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Soil fungal-arthropod responses to *Populus tremuloides* grown under enriched atmospheric CO_2 under field conditions

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Abstract

We investigated the influence of elevated CO_2 and soil N availability on the growth of arbuscular mycorrhizal and non-mycorrhizal fungi, and on the number of mycophagous soil microarthropods associated with the roots of *Populus tremuloides*. CO_2 concentration did not significantly affect percentage infection of *Populus* roots by mycorrhizal or non-mycorrhizal fungi. However, the extra-radical hyphal network was altered both qualitatively and quantitatively, and there was a strong interaction between CO_2 and soil N availability. Under N-poor soil conditions, elevated CO_2 stimulated hyphal length by arbuscular mycorrhizal fungi, but depressed growth by non-mycorrhizal fungi. There was no CO_2 effect at high N availability. High N availability stimulated growth by opportunistic saprobic/pathogenic fungi. Soil mites were not affected by any treatment, but collembolan numbers were positively correlated with the increase in non-mycorrhizal fungi. Results indicate a strong interaction between CO_2 concentration and soil N availability on mycorrhizal functioning and on fungal-based soil food webs.

Keywords: *arbuscular mycorrhiza,* global change, *Glomales,* microarthropod, soil fungi

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Introduction

Rhizosphere fungi are important regulators of plant productivity and of nutrient cycles in terrestrial ecosystems (Allen 1991). They are a diverse and ubiquitous set of heterotrophic microorganisms which can function as decomposers, pathogens, parasites, and mutualistic symbionts (Kendrick 1992), and as such they can provide both positive and negative feedback effects on plant growth. It is hypothesized that rhizosphere fungi will be greatly affected by an increasing atmospheric CO_2 concentration (Allen *et al*. 1995), since elevated CO_2 increases carbon allocation below-ground in the form of increased root growth and rhizodeposition (Zak *et al*. 1993; Rogers *et al*. 1994). As the soil microbial community is typically carbon-limited (Zak *et al*. 1994), an increase in substrate is expected to increase fungal activity and/or abundance. Also, since fungi are the main food source for many common soil invertebrates (Visser 1985) it is expected that elevated CO_2 will stimulate carbon cycling in below-ground food webs.

Various fungal groups do not exist independently in soil and when considering the enormous functional diversity within the fungal kingdom (Kendrick 1992), extrapolating behaviour of one fungus to the entire kingdom is misleading. For example, in a recent pot study, plant-mediated changes resulting from elevated atmospheric CO_2 did not affect total microbial biomass, but dramatically altered the balance between mycorrhizal and non-mycorrhizal fungi and bacteria (Klironomos *et al*. 1996). Although rhizosphere fungi have been previously studied in response to atmospheric CO_2 fertilization (see O'Neill 1994), most studies have focused on specific types of organisms under controlled conditions (either mycorrhizal symbionts or specific plant pathogens). Alternatively, total microbial biomass has been reported (Zak *et al*. 1993), but this measure lacks enough resolution to make any conclusions at the functional level.

Functionally and structurally, rhizosphere fungi can be

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divided into two groups: (i) mycorrhizal symbionts, which have direct access to plant photosynthates because of their intimate relationship with plant roots, and (ii) non-mycorrhizal opportunists, which have a looser structural relationship with roots, and thus a less direct access to plant-derived substrates. In this study, we grew *Populus tremuloides* (trembling aspen) saplings at ambient and twice-ambient atmospheric CO_2, and at two levels of N availability in a factorial experiment in the field. We investigated fungal and arthropod responses in the soil, to test the following hypotheses:

1 Percent root infection by mycorrhizal and non-mycorrhizal fungi will increase in response to elevated CO_2.

2 Extra-radical fungal growth by mycorrhizal and non-mycorrhizal fungi will increase in response to elevated CO_2.

3 A CO_2-induced increase in fungal standing crop will stimulate fungal-feeding animal populations in soil.

Methods

Our study was located at the University of Michigan Biological Station, near Pellston, Michigan, USA (45°34′ N, 84°40′ W). Eight open-top chambers (Curtis & Teeri 1992) were used for each of the four treatments of a 2 × 2 factorial experiment (total 32 chambers). The two factors were atmospheric CO_2 (350 ppm and 700 ppm) and soil N availability (high and low). Saplings were grown in open-bottom root boxes from cuttings (Zak *et al*. 1993). Two soil fertility levels were established by filling the boxes with either 100% locally excavated Kalkaska series topsoil (Typic haplorthod, high N treatment) or a homogenized mixture of 20% topsoil, and 80% native Rubicon sand (Entic Haplorthod, low treatment). Net N mineralization was significantly higher in the high N treatment (348 μg N $g^{-1}d^{-1}$) than in the low-N treatment (45 μg N $g^{-1}d^{-1}$) (Pregitzer *et al*. 1995). These rates of N mineralization are typical of the range that occurs in the upper Great Lakes forest ecosystems (Zak & Pregitzer 1990).

