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Abrupt rise in atmospheric CO_2 overestimates community response in a model plant–soil system

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Attempts to understand the ecological effect of increasing atmospheric CO_2 concentration, $[CO_2]$, usually involve exposing today's ecosystems to expected future $[CO_2]$ levels[1,2]. However, a major assumption of these approaches has not been tested—that exposing ecosystems to a single-step increase in $[CO_2]$ will yield similar responses to those of a gradual increase over several decades[3]. We tested this assumption on a mycorrhizal fungal community over a period of six years. $[CO_2]$ was either increased abruptly, as is typical of most $[CO_2]$ experiments, or more gradually over 21 generations. The two approaches resulted in different structural and functional community responses to increased $[CO_2]$. Some fungi were sensitive to the carbon pulse of the abrupt $[CO_2]$ treatment. This resulted in an immediate decline in fungal species richness and a significant change in mycorrhizal functioning. The magnitude of changes in fungal diversity and functioning in response to gradually increasing $[CO_2]$ was smaller, and not significantly different to those with ambient $[CO_2]$. Our results suggest that studies may overestimate some community responses to increasing $[CO_2]$ because biota may be sensitive to ecosystem changes that occur as a result of abrupt increases.

A major goal in climate change research is to predict the structure and functioning of ecological systems at a future date when the climate will be significantly different from that of today[4]. For example, atmospheric $[CO_2]$ is expected to continue rising during the next century to concentrations of 550 p.p.m. or more[4]. A major research effort is under way to understand the changes that will occur in population, community and ecosystem structure and function in response to this increase in $[CO_2]$[5].

A typical experimental approach used to predict the ecological effect is to expose current ecosystems to ambient and elevated atmospheric $[CO_2]$ and compare the responses[1,2,5]. A major assumption in such $[CO_2]$ experiments is that exposing today's ecosystems to an abrupt increase in $[CO_2]$ will yield structural and functional responses similar to those that would be observed by exposing the same ecosystems to a gradual increase of the same magnitude over several decades. There is evidence that plants undergo microevolutionary changes in response to elevated $[CO_2]$[6,7], and that there are compositional changes in communities[5,6]. Many organisms, especially those that grow quickly, will have gone through many generations, each one exposed to an incrementally higher $[CO_2]$. Subjecting organisms to a step increase in $[CO_2]$ may exert a selective pressure on biota very different from the selective pressure exerted when each generation is subjected to an incrementally increased $[CO_2]$. Theory indicates that gradual increases in $[CO_2]$ over many generations may elicit responses that are different from those following abrupt increases[3]. However, experimental support for this claim has been lacking.

Using a model experimental system, we tested the hypothesis that biodiversity and function would respond differently depending on whether a community is exposed to an abrupt or a gradual increase in $[CO_2]$. The arbuscular mycorrhizal symbiosis is ideal for testing this hypothesis for two main reasons. First, arbuscular mycorrhizal fungi (AMF) are affected indirectly by elevated atmospheric $[CO_2]$, responding to changes in plant physiology and growth[8–10]. These fungi are obligate biotrophs that are intimately associated with plant roots and depend directly on plant photosynthate as a source of carbon[11,12]. Increasing atmospheric $[CO_2]$ often results in increased allocation of carbon to roots. This increased carbon availability can influence microbial interactions in the rhizosphere and the structure of the AMF community[8,13–15]. Second, AMF and many of their plant hosts grow and reproduce quickly. This allows us to study the responses of several generations over a reasonably short period of time.

The experiment was conducted using *Bromus inermis* (a perennial C3 grass) and its associated mycorrhizal community from the Long-Term Mycorrhiza Research Site in Guelph, Ontario, Canada[16]. *B. inermis* was chosen because it is a common plant at the site, shows significant growth responses when associated with AMF[16], and preliminary studies have demonstrated that the plant significantly increases its dependency on AMF for growth in an elevated atmospheric $[CO_2]$ environment (data not shown). Plants and fungi were grown for 21 generations, each generation lasting 15 weeks (about 6 yr in total). Plants were subjected to one of three treatments: (1) 350 p.p.m. $[CO_2]$ at each generation (here referred to as 'ambient'); (2) 550 p.p.m. $[CO_2]$ at each generation (here referred to as 'abrupt'); or (3) 350 p.p.m. $[CO_2]$ at the first generation, increasing by 10 p.p.m. at each subsequent generation, with the final generation exposed to 550 p.p.m. (here referred to as 'gradual') (see Methods for more details).

