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combined metal contamination of soil. *Journal of Plant Nutrition* 28: 2065–2077.

## Supporting Information

Additional supporting information may be found in the online version of this article.

**Methods S1** All the techniques used to obtain results shown in this paper, including established method for AMF nuclei isolation and their analysis by FC and FISH.

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**Key words:** arbuscular mycorrhizal fungi, flow cytometry, genome size, *Glomus intraradices*, nuclear DNA content.

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## Meetings

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# Multi-factor global change experiments: what have we learned about terrestrial carbon storage and exchange?

**Organized Session – 96th Annual Meeting, Ecological Society of America in Austin, Texas, August 2011**

Interest in understanding the mechanisms controlling carbon exchange in terrestrial ecosystems has increased recently as these ecosystems sequester one quarter of anthropogenic CO<sub>2</sub> emissions (IPCC, 2007). However, this carbon sink is vulnerable to global change. For example, climate projections indicate that temperatures will warm and precipitation patterns will be altered across the globe over the next century (IPCC, 2007). Simultaneously, terrestrial ecosystems are subject to increased nitrogen availability, tropospheric ozone, and atmospheric CO<sub>2</sub> concentrations. Numerous studies have shown that single factor manipulations lead to dramatic shifts in ecosystem

carbon dynamics (Lin *et al.*, 2010). However, it is not always possible to predict the impacts of environmental change on the magnitude and direction of ecosystem carbon exchange based on manipulation of individual factors due to additive and synergistic effects (Heimann & Reichstein, 2008).

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*‘Global change experiments should consider not only the complexity of changes in the environment, but also the underlying structure of ecosystems ...’*

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We led an organized oral session at the 96th Annual Meeting of the Ecological Society of America in Austin, Texas, to bring together multi-factor ecosystem-scale experiments and modeling efforts that have evaluated the effects of global change on carbon storage and exchange. Three major themes about global change experiments emerged in the session: trade-offs between capturing complexity and maintaining sensitivity to detect differences,

the importance of understanding temporal variation and non-linear responses, and the importance of using different models for autotrophic compared to heterotrophic respiration for predicting CO<sub>2</sub> losses.

### Capturing complexity vs detecting differences

Multi-factor experiments have been increasingly utilized to examine interactions among changes in the environment. However, in conducting complex experiments, researchers may not have sufficient replication to detect significant differences between experimental treatments. While more complex experiments may allow us to examine multiple factors, teasing apart their interactions may prove to be challenging. Rich Norby (Oak Ridge National Laboratory, TN, USA) presented results from the 'Old Field Community Climate and Atmosphere Manipulation' (OCCAM) experiment, in which old-field assemblages in open-top chambers were exposed to combinations of ambient or elevated CO<sub>2</sub> and temperature, and low or high soil moisture. Using examples from recent research (e.g. Wan *et al.*, 2007; Classen *et al.*, 2010; Kardol *et al.*, 2010), he showed that drought induced the largest effect on productivity and carbon inputs to the soil, with few significant treatment interactions. He stressed that we should not sacrifice replication for complexity of experimental design. Experimental resources could be better spent by reducing treatment combinations, increasing replication, and analyzing interactions with naturally variable environmental factors.

Global change experiments should consider not only the complexity of changes in the environment, but also the underlying structure of ecosystems, including soil type in mediating the response of soil carbon pools to environmental perturbations. For example, William Hockaday (Baylor University, TX, USA) described the use of chemical inventories to evaluate different organic compounds to provide insight into the response of carbon pools to global change. Using results from the CO<sub>2</sub> Tunnel Experiment (Fay *et al.*, 2009) in Temple, Texas, he showed that the stability and response of soil organic matter (SOM) to global change varies across soil types, thus providing an example of incorporating complexity in a way that informs results for multi-factor experiments.