In July 1995, after the treatments were running for 14 months, two soil cores (20 cm deep × 5 cm diameter) were taken from each chamber, one for fungal parameters and the other for invertebrates.

Extra-radical fungal standing crop

Total hyphal lengths were estimated by extracting hyphae from the two 5-g portions of soil (Miller *et al*. 1995) and measuring lengths by a gridline-intersect method. Hyphal length (m g^{-1} dry soil) was calculated as in Newman (1966). The hyphae of non-mycorrhizal fungi were distinguished from those of arbuscular mycorrhizal fungi by careful observation of characters normally missing in the latter, such as melanization, clamp connections or regularly septate hyphae. Spore abundance was calculated directly by extracting arbuscular mycorrhizal fungal spores from the 10 grams of soil using a wet-sieving technique (Klironomos *et al*. 1993). Spores were identified to genus under a dissecting microscope.

Intra-radical infection

Fine roots were obtained by seiving each core through a 75-μm screen, and subsequently stored in FAA for at least 24 h. Roots were then cleared by autoclaving for 15 min in 10% potassium hydroxide, acidified in formalin acetic acid alcohol for 5 min and stained using Chlorazol Black E (Brundrett *et al*. 1984). Fungal infection was quantified using the magnified-intersections method (McGonigle *et al*. 1990) by inspecting intersections between the microscope eyepiece cross-hair and roots at 200x magnification. The proportion of root length containing arbuscules, vesicles and hyphae was determined. The hyphae of non-mycorrhizal fungi were distinguished from those of AM as described above for extra-radical hyphae.

Arthropods

Mites and collembolans were extracted onto dishes containing picric acid, using a high efficiency canister-type soil arthropod extractor (Lussenhop 1971). They were sorted and stored in 70% ethanol. The mites were further divided into predators and microbe-feeders, using mouthpart morphologies (Dindal 1990). It is not an easy task to further differentiate the microbe-feeders into fungal- and bacterial-, so they were lumped into one category.

Statistical analyses

A 2 × 2 multivariate analysis of variance (MANOVA) with the use of Wilk's criterion was performed on the dependent variables (infection by arbuscules, vesicles, mycorrhizal hyphae, clamped hyphae, other hyphae; extra-radical hyphal length of mycorrhizal, clamped and other soil fungi; spore numbers by *Glomus* and *Acaulospora* spp.; and numbers of collembolans, microbial-feeding mites and predatory mites. Root infection and arthropod variables were log transformed prior to statistical analysis. All variables were also analysed further using univariate factorial ANOVA to help determine which variables contribute to any significant differences observed in the multivariate analyses. The Tukey post-hoc test was subsequently used to test differences among treatment means.

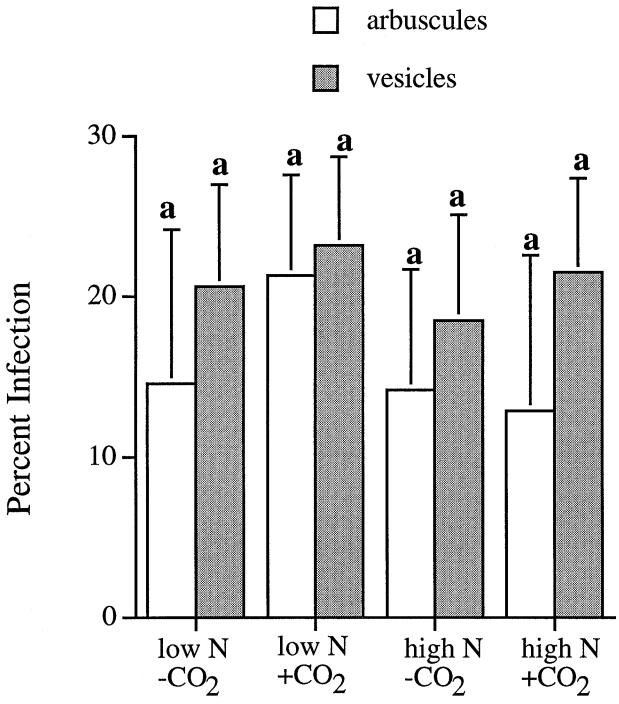

Fig. 1 Effects of elevated atmospheric CO_2 and soil N availability on arbuscular and vesicular infection of *Populus tremuloides* roots. Values are the mean + 1 SE. The letter 'a' represents no differences at the significance level $P < 0.05$.