After each generation we determined the biodiversity and functioning of the resulting fungal community. For biodiversity, we identified AMF taxa following a trap-culture bioassay and determined AMF species richness. For functioning, we determined the ability of the AMF community to influence plant biomass. On the first and final generations, we also measured a number of other variables that are highly influenced by AMF, including the concentration of phosphorus in plant tissue, [P], root length, per cent mycorrhizal colonization, mycelium production, and soil aggregate size distribution and aggregate water stability (both measures of soil aggregation).

We did not find any evidence of plant genotype selection over the course of the experiment. We grew seedlings from all generations in a common environment (on a field-collected soil under greenhouse conditions) and did not observe significant differences in gross photosynthesis rates, above- or below-ground plant biomass, or their mycorrhization (data not shown). Furthermore, measures of plant photosynthesis under 550 p.p.m. $[CO_2]$ did not differ between gradual and abrupt treatments (data not shown). However, we did observe significantly higher below-ground plant production in the abrupt treatment, which suggests that the plants were responding to changes in the soil environment. The plants were most probably responding to changes in the composition of the mycorrhizal

Table 1 **Influence of increased [CO_2] on mycorrhizal structure and function**

Response variables	Treatment		
	Ambient	Abrupt	Gradual
Generation 1			
Total plant biomass (g)	7.1 (1.8)^{a}	11.6 (2.5)^{a}	8.2 (1.3)^{a}
Leaf [P]	30.3 (2.9)^{a}	17.1 (1.8)^{b}	29.9 (2.0)^{a}
Root length (m)	297 (42)^{a}	329 (45)^{a}	290 (36)^{a}
AMF root colonization (%)	42.7 (4.7)^{a}	38.8 (3.3)^{a}	37.8 (3.8)^{a}
Hyphal length (m g^{-1} soil)	5.4 (0.4)^{a}	2.9 (0.2)^{b}	4.1 (0.4)^{ab}
Glomus hyphae (m g^{-1} soil)	1.7 (0.4)^{a}	1.4 (0.3)^{a}	1.3 (0.2)^{a}
Acaulospora hyphae (m g^{-1} soil)	0.2 (0.1)^{a}	0.3 (0.1)^{a}	0.1 (0.1)^{a}
Gigaspora hyphae (m g^{-1} soil)	0.8 (0.1)^{a}	0.2 (0.05)^{b}	0.7 (0.1)^{a}
Scutellospora hyphae (m g^{-1} soil)	0.6 (0.2)^{a}	0.0 (0.0)^{b}	0.9 (0.2)^{a}
MWD (mm)	1.53 (0.04)^{a}	1.52 (0.02)^{a}	1.52 (0.03)^{a}
WSA (%)	92.6 (0.8)^{a}	90.1 (1.3)^{a}	89.7 (1.1)^{a}
Generation 21			
Total plant biomass (g)	6.8 (1.2)^{a}	10.7 (2.0)^{a}	7.8 (1.6)^{a}
Leaf [P]	24.1 (1.9)^{ab}	16.7 (2.0)^{a}	28.3 (2.1)^{b}
Root length (m)	250 (38)^{a}	394 (42)^{b}	282 (35)^{a}
AMF root colonization (%)	38.2 (2.5)^{a}	36.3 (3.1)^{a}	34.9 (2.9)^{a}
Hyphal length (m g^{-1} soil)	4.9 (0.4)^{a}	2.4 (0.3)^{b}	4.9 (0.3)^{a}
Glomus hyphae (m g^{-1} soil)	2.1 (0.4)^{a}	1.5 (0.3)^{a}	1.5 (0.4)^{a}
Acaulospora hyphae (m g^{-1} soil)	0.1 (0.1)^{a}	0.2 (0.1)^{a}	0.2 (0.1)^{a}
Gigaspora hyphae (m g^{-1} soil)	0.5 (0.1)^{a}	0.1 (0.03)^{b}	0.8 (0.1)^{a}
Scutellospora hyphae (m g^{-1} soil)	1.0 (0.03)^{a}	0.2 (0.03)^{b}	0.9 (0.2)^{a}
MWD (mm)	1.53 (0.02)^{a}	1.75 (0.05)^{b}	1.45 (0.02)^{a}
WSA (%)	91.9 (1.8)^{a}	92.0 (0.7)^{a}	91.0 (1.6)^{a}

