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Title: Mycorrhizal Association as a Primary Control of the CO₂ Fertilization Effect.

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Abstract: Plants buffer increasing atmospheric CO₂ 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 CO₂ 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 ($30 \pm 3\%$, $P < 0.001$) in response to elevated CO₂ regardless of nitrogen availability, whereas low nitrogen availability limits CO₂ fertilization ($0 \pm 5\%$, $P = 0.946$) 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 CO₂ fertilization effect.

Main Text: Terrestrial ecosystems sequester annually about a quarter of anthropogenic CO₂ emissions (1), slowing climate change. Will this effect persist? Two contradictory hypotheses have been offered: the first is that CO₂ will continue to enhance plant growth, partially mitigating anthropogenic CO₂ emissions (1, 2), while the second is that nitrogen (N) availability will limit the CO₂ fertilization effect (3, 4), reducing future CO₂ uptake by the terrestrial biosphere (5-7). Plants experimentally exposed to elevated levels of CO₂ (eCO₂) show a range of responses in biomass, from large and persistent (8, 9) to transient (6), to non-existent (10), leaving the question of CO₂ 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 CO₂

enrichment (FACE) experiments with trees, eCO₂ 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 eCO₂: age of the vegetation (13), water limitation (14), temperature (15), type of vegetation (12), or even the eCO₂ 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 CO₂ (22, 23). Yet, their impact on the N-dependence of the CO₂ fertilization effect has not been tested, despite the increasing evidence that N limitation constrains the CO₂ 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 eCO₂ to sustain CO₂ 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 eCO₂ 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 CO₂.

We synthesized data (overview in Table S1) on total plant biomass (g m⁻²) from 83 eCO₂ 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 CO₂ concentration (Δ CO₂), mean annual precipitation (MAP), mean annual temperature (MAT), age of the vegetation at the start of the experiment, vegetation type (e.g. grassland, forest), CO₂ 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 Δ CO₂ ($P < 0.001$). 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 CO₂ effect on biomass (e.g. age of the vegetation), whereas others were associated with an increased CO₂ effect (e.g. ECM, Δ CO₂, high N availability) (Fig. S4).

The response of total biomass to an increase of CO₂ from 400 to 650 μmol mol⁻¹ was larger ($P < 0.001$) in ECM ($30 \pm 3\%$, $P < 0.001$) than in AM-dominated ($7 \pm 4\%$, $P = 0.089$) ecosystems (mean $\pm$ SE, mixed effects meta-regression). The overall response of total biomass was $20 \pm 3\%$ ($P < 0.001$), similar to previous meta-analyses (e.g., 15), with a larger effect under high ($27 \pm 4\%$, $P < 0.001$) than low N availability ($15 \pm 4\%$, $P < 0.001$), as expected (5, 7, 11). Furthermore, we found a strong interaction between mycorrhizal type and N-status ($P < 0.001$): under low N availability, eCO₂ had no effect on total biomass of AM-dominated species ($0 \pm 5\%$, $P = 0.946$) but increased biomass by $28 \pm 5\%$ in ECM-dominated species ($P < 0.001$) (Fig. 2A). Under high N availability, the CO₂ effect on total biomass in both AM- and ECM-dominated species was significant: $20 \pm 6\%$ ($P = 0.002$) for AM and $33 \pm 4\%$ ($P < 0.001$) for ECM (Fig. 2A), with no significant differences between the two groups ($P = 0.139$). Hence, high N availability significantly increased the CO₂ 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, eCO₂ stimulated aboveground biomass significantly in ECM plants ($P < 0.001$), with no effect in AM plants ($P = 0.584$) (Fig. 2B). Similarly, eCO₂ 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 CO₂ 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 eCO₂ 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 eCO₂ can thus be used to isolate the influence of mycorrhizal type from that of vegetation growth form. These three sensitivity analyses confirmed that the CO₂ 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 CO₂ experiments in our study contained N-fixing species, which might have increased N availability (Table S3). eCO₂ stimulated aboveground biomass in AM species under low N by $8 \pm 3\%$ ($P = 0.019$) in this subgroup of experiments that included N-fixing species, whereas the remaining AM experiments under low N availability showed no biomass response to eCO₂ ($1 \pm 10\%$, $P = 0.893$). But even with the additional N input from N₂ fixation, the 8% biomass increase in AM plants under low N was considerably smaller than the $28 \pm 5\%$ increase found for ECM plants.

Most CO₂ 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 eCO₂. The role of nutrients on the CO₂ fertilization effect in these P-limited forests has yet to be explored (26).

Responses of plants to rising CO₂ 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 eCO₂. Several mechanisms could explain these responses. First, ECM-associated plants typically allocate more C to support mycorrhizae than AM plants, particularly under eCO₂ (23). Moreover, because ECM fungi, unlike AM fungi, produce extracellular enzymes that degrade organic N compounds (27), increased allocation to ECM fungi under eCO₂ may supply host plants with the N needed to sustain their growth response to eCO₂. This may explain why eCO₂ 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 where N is more tightly protected. Both factors would presumably limit the enhancement of AM plant growth in response to eCO₂.

Mycorrhizal symbioses are not accounted for in most global vegetation models (but see ref. 24). Thus, the projected CO₂ 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 CO₂ fertilization effect (4) likely underestimate responses of ECM plants to eCO₂, 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 CO₂ fertilization (33). These results may also partly explain past findings that forests (commonly ECM) show stronger responses to eCO₂ compared to grasslands (AM) (12). We propose that the CO₂ 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 eCO₂. 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ effects when the zero line is not crossed.

Supplementary Materials:

Materials and Methods

Figures S1-S8

Tables S1-S4

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