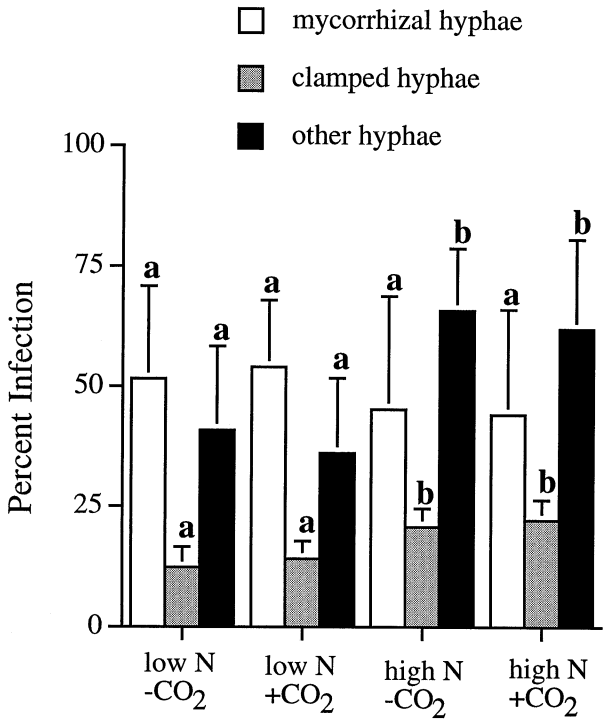

Fig. 2 Effects of elevated atmospheric CO_2 and soil N availability on the hyphal infection of *Populus tremuloides* roots. Values are the mean + 1 SE. Different letters (a,b) within dependent variables represent differences at the significance level $P < 0.05$.

Results

The combined dependent variables were significantly affected by both CO_2 (MANOVA, $P < 0.001$) and N availability (MANOVA, $P < 0.0001$) and by their interaction ($P < 0.01$). Percentage infection of *P. tremuloides* roots by mycorrhizal fungi (Figs 1, 2), in the form of the proportion of root length containing arbuscules, vesicles, and mycorrhizal hyphae, was not significantly altered by CO_2 or N availability. However, infection of the roots by basidiomycetous hyphae (with clamp connections) and other hyphae did change, although it did in response to N availability (Fig. 2). Percentage infection by hyphae with clamp connections increased 61% ($P < 0.001$), and infection by other non-mycorrhizal fungi increased by 66% ($P < 0.01$) in that high N availability soil.

With the extra-radical network, however, the results were very different (Fig. 3). In the low N soil, mycorrhizal fungi responded positively to increased CO_2. Hyphal length of arbuscular mycorrhizal fungi was significantly higher (77%, $P < 0.001$) in elevated CO_2, whereas clamped fungi and other non-mycorrhizal fungi showed decreased hyphal lengths (-75%, $P < 0.001$ and – 78%, $P < 0.001$, respectively). High N availability had a negative effect on mycorrhizal hyphal length (–71%, $P < 0.001$), whereas the hyphal length of clamped hyphae (220%, $P < 0.001$) and other hyphae (177%, $P < 0.001$) increased. Mycorrhizal fungi belonging to *Glomus* spp. also sporulated in greater amounts (Fig. 4) under high soil N ($P < 0.05$). There was no significant CO_2 effect under low or high N conditions ($P > 0.05$).

Soil arthropods were also affected by atmospheric CO_2 and soil N availability (Fig. 5). More collembolans were found under high N conditions ($P < 0.01$). Under low N conditions, collembolans were negatively affected by elevated CO_2 ($P < 0.05$), but CO_2 had no effect at high N conditions ($P > 0.05$). Microbial-feeding and predatory mites were not affected by any treatment ($P > 0.05$).