Values represent the mean, followed by standard error in parentheses. Different superscript letters (a, b) represent significant differences ($P < 0.05$) after ANOVA and Tukey post-hoc tests.

community (see below). The most carbon-demanding fungal taxa were lost in the abrupt treatment, possibly releasing the plants from a heavy carbon drain.

Per cent root colonization by AMF did not change in response to any [CO_2] treatment (Table 1). However, we detected significant treatment effects on AMF species richness. Average AMF species richness across the entire ambient treatment was significantly higher than the average species richness in the abrupt treatment (Fig. 1). Average species richness across generations in the gradual treatment did not significantly differ from that in the ambient, but did differ from the abrupt treatment. At the end of the experiment (final generation 21), AMF species richness in the ambient and gradual treatments remained similar, and contained twice as many AMF taxa compared with the abrupt treatment.

More AMF taxa were lost when [CO_2] was raised abruptly than when it was raised gradually. However, not all taxa were equally vulnerable. The number of species observed from the genus *Glomus* was similar under the three treatments, but the number of species in the other genera (mainly *Gigaspora* and *Scutellospora*) declined sharply. On average, *Glomus* spp. represented 62% of all taxa in the ambient treatment and 67% of taxa in the gradual treatment. However, species of *Glomus* made up 93% of taxa in the abrupt treatment. In this latter treatment, the length of living hyphae in the soil also declined significantly for species of *Gigaspora* and *Scutellospora*, but remained constant for species of *Glomus* (Table 1). The decline in fungal species diversity in the abrupt treatment was observed after generation 1, indicating that species of *Gigaspora* and *Scutellospora* are sensitive to the abrupt carbon pulse.

The change in AMF species composition under the different [CO_2] treatments also resulted in differences in functioning. In the abrupt treatment, the elimination of many *Gigaspora* and *Scutellospora* species, and the reduction in hyphal biomass through the loss of these taxa, resulted in an AMF community that was better able to stimulate plant biomass compared with the other two treatments (Fig. 2). Also, total P, determined by multiplying [P] with plant biomass, did not differ significantly among treatments, suggesting the P available to plants was diluted in larger plants. Such a dilution of P may not necessarily indicate higher P demand by the plant. However, it does indicate that the resulting AMF community was less capable of stimulating plant tissue [P]. Species of *Gigaspora* and *Scutellospora* are known to produce the most extensive extra-radical mycelia[17,18]. In the present study, the highest mycelial production in the soil was observed in the ambient and gradual treatments, in which *Gigaspora* and *Scutellospora* species had their

