 (2011).

60. M. J. Hovenden, P. C. D. Newton, K. E. Wills, Seasonal not annual rainfall determines grassland biomass response to carbon dioxide. *Nature*. **511**, 583–586 (2014).

61. M. Lee, P. Manning, J. Rist, S. A. Power, C. Marsh, A global comparison of grassland biomass responses to CO2 and nitrogen enrichment. *Philosophical Transactions of the Royal Society B: Biological Sciences*. **365**, 2047–2056 (2010).

62. P. B. Reich, S. E. Hobbie, T. D. Lee, Plant growth enhancement by elevated CO2 eliminated by joint water and nitrogen limitation. *Nature Geosci*. **7**, 920–924 (2014).

63. A. A. M. Cantarel, J. M. G. Bloor, J.-F. Soussana, Four years of simulated climate change reduces above-ground productivity and alters functional diversity in a grassland ecosystem. *Journal of Vegetation Science*. **24**, 113–126 (2013).

64. S. Piao *et al.*, Evaluation of terrestrial carbon cycle models for their response to climate variability and to CO2 trends. *Global Change Biology*. **19**, 2117–2132 (2013).

65. D. J. Griggs, M. Noguer, Climate change 2001: the scientific basis. Contribution of working group I to the third assessment report of the intergovernmental panel on climate change. *Weather*. **57**, 267–269 (2002).

66. H. ter Steege *et al.*, Continental-scale patterns of canopy tree composition and function across Amazonia. *Nature*. **443**, 444–447 (2006).

67. C. L. Quéré *et al.*, Global Carbon Budget 2015. *Earth System Science Data*. **7**, 349–396 (2015).

68. R. Norby *et al.*, Forest response to elevated CO2 is conserved across a broad range of productivity. *Proceedings of the National Academy of Sciences*. **102**, 18052–18056 (2005).

69. C. Terrer *et al.*, Response to Comment on “Mycorrhizal association as a primary control of the CO2 fertilization effect.” *Science*. **355**, 358–358 (2017).

70. P. M. Vitousek, Litterfall, Nutrient Cycling, and Nutrient Limitation in Tropical Forests. *Ecology*. **65**, 285–298 (1984).

71. S. Alvarez-Clare, M. C. Mack, M. Brooks, A direct test of nitrogen and phosphorus limitation to net primary productivity in a lowland tropical wet forest. *Ecology*. **94**, 1540–1551 (2013).

72. S. E. Smith, D. J. Read, *Mycorrhizal symbiosis* (Academic press, Cambridge, UK, 2008).

73. C. Plassard, B. Dell, Phosphorus nutrition of mycorrhizal trees. *Tree Physiol*. **30**, 1129–1139 (2010).

74. O. Franklin, T. Näsholm, P. Högberg, M. N. Högberg, Forests trapped in nitrogen limitation - an ecological market perspective on ectomycorrhizal symbiosis. *New Phytol*. **203**, 657–666 (2014).

75. B. D. Sigurdsson, J. L. Medhurst, G. Wallin, O. Eggertsson, S. Linder, Growth of mature boreal Norway spruce was not affected by elevated [CO2] and/or air temperature unless nutrient availability was improved. *Tree Physiol*. **33**, 1192–1205 (2013).

76. R. J. Norby *et al.*, Model-data synthesis for the next generation of forest free-air CO2 enrichment (FACE) experiments. *New Phytol*. **209**, 17–28 (2015).

77. G. J. O'Leary *et al.*, Response of wheat growth, grain yield and water use to elevated CO2 under a Free-Air CO2 Enrichment (FACE) experiment and modelling in a semi-arid environment. *Global Change Biology*. **21**, 2670–2686 (2015).

78. A. R. Smith, M. Lukac, M. Bambrick, F. MIGLIETTA, D. L. Godbold, Tree species diversity interacts with elevated CO2 to induce a greater root system response. *Global Change Biology*. **19**, 217–228 (2013).

79. M. R. Hoosbeek, M. Lukac, E. Velthorst, A. R. Smith, D. L. Godbold, Free atmospheric CO2 enrichment increased above ground biomass but did not affect symbiotic N2-fixation and soil carbon dynamics in a mixed deciduous stand in Wales. *Biogeosciences*. **8**, 353–364 (2011).