Discussion

Our data illustrate that mycorrhizal and non-mycorrhizal fungi associated with plant roots can be greatly affected by atmospheric CO_2 fertilization, but the extent and direction of this response depends on soil N availability. Overall, carbon flow within the plant–soil system was shifted to a more mutualistic-closed, mycorrhizal dominated food web under high CO_2 and low N availability, and to a more opportunistic-open, saprobe/pathogen

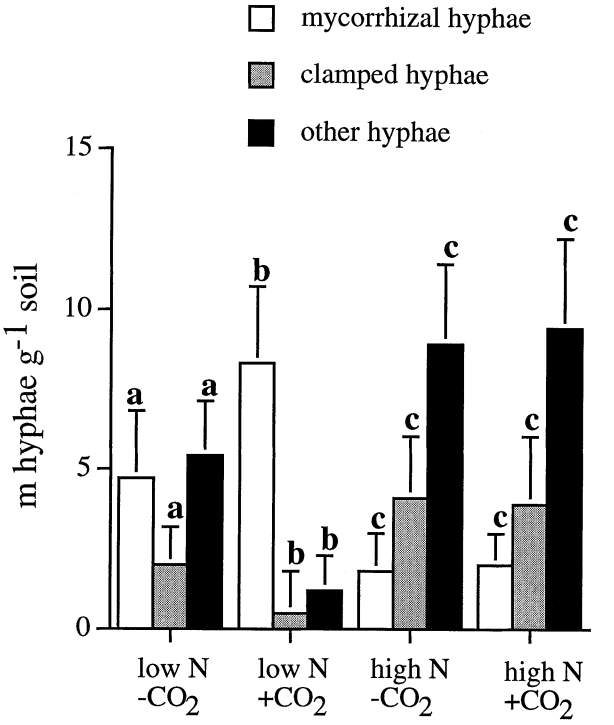

Fig. 3 Effects of elevated atmospheric CO_2 and soil N availability on the extra-radical hyphal length. Values are the mean + 1 SE. Different letters (a,b,c) within dependent variables represent differences at the significance level $P < 0.05$.

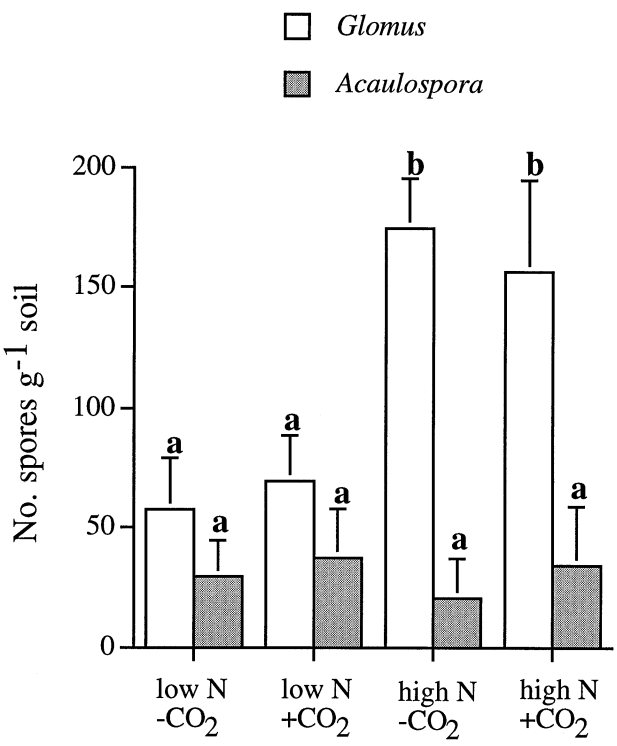

Fig. 4 Effects of elevated atmospheric CO_2 and soil N availability on arbuscular mycorrhizal spore production. Values are the mean + 1 SE. Different letters (a,b) within dependent variables represent differences at the significance level $P < 0.05$.

dominated one under high N availability. Though percentage root infection did not change with the treatments, fungal responses in the soil around *Populus* roots responded dramatically. Extra-radical mycorrhizal hyphae were stimulated under combined high CO_2 and low N availability, but decreased sharply under high N availability. The opposite trend was found for non-mycorrhizal fungi, as they were less abundant under a combination of high CO_2 and low N availability, and were most abundant under high N availability.

The strong interactive effects between CO_2 and soil N may explain the disparity in results from other CO_2 fertilization experiments on arbuscular mycorrhizae. Experiments have been carried out on trees (O'Neill *et al.* 1991), shrubs (Klironomos *et al.* 1996), crops (Rogers *et al.* 1992; Runion *et al.* 1994), and grasses (Whitbeck 1993; Monz *et al.* 1994), growing under very different conditions. Only Monz *et al.* (1994) working with *Bouteloua gracilis*, and Klironomos *et al.* (1996) with *Artemisia tridentata* showed increases in percentage infection by mycorrhizal structures in response to elevated CO_2. Both studies demonstrate that CO_2 fertilization interacted strongly with other factors; in the former it was temperature and water availability, and in the latter it was soil nutrient availability. These studies, as well as the present one, suggest that fungal communities will respond very differently to elevated atmospheric CO_2 depending on the status of other environmental parameters. Also, because of these strong interactive effects, changes in other biogeochemical cycles, in particular nitrogen and water, need to be considered within CO_2 research. Global change is not a single-factor phenomenon, so the study of interactions of changing parameters, such as in this study, will be very valuable for predicting the effects of global change.