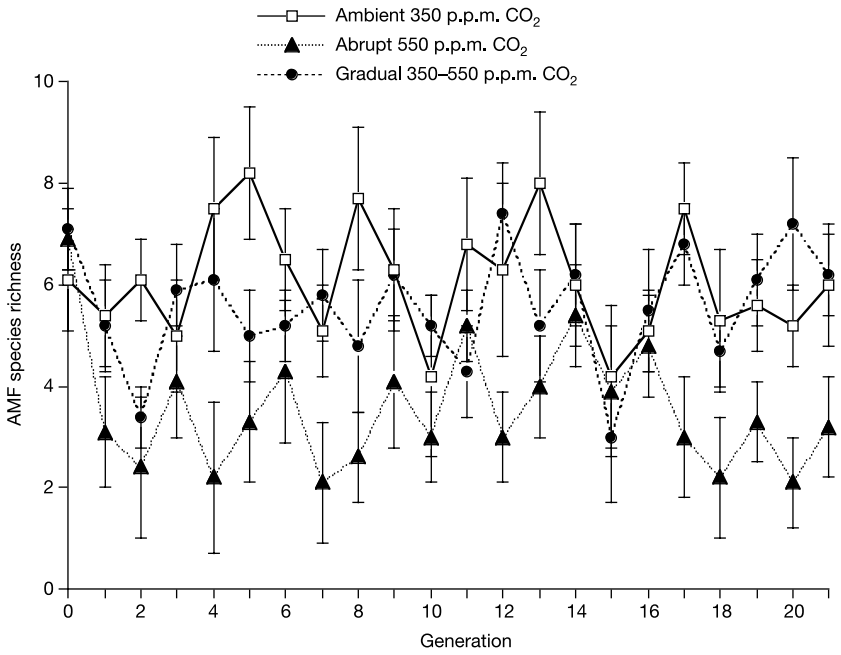

Figure 1 The effect of atmospheric [CO_2] on AMF species richness. Overall, mean richness was significantly higher (repeated-measures ANOVA (Tukey), $P = 0.001$) in the 'ambient' compared with the 'abrupt' treatment. Richness in the 'gradual' treatment did not significantly differ from the ambient treatment (repeated-measures ANOVA (Tukey), $P = 0.06$), but did differ from the abrupt treatment (repeated-measures ANOVA (Tukey), $P = 0.001$). AMF species richness at generation 21 in the ambient and gradual treatments remained similar (ANOVA (Tukey), $P = 0.879$), and contained twice as many AMF taxa compared with the abrupt treatment (ANOVA (Tukey), $P = 0.001$). Points represent the mean and bars represent the standard error.

highest occurrence (Table 1). High investment in mycelial production may result in an improved ability to extract P from the soil and translocate it to the plant host, but it also results in a higher demand for carbon from the plant.

The different CO_2-mediated changes in AMF species composition had other functional consequences as well, such as in the formation of soil aggregates. AMF play a central role in this process[19], which reduces soil losses associated with wind and water erosion and greatly influences other biological, physical and chemical soil processes. The soil of the *Glomus*-dominated AMF communities found in the abrupt treatment produced higher mean weight diameter (MWD) soil aggregates in conjunction with plant roots (Table 1), indicating a shift in soil aggregates towards greater mass in larger aggregate size classes (as had been found previously with step-increase experiments[19]). The absence of change in the percentage of water-stable aggregates (WSA, Table 1) indicated that this was not a transient response, but that aggregates had similar water stability irrespective of $[CO_2]$ treatments. The soil aggregation results are most probably a response to the decrease in the relative abundance of *Gigaspora* and increased root lengths. *Gigaspora* has been shown to be the least effective AMF group at stimulating soil aggregation when associated with *B. inermis*[20].

The present results show that exposing plants to elevated $[CO_2]$ could influence the structure and functioning of their associated AMF. This phenomenon has been shown in many other studies[8–10,15] that have instantaneously raised the $[CO_2]$ from ambient to $\geq$550 p.p.m. However, the present data also support the hypothesis that AMF responses to an abrupt increase in $[CO_2]$ could differ from responses to a gradual increase if an initial loss of fungal taxa is not replaced. Global atmospheric $[CO_2]$ is increasing at a rate (around 1.5 p.p.m. per year)[4] that is much lower even than that of the gradual treatment. Under that treatment, AMF diversity and functioning were not significantly different from those under ambient conditions. As a result, we conclude that mycorrhizal functioning may not be as profoundly affected by increasing atmospheric $[CO_2]$ as is generally reported.