80. P. B. Reich, S. E. Hobbie, Decade-long soil nitrogen constraint on the CO2 fertilization of plant biomass. *Nature Climate change*. **3**, 278–282 (2013).

81. D. Spinnler, P. Egli, C. Körner, Four-year growth dynamics of beech-spruce model ecosystems under CO2 enrichment on two different forest soils. *Trees*. **16**, 423–436 (2002).

82. J. Kongstad *et al.*, High Resilience in Heathland Plants to Changes in Temperature, Drought, and CO2 in Combination: Results from the CLIMAITE Experiment. *Ecosystems*. **15**, 269–283 (2012).

83. T. Kou, J. ZHU, Z. Xie, T. Hasegawa, K. Heiduk, Effect of elevated atmospheric CO2 concentration on soil and root respiration in winter wheat by using a respiration partitioning chamber. *Plant Soil*. **299**, 237–249 (2007).

84. X. Hao *et al.*, Effects of free-air CO2 enrichment (FACE) on N, P and K uptake of soybean in northern China. *Agricultural and Forest Meteorology*. **218-219**, 261–266 (2016).

85. S. K. Lam, X. Han, E. Lin, R. Norton, D. Chen, Does elevated atmospheric carbon dioxide concentration increase wheat nitrogen demand and recovery of nitrogen applied at stem elongation? *Agriculture, Ecosystems & Environment*. **155**, 142–146 (2012).

86. H. R. McCarthy *et al.*, Re-assessment of plant carbon dynamics at the Duke free-air CO2 enrichment site: interactions of atmospheric [CO2] with nitrogen and water availability over stand development. *New Phytol*. **185**, 514–528 (2010).

87. P. Ineson, M. F. Cotrufo, R. Bol, D. D. Harkness, H. Blum, Quantification of soil carbon inputs under elevated CO2: C3 plants in a C4 soil. *Plant Soil*. **187**, 345–350 (1996).

88. D. S. Ellsworth *et al.*, Elevated CO2 does not increase eucalypt forest productivity on a low-phosphorus soil. *Nature Climate change*. **320**, 1444 (2017).

89. M. Liberloo *et al.*, Woody biomass production during the second rotation of a bio-energy Populus plantation increases in a future high CO2 world. *Global Change Biology*. **12**, 1094–1106 (2006).

90. A. F. Talhelm *et al.*, Elevated carbon dioxide and ozone alter productivity and ecosystem carbon content in northern temperate forests. *Global Change Biology*. **20**, 2492–2504 (2014).

91. J. M. G. Bloor, P. Pichon, R. Falcimagne, P. Leadley, J.-F. Soussana, Effects of Warming, Summer Drought, and CO2 Enrichment on Aboveground Biomass Production, Flowering Phenology, and Community Structure in an Upland Grassland Ecosystem. *Ecosystems*. **13**, 888–900 (2010).

92. L. C. Andresen *et al.*, *Global Change Biology*, in press, doi:10.1111/gcb.13705.

93. A. Rey, P. G. Jarvis, Growth Response of Young Birch Trees (Betula pendulaRoth.) After Four and a Half Years of CO2Exposure. *Annals of Botany*. **80**, 809–816 (1997).

94. V. M. Temperton, The effects of nitrogen uptake and nitrogen fixation on trees grown in elevated [CO2]: Alnus glutinosa and Pinus sylvestris (1998).

95. B. D. Sigurdsson, H. Thorgeirsson, S. Linder, Growth and dry-matter partitioning of young Populus trichocarpa in response to carbon dioxide concentration and mineral nutrient availability. *Tree Physiol*. **21**, 941–950 (2001).

96. J. Franzaring, P. Högy, A. FANGMEIER, Effects of free-air CO2 enrichment on the growth of summer oilseed rape (Brassica napus cv. Campino). *Agriculture, Ecosystems & Environment*. **128**, 127–134 (2008).

97. J. Franzaring, P. Högy, M. Erbs, A. FANGMEIER, Responses of canopy and soil climate in a six year free-air CO2 enrichment study with spring crops. *Agricultural and Forest Meteorology*. **150**, 354–360 (2010).

98. G. J. Fitzgerald *et al.*, Elevated atmospheric [CO2] can dramatically increase wheat yields in semi-arid environments and buffer against heat waves. *Global Change Biology*. **22**, 2269–2284 (2016).