Empirical multi-factor experiments provide understanding of short-term dynamics, yielding a glimpse of ecosystem responses to global change. By contrast, modeling efforts can be used to project the future response of ecosystems to environmental change, which can inform future empirical work and allow compromise between capturing complexity and having the statistical power to detect significant effects between experimental treatments. Paul Hanson (Oak Ridge National Laboratory, TN, USA) presented results from a multi-factor simulation experiment he and colleagues per-

formed to examine the impacts of elevated CO<sub>2</sub>, ozone (O<sub>3</sub>), air temperatures, and precipitation on a variety of ecosystem processes (Hanson *et al.*, 2005). Initial findings showed that the combined effects of these environmental changes resulted in a simulated 29% reduction in mean annual net ecosystem exchange of carbon. However, when they took into account physiological adjustments (e.g. acclimation of leaf respiration to warming), the combined influence resulted in a 20% increase in net ecosystem exchange, rather than a decrease. Without the use of both models and understanding of fine-scale physiological processes revealed from experiments, Hanson and colleagues would not have been able to accurately simulate the future response of net ecosystem carbon exchange to multiple changes in the environment.

Adrien Finzi (Boston University, MA, USA) urged ecologists to increase their consideration of biophysical principles and enzyme kinetics in order to deal with the 'contingency' inherent in biogeochemistry. Contingent outcomes refer to the variability in ecosystem responses to, for example, environmental perturbations and arise 'when the nature and strength of ecosystem functioning in different locations are different realizations of the same underlying process' (Schmitz, 2010). Using the temperature sensitivity of SOM decomposition as an example, Finzi argued that the 'standard model' (typically three soil pools based on SOM turnover time with first-order, temperature-dependent decay dynamics, and a soil moisture multiplier) is not sufficiently rooted in fundamental principles of biophysics and microbial ecology. It is therefore unlikely to accurately reflect changes in terrestrial carbon balance associated with global change. New approaches that base models on fundamental principles, and can be used to structure algorithms to predict SOM decomposition in ecosystem and earth-system models, include the Davidson *et al.* (2011) 'Dual Arrhenius Michaelis–Menton' model of enzyme kinetics and the work of Schimel & Weintraub (2003) and Allison *et al.* (2010).

### Understanding temporal variation and non-linear responses of terrestrial ecosystems to global change

The response of carbon cycling in terrestrial ecosystems may vary among seasons and years. Hugh Henry (University of Western Ontario, Ontario, Canada) presented results from an old-field experiment in Ontario, Canada, where nitrogen and temperature were artificially elevated. Henry discussed the cyclical nature of feedbacks between changes in nitrogen availability and aboveground productivity that became apparent every other year. Results were presented by Rebecca McCulley (University of Kentucky, KY, USA) from a multi-factor experiment in which she and colleagues examined the combined effects of

elevated air temperature and precipitation on pastureland in Kentucky. They found that warmer temperatures and increases in precipitation alone did not alter annual rates of net primary productivity (NPP), but the combined effects of these changes led to an increased annual NPP (although no change in soil respiration), suggesting an increase in carbon storage.

Vidya Suseela (Purdue University, IN, USA) presented results from an experiment with four temperature and three precipitation treatments in an old-field ecosystem in Massachusetts (the Boston Area Climate Change Experiment). Results from this experiment show that warmer and drier climatic conditions in this region could lead to higher concentrations of highly reactive tannins in *Acer rubrum* foliage, which has the potential to slow rates of decomposition and nutrient loss (Tharayil *et al.*, 2011). Nonlinear responses and thresholds to global change must also be accounted for, since the temperature sensitivity of soil respiration (i.e.  $Q_{10}$  values) can vary inter- and intra-seasonally, indicating that a fixed value for  $Q_{10}$  may not adequately explain the response of soil carbon to warming.

### Separating autotrophic vs heterotrophic respiration and their relationship with $Q_{10}$

When quantifying the response of soil respiration to global change, heterotrophic and autotrophic respiration should be considered separately. For example, results presented by Jim Tang (Marine Biological Laboratory, MA, USA), from a multi-factor experiment in a temperate forest in Massachusetts, indicate that microbial respiration is more sensitive than root respiration to changes in temperature. In an old-field ecosystem, Vidya Suseela observed that warming increased rates of autotrophic soil respiration in the winter, while drought in the growing season reduced annual rates of heterotrophic soil respiration (Suseela *et al.*, 2011).