The structure and functioning of communities and ecosystems will alter in response to environmental change, such as increasing atmospheric $[CO_2]$, warming and nitrogen enrichment. Determining the direction and magnitude of such responses is important because they are used to make decisions regarding current and future management of ecosystems[4]. Experimental efforts to determine the nature and magnitude of such responses typically use the abrupt step-increase approach. We cannot be certain that the differential responses to abrupt versus gradual increase in $[CO_2]$ detected here, with this model system, will also be observed in other ecosystems. However, given that mycorrhizal fungi are ubiquitous[11,12] and that their presence and composition in natural ecosystems can have profound effects on the structure and function of plant communities[21], the structure of higher trophic levels[22,23] and various ecosystem processes[24], there is little reason to think that such responses will be restricted to this model community. Also, the model system used here was fundamentally that of a plant–microbe trophic interaction. The observed responses were primarily a consequence of the sensitivity of certain AMF to a pulse of carbon at the start of the experiment. Will such a pulse also influence other interactions, such as plant–parasite and plant–insect interactions? At present, we cannot tell.

In conclusion, the abrupt approach resulted in a significant change in mycorrhizal diversity and functioning in the first generation, with little change after additional generations. It is not known whether the effects would be similar in an intact field experiment where fungal meta-community dynamics may come into play and mediate any local species extinctions. We therefore encourage research in other model systems and in intact ecosystems to quantify the proportion of observed responses that is a result of exposure to an abrupt increase in $[CO_2]$. Finally, our present experimental study arrives at evidence similar to the model predictions of ref. 3, in which plant carbon allocation would be overestimated using the abrupt approach. Both studies suggest that caution should be exercised in interpreting the magnitude of

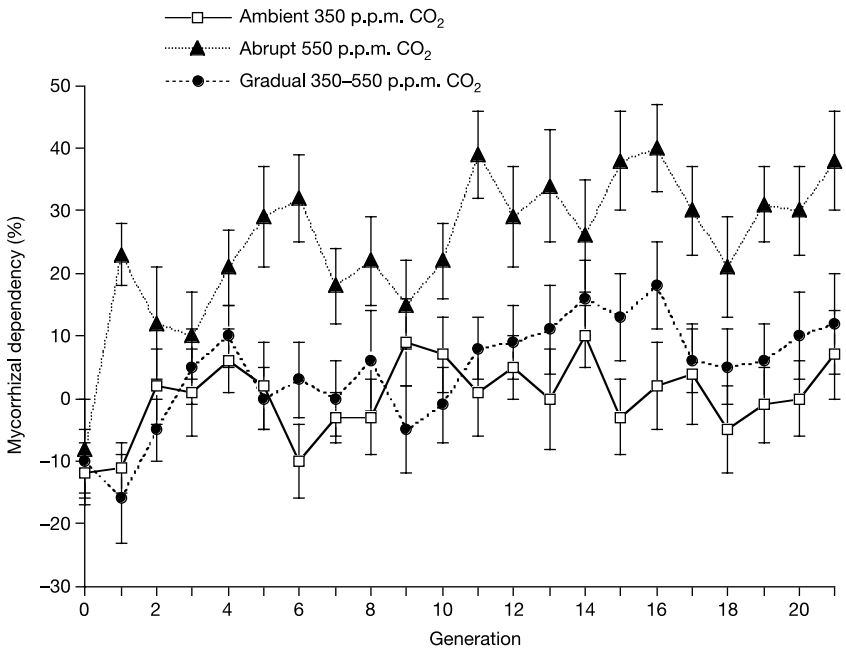

Figure 2 The effect of atmospheric $[CO_2]$ on mycorrhizal dependency of *B. inermis*. Points represent the mean and bars represent the standard error. Mycorrhizal dependency was similar before the treatments (generation 0). Overall, mean mycorrhizal dependency was significantly higher (repeated-measures ANOVA (Tukey), $P = 0.001$) in the abrupt compared with the ambient and gradual treatments. Mean mycorrhizal dependency did not differ between the latter two treatments (repeated-measures ANOVA (Tukey), $P = 0.71$). At generation 21, mycorrhizal dependency was significantly higher in the 'abrupt' treatment (ANOVA (Tukey), $P = 0.001$) but no difference was observed between ambient and gradual treatments (ANOVA (Tukey), $P = 0.53$).