99. A. R. Reddy, G. K. Rasineni, A. S. Raghavendra, The impact of global elevated CO2 concentration on photosynthesis and plant productivity. *Current Science*. **99**, 46–57 (2010).

100. K. Zhu, N. R. Chiariello, T. Tobeck, T. Fukami, C. B. Field, Nonlinear, interacting responses to climate limit grassland production under global change. *Proceedings of the National Academy of Sciences*. **113**, 10589–10594 (2016).

101. B. A. Hungate *et al.*, The fate of carbon in grasslands under carbon dioxide enrichment. *Nature*. **388**, 576–579 (1997).

102. P. A. T. Higgins, R. B. Jackson, J. M. Des Rosiers, C. B. Field, Root production and demography in a california annual grassland under elevated atmospheric carbon dioxide. *Global Change Biology*. **8**, 841–850 (2002).

103. C. Cai *et al.*, Responses of wheat and rice to factorial combinations of ambient and elevated CO2 and temperature in FACE experiments. *Global Change Biology*. **22**, 856–874 (2016).

104. C. W. Wood, H. A. Torbert, H. H. Rogers, G. B. Runion, S. A. Prior, Free-air CO2 enrichment effects on soil carbon and nitrogen. *Agricultural and Forest Meteorology*. **70**, 103–116 (1994).

105. M. J. Ottman *et al.*, Elevated CO2 increases sorghum biomass under drought conditions. *New Phytol*. **150**, 261–273 (2001).

106. B. A. Kimball *et al.*, Data from the Arizona FACE (Free-Air CO2 Enrichment) Experiments on Wheat at Ample and Limiting Levels of Water and Nitrogen. *Open Data Journal for Agricultural Research*. **3** (2017).

107. B. A. Hungate *et al.*, Cumulative response of ecosystem carbon and nitrogen stocks to chronic CO2 exposure in a subtropical oak woodland. *New Phytol*. **200**, 753–766 (2013).

108. P. Kardol *et al.*, Climate change effects on plant biomass alter dominance patterns and community evenness in an experimental old-field ecosystem. *Global Change Biology*. **16**, 2676–2687 (2010).

109. R. J. Norby, J. M. Warren, C. M. Iversen, B. E. Medlyn, R. E. McMurtrie, CO2 enhancement of forest productivity constrained by limited nitrogen availability. *Proc Natl Acad Sci U S A*. **107**, 19368–19373 (2010).

110. K. E. Mueller *et al.*, Impacts of warming and elevated CO2 on a semi-arid grassland are non-additive, shift with precipitation, and reverse over time. *Ecol Lett*. **19**, 956–966 (2016).

111. B. Gielen *et al.*, Net carbon storage in a poplar plantation (POPFACE) after three years of free-air CO2 enrichment. *Tree Physiol*. **25**, 1399–1408 (2005).

112. M. E. Gavito, P. Schweiger, I. Jakobsen, P uptake by arbuscular mycorrhizal hyphae: effect of soil temperature and atmospheric CO2 enrichment. *Global Change Biology*. **9**, 106–116 (2003).

113. Y. WATANABE *et al.*, Response of tree growth and wood structure of Larix kaempferi, Kalopanax septemlobus and Betula platyphylla saplings to elevated CO2 concentration for 5 years exposure in a FACE system. *Trees*. **30**, 1569–1579 (2016).

114. M. Watanabe *et al.*, Elevated CO2 enhances the growth of hybrid larch F1 (Larix gmelinii var. japonica × L. kaempferi) seedlings and changes its biomass allocation. *Trees*. **27**, 1647–1655 (2013).

115. Q. Deng *et al.*, Responses of soil respiration to elevated carbon dioxide and nitrogen addition in young subtropical forest ecosystems in China. *Biogeosciences*. **7**, 315–328 (2010).

116. J. Riikonen *et al.*, Silver birch and climate change: variable growth and carbon allocation responses to elevated concentrations of carbon dioxide and ozone. *Tree Physiol*. **24**, 1227–1237 (2004).

117. M. K. Schneider *et al.*, Ten years of free-air CO2 enrichment altered the mobilization of N from soil in Lolium perenne L. swards. *Global Change Biology*. **10**, 1377–1388 (2004).

118. A. Lüscher, U. A. Hartwig, D. Suter, J. Nösberger, Direct evidence that symbiotic N2 fixation in fertile grassland is an important trait for a strong response of plants to elevated atmospheric CO2. *Global Change Biology*. **6**, 655–662 (2000).

