Title: Mycorrhizal Association as a Primary Control of the CO_2 Fertilization Effect.

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Abstract: 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.

One Sentence Summary: Only plants that associate with ectomycorrhizal fungi can overcome nitrogen limitation, and thus take full advantage of the CO_2 fertilization effect.

Main Text: 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.

We synthesized data (overview in Table S1) on total plant biomass ($g\ m^{-2}$) from 83 eCO_2 experiments (Fig. S1), separating responses into aboveground biomass (n=83, Fig. S2) and belowground biomass (n=82, Fig. S3) 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 S2).

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. 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. S4).

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. 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. 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-$P=0.999$).

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. 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. 2C).

We conducted a sensitivity analysis to ensure the findings were robust. First, we added an intermediate level of N availability (Table S2) by assigning some ecosystems that were initially classified as "low" to a "medium" class (e.g. Duke, Aspen, ORNL) (Figure S5). 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 (Figure S6), 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 (Figure S7). 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 S3). 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.

Most CO_2 experiments have been carried out in the Northern Hemisphere (Fig. S8, 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 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.

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Fig. 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 0.8 (dashed line) to differentiate among the most important predictors.

Fig. 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.

Supplementary Materials:

Materials and Methods

Figures S1-S8

Tables S1-S4

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