responses to environmental changes that are significantly more abrupt than those that would occur in nature. □

Methods

Each experimental unit consisted of a single plant growing in an eight-inch pot containing field-collected soil. Plants were initially collected as seed from the field, germinated in the laboratory and added to the experimental units as one-week-old seedlings. After each 15-week growth period, plant shoots were removed and new seedlings were added to each pot. After the first generation, the source of seed was from plants of the previous generation. *B. inermis* is an obligate cross-pollinated plant. Some inbreeding can occur, but this results in seed that is significantly smaller and distorted. Therefore only out-crossed seed was used for the establishment of new generations.

The experiment consisted of 15 experimental units per treatment. The three [CO_2] treatments were imposed using nine environment-controlled growth chambers. Throughout the experiment, other environmental variables were recorded for each chamber, including air temperature, relative humidity and light intensity. Apart from [CO_2], we did not detect any significant difference in any measured environmental variable among treatments. Plants were randomly assigned to a different growth chamber after every five weeks, and the appropriate [CO_2] was re-set. Plants were watered (400 ml) on a weekly basis and fertilized with 250 ml of low-phosphorus, Long-Ashton solution every two weeks.

At harvest, soil was collected using a 10-cm diameter corer. This soil core was then used to trap AMF for determination of AMF species richness and mycorrhizal dependency. This was achieved by mixing the soil with silica sand, adding this mix to a new eight-inch pot, planting a new seedling of *B. inermis*, letting it grow for three months, cutting off the shoots, planting a new seedling and letting it grow for two more months, and finally collecting the resulting AMF spores from a 50-g core sample. A subsample of the spores (200 randomly chosen spores) was used for species identification. The remainder (average = 548 spores) were mixed into a sterile soil/sand medium in a new eight-inch pot that was then used to grow a seedling of *B. inermis* for 12 weeks. Mycorrhizal dependency was calculated as (the biomass of *B. inermis* grown with AMF minus the biomass of plants grown without AMF) divided by the biomass of *B. inermis* grown with AMF. Plant biomass was determined after drying at 60 °C for 36 h. For [P], plant shoot tissue was ashed at 500 °C for 4 h, dissolved in aqua regia and then determined using a mass spectrometer. Root lengths were determined using an image analysis system (WinRhizo, Regent Instruments Inc.). A sub-sample of roots was collected, cleaned of soil, stained with Chlorazol Black E[25] and assessed for mycorrhizal colonization[26]. The total length of fungal hyphae was determined after extraction from a subsample of soil using the filtration method[27]. The length of living hyphae belonging to each of the different genera was assessed using direct immunofluorescence[14]. Finally, as an overall assessment of soil aggregate size distribution we measured the MWD and WSA of the 1–2 mm aggregate size class using a wet-sieving protocol[28].

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Directed aerial descent in canopy ants

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Numerous non-flying arboreal vertebrates use controlled descent (either parachuting or gliding *sensu stricto*[1,2]) to avoid predation or to locate resources[3–7], and directional control during a jump or fall is thought to be an important stage in the evolution of flight[3,8,9]. Here we show that workers of the neotropical ant *Cephalotes atratus* L. (Hymenoptera: Formicidae) use directed aerial descent to return to their home tree trunk with >80% success during a fall. Videotaped falls reveal that *C. atratus* workers descend abdomen-first through steep glide trajectories at relatively high velocities; a field experiment shows that falling ants use visual cues to locate tree trunks before they hit the forest floor. Smaller workers of *C. atratus*, and smaller species of *Cephalotes* more generally, regain contact with their associated tree trunk over shorter vertical distances than do larger workers. Surveys of common arboreal ants suggest that directed descent occurs in most species of the tribe Cephalotini and arboreal Pseudomyrmecinae, but not in arboreal ponerimorphs or Dolichoderinae. This is the first study to document the mechanics and ecological relevance of this form of locomotion in the Earth's most diverse lineage, the insects.