119. T. Zhang, Y. Huang, X. Yang, Climate warming over the past three decades has shortened rice growth duration in China and cultivar shifts have further accelerated the process for late rice. *Global Change Biology*. **19**, 563–570 (2013).

120. Y. Usui *et al.*, Rice grain yield and quality responses to free-air CO2 enrichment combined with soil and water warming. *Global Change Biology*. **22**, 1256–1270 (2016).

121. K. S. Pregitzer *et al.*, Atmospheric CO2, soil nitrogen and turnover of fine roots. *New Phytol*. **129**, 579–585 (1995).

122. D. R. Zak *et al.*, Elevated atmospheric CO2 and feedback between carbon and nitrogen cycles. *Plant Soil*. **151**, 105–117 (1993).

123. C. J. Mikan, D. R. Zak, M. E. Kubiske, K. S. Pregitzer, Combined effects of atmospheric CO2 and N availability on the belowground carbon and nitrogen dynamics of aspen mesocosms. *Oecologia*. **124**, 432–445 (2000).

124. R. F. Walker, D. R. Geisinger, D. W. Johnson, J. T. Ball, Elevated atmospheric CO2 and soil N fertility effects on growth, mycorrhizal colonization, and xylem water potential of juvenile ponderosa pine in a field soil. *Plant Soil*. **195**, 25–36 (1997).

Chapter V: Conclusions

5.1. Conclusions

The results from the chapters that comprise the thesis point to the importance of plant mechanisms to increase N availability as a key mediator of the fate of C in a CO_2-enriched atmosphere.

A large dataset of elevated CO_2 experiments was assembled and synthesised in order to study the factors that statistically best explain the varying magnitude of the CO_2 effect on plant biomass. We found that the type of mycorrhizal fungi that associate with the plant's roots was the "missing link" required to explain observed large differences in CO_2-driven biomass accumulation under N limitation (Chapter II). When N availability was low, only plants associated with ECM fungi accumulated more C in biomass in response to elevated CO_2, whereas the response of AM-associated plants was generally slight. This effect was consistent for total, aboveground and below ground biomass (Chapter II), as well as for aboveground productivity (Chapter II-Addendum). When AM plants were grown together with plants with N_2-fixing capacity, however, the result was a small but significant enhancement in biomass by eCO_2.

The most immediate hypothesis that follows from this work is that ECM and N-fixing plants can acquire the N required to satisfy increased N demand for plant growth, to a greater extent than AM plants. In Chapter III we tested this hypothesis and found that, indeed, ECM plants efficiently invest part of the extra C provided by eCO_2 in N acquisition through mycorrhizal uptake, which returns an equivalent amount of N in exchange for this investment. As N is the most important limiting nutrient in many or most ecosystems (LeBauer & Treseder, *Ecology*, **89**, 371–379, 2008), mycorrhizal type may explain the degree to which ecosystems can accumulate extra C as biomass under elevated CO_2. The AM association, on the other hand, does not result in a long-term enhancement in N uptake by the plant in response to elevated CO_2 (Chapter III), explaining the low effects of CO_2 on biomass or productivity in AM Plants (Chapters II).

I expanded the dataset of studies further and scaled up the effects of elevated CO_2 on biomass, from the stand-scale of CO_2 experiments to the globe (Chapter IV). As opposed to Chapter II where experiments were classed as high versus low N availability, this analysis required a quantitative indicator of N availability. It emerged that the effect of elevated CO_2

on aboveground biomass in AM plants is best predicted by soil C:N ratio, with a decreasing effect with increasing soil C:N. This finding provides further support for the conclusions from Chapter II and III – that AM plants cannot sustain high rates of biomass accumulation when N availability is low – but now using a quantitative, continuous metric of N availability. The effect in ECM plants was best predicted by temperature, precipitation and the intensity of the CO_2 fumigation. The effect of CO_2 on biomass in ECM was generally positive and high regardless of soil C:N, supporting the conclusion of Chapters II and III that ECM plants can overcome the N limitation of CO_2 fertilisation to a greater extent than AM plants. By using continuous predictors with available data at a global scale, I could then develop an equation to predict the potential effect of elevated CO_2 on aboveground biomass, quantitatively and geographically. The results showed an overall ~8% CO_2 effect on aboveground biomass, with greater values found in hotspots in tropical Asia and Africa as well as in temperate coniferous forests. The modelled CO_2 effect on aboveground biomass is constrained by temperature in boreal forests, and by N availability in grasslands.