The  $Q_{10}$  values are often used to characterize the response of soil carbon to global change. However, many speakers, including Adrien Finzi, Rebecca McCulley, Jim Tang, and Vidya Suseela, demonstrated that rates of soil respiration are influenced by many factors including different responses of autotrophic and heterotrophic respiration, seasonality, and changes in soil moisture. The complexity of soil carbon dynamics reinforces the need for finer-scale mechanistic understanding of SOM turnover dynamics, which corroborates earlier suggestions to move beyond  $Q_{10}$  alone as a means to model soil respiration (Davidson *et al.*, 2006).

### Conclusions and future challenges to understanding global change

This organized oral session highlighted the importance of multi-factor experiments in furthering our understanding of

the impacts of global change on the terrestrial carbon cycle. The session acknowledged that the tendency toward highly complex multi-factor experiments should be tempered with parsimony to maximize the sensitivity and applicability of these experiments. 'Ockham's razor' dictates that one should select the simplest explanation when competing hypotheses are similar in other respects. In doing so, one can minimize the number of assumptions that need to be made to draw conclusions. Striking the appropriate balance between complexity and replication can get us closer to understanding how many facets of global change will impact carbon storage and exchange in terrestrial ecosystems. As we move forward, we encourage ecologists to consider the following when designing multi-factor experiments: strike the appropriate balance between complexity and sufficient statistical power, conduct the iterative process of empirical and modeling work, consider temporal variation and nonlinear responses, and develop contingencies when possible.

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**Key words:** carbon exchange, carbon storage, global change, modeling, multi-factor experiments, Q<sub>10</sub>, terrestrial ecosystems, terrestrial carbon cycle.

## Mechanistic causes of tree drought mortality: recent results, unresolved questions and future research needs

### 96th Annual Meeting, Ecological Society of America in Austin, Texas, August 2011

There is evidence that drought is occurring more frequently (Huntington, 2006), with reports of forest productivity declines (Ciais *et al.*, 2005; Phillips *et al.*, 2009) and widespread forest mortality from drought (McDowell *et al.*, 2008; van Mantgem *et al.*, 2009; Allen *et al.*, 2010). Furthermore, drought frequency and severity will likely increase in many regions in the future (IPCC, 2007). Reports of tree mortality appear to be on the rise globally and may be related to increased temperatures elevating tree drought stress and the populations of tree pests and pathogens (Allen *et al.*, 2010). The consequences of tree mortality range from changes in community structure and

species interactions to landscape effects on water, carbon, and energy budgets that could disrupt biosphere–atmosphere feedbacks which are important for climate regulation (Bonan, 2008; McDowell *et al.*, 2008).

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*‘... causes of drought mortality may be interrelated for some species, but not related for others.’*

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In August 2011, at the Ecological Society of America meeting in Austin, Texas, researchers investigating drought mortality presented results from experimental, modelling, and observational studies. Research included investigations of physiological mechanisms, patterns of mortality across landscapes, potential contributions of tree stress and biotic agents, and how models could incorporate mortality mechanisms. Experimental results suggest that causes of drought mortality may be interrelated for some species, but not related for others.

Despite these potential ecological impacts, the causes and physiological mechanisms of drought-induced tree mortality remain unresolved (McDowell *et al.*, 2008; Adams *et al.*, 2009; Sala, 2009; McDowell, 2011). The absence of a mechanistic understanding means that most models of global change effects on climate and vegetation do not mechanistically model tree mortality in response to drought (McDowell *et al.*, 2011). Rather, they assume tree mortality can be captured through other modelled processes such as declines in productivity, which may not accurately model the thresholds leading to death, as well as consequences of forest mortality. Clearly, there is a need to include mortality processes in coupled vegetation–climate models to more accurately predict future global carbon and water budgets.

Multiple hypotheses for the mechanisms of drought-induced forest mortality have been proposed, yet the debate over mechanisms in current literature is ongoing. Hydraulic failure and carbon starvation have been the focus of early research, though additional mechanisms of carbon mobilization and transport failure, and carbon–hydraulic interactions, among others, could occur as well (Sala *et al.*, 2010; McDowell *et al.*, 2011). Tree death via hydraulic failure occurs from drought when xylem tension is sufficient to cause lethal levels of cavitation by air embolism (McDowell *et al.*, 2008). Carbon starvation would occur for trees that regulate stomatal conductance to prevent cavitation, but with closed stomata photosynthesis is curtailed. These trees would die by depleting stored carbohydrates during