

Response to comment on "Mycorrhizal association as a primary control of the CO₂ fertilization effect."

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Abstract: Norby *et al.* centre their critique on the design of the dataset and the response variable used. We address these criticisms in our Response, which reinforces the conclusion that plants that associate with ectomycorrhizal (ECM) fungi exhibit larger biomass and growth responses to elevated CO₂ compared to plants that associate with arbuscular mycorrhizae (AM).

Main text

In their Comment, Norby *et al.* question the robustness of the conclusions in Terrer *et al.* (1). We 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 CO₂, so we used a database of experiments that measured plant biomass responses to elevated CO₂. 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 CO₂ response (see Fig S4 of (1)). 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. S2 of (1)), 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% CO₂ 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 - CO₂ 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 CO₂ experiments. Nonetheless, here we have performed a meta-analysis of aboveground productivity (ANPP) responses to CO₂ in N-limited studies (Fig. 1). Despite the small sample size, results support our original conclusions (Fig. 2 of (1)). Norby *et al.* argue that leaf area normalization should be used to control for CO₂ 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 CO₂ 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 CO₂ and N additions affect both leaf area and growth efficiency (5), both need to be included in evaluating effects on biomass or productivity.

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. 1 in (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 CO₂ 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 CO₂ experiments under low N (5 ECM, 5 AM), we found that the CO₂ 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 CO₂, the much larger capacity of ECM than AM plants to increase N uptake in response to elevated CO₂ likely helps explain the observed difference in growth responses to elevated CO₂.

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 CO₂ effect over time, to explain the observed differences. Such comparison between 2 sites cannot be directly compared to the outcome of a meta-analysis with 83 sites. 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 CO₂ responses across the full range of enhanced CO₂ experiments. Furthermore, we showed that the length of the treatment was not among the most important predictors (Fig. 1 in (1)) indicating that CO₂ 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 CO₂, 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.

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Fig. 1 Meta-analysis of CO₂ 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 CO₂ 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 S1 of Terrer *et al.* (1).