Elevated CO_2 stimulates photosynthesis in both AM and ECM plants under low N availability (Chapter III). Therefore, the lack of biomass accumulation in AM plants raises the question: “where does the C go?”. In Chapter III I explored different potential hypotheses and proposed a data-driven conceptual framework, in which the increase in N acquisition in ECM leads to a loss of soil C, via priming – whereas soil C storage may increase in AM systems, via rhizodeposition. In order to fully account for the magnitude of the land C sink now and to predict it in the future, research should focus also on synthesising data on the effects of elevated CO_2 on soil C storage.

I found evidence for the importance of mycorrhizal N-acquisition, and potentially N-fixation (Chapter III), as key mediators in shaping plant responses to elevated CO_2. I proposed a conceptual model based on the C cost of N acquisition and suggested several lines of further research which, if explored, would improve the representation of the N cycle in C models and thereby the simulation of the CO_2 fertilisation effect.

5.2. Future perspectives

The fate of the terrestrial carbon sink under rising CO_2 has two major components: plant biomass and soil C storage.

This thesis has made progress in disentangling the factors that drive the CO_2 fertilisation effect on plant biomass. Norby *et al.* [Science, **355**, 358–358, 2017] suggested that in order to advance in this direction, future research should study the responses of both AM and ECM trees within the same experimental setup, with the same soil and same climate. I agree that, given the evidence we show here about the different dynamics triggered by elevated CO_2 across N acquisition strategies, future research should continue working in this direction. Furthermore, I highlighted two important research gaps in the current body of CO_2 observational studies. First, tropical ecosystems are not represented by any CO_2 experiment. This is especially alarming because tropical forests are the most important reservoir of forest biomass, and thought to be the main ecosystem type responsible for the current terrestrial C sink. Second, boreal forests, and cold ecosystems in general, are underrepresented in CO_2 experiments. Future experimental strategies should ideally aim to cover a wider range of ecosystems, prioritising currently underrepresented ecosystems: tropical forests, boreal forests, deserts and savannas.

The uncertainties surrounding the effects of elevated CO_2 on soil C storage remain large. Although not the main goal of this thesis, I found some evidence for a potential mechanism by which soil C storage under elevated CO_2 is regulated by the type of N acquisition strategy. This hypothesis needs to be investigated further. I propose that future research should test the hypothesis that the effects of elevated CO_2 on plant biomass and soil C storage are negatively correlated due to priming effects. The analyses to be undertaken should accomplish an inventory of the amount of CO_2 that is removed from the atmosphere, the fluxes of C among the different pools, and the final magnitude of the C sink, both as biomass and soil organic matter, with their different turnover times. The goal would be to quantify ecosystems' capacity to absorb CO_2, and therefore reduce the large uncertainties in future predictions of climate change (Booth *et al.*, *Environ. Res. Lett.* **7**, 2012).

A final question that emerges from the conclusions of this thesis is: for how long might the association of ECM fungi and plants be able to sustain higher rates of growth under elevated CO_2 through enhanced N acquisition? As N in the soil is finite, the answer to this question likely depends on the size of the pool, the capacity of soil microbes to access N, the degree to which N is recycled in the system, leaching, as well as N inputs from N deposition or fertilization.

As opposed to enhanced CO_2 experiments, increases in atmospheric CO_2 in the real world increase gradually, allowing other processes such as N deposition and SOM decomposition to evolve slowly over the time scale of decades or centuries. Increase N deposition, for example, will likely play an important role in these dynamics, as it may more strongly benefit AM than ECM plants (Thomas *et al.*, *Nature Geoscience* **3**, 2010), potentially leading to changes in species composition of opposite sign to those of elevated CO_2. Furthermore, the capacity of N_2-fixing bacteria to acquire N from a virtually infinite pool (i.e. the atmosphere) adds more uncertainty to this matter. As elevated CO_2 experiments are short-term and simulate an abrupt increase in CO_2 concentration without letting ecological dynamics to adapt accordingly, dynamic vegetation models should account for the long-term effects of SOM decomposition, N deposition, N leaching, climate, species composition or disturbances to fully unravel the long-term capacity of terrestrial ecosystems to absorb CO_2.