Our results also illustrate the importance of considering the entire life-cycle of arbuscular mycorrhizal fungi in experimental studies. Intra-radical infection is only one phase of these fungal mutualists. The other component, the extra-radical phase, which is most often neglected in mycorrhizal research, extends hyphae away from the plant root which branch profusely and penetrate tiny soil crevices (Friese & Allen 1991). In the past, too much emphasis has been placed on root infection and not enough on other manifestations of arbuscular mycorrhizal fungi. These extra-radical hyphae have a direct pipeline

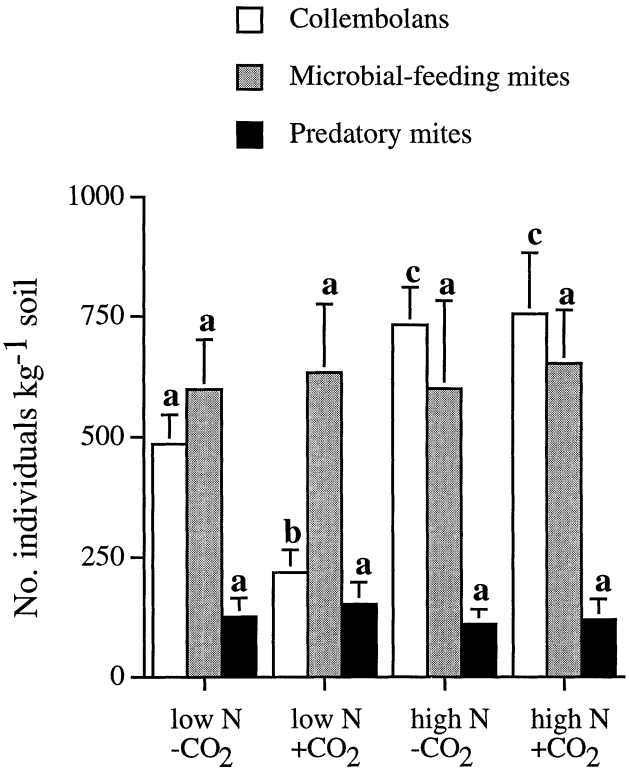

Fig. 5 Effects of elevated atmospheric CO_2 and soil N availability on soil animal densities. Values are the mean + 1 SE. Different letters (a,b,c) within dependent variables represent differences at the significance level $P < 0.05$.

to the plant and transfer nutrients directly to their host (Allen 1991). Functionally, they act more as an extension to the root system than as part of the remaining 'microbial biomass.' In this study, arbuscular mycorrhizal fungi under low N conditions, added more energy into this extra-radical 'pipeline', an effect which was further stimulated by an increase in atmospheric CO_2. This indicated a shift towards increased production of extra-radical fungal mass per volume soil. The implications of this activity are interesting, especially in soils with low N and P availability, and where mycorrhizal activity is high, which was the case here. By immobilizing N and P, mycorrhizal fungi may reduce the availability of those nutrients so that the N and P limits growth of saprophytic microbes and thereby retards decomposition. When soil N was high, it was the non-mycorrhizal opportunistic fungi which were favoured. This trend is not surprising since mycorrhizal associations are known to better function under low soil nutrient conditions (Harley & Smith 1983; Allen 1991).

Mycorrhizal fungi can utilize from 5 to 85% of the host plant's photosynthate (Allen 1991). Thus changes in the amount of mycorrhizal activity has a great potential to affect C sink-source relationships in plants. Increased arbuscular mycorrhizal fungal biomass per volume soil in the high CO_2, low N availability treatment is evidence for an increased sink activity of the fungal symbiont. This may relieve possible negative feedbacks as a result of inhibition of photosynthesis by sink-limited host plants growing under elevated atmospheric CO_2 (Bazzaz 1990; Strain & Thomas 1995). Since hyphae may compete for nutrients with both host roots and rhizosphere soil microorganisms (Azcon-Aguilar & Barea 1992; Jakobsen *et al*. 1994), the plant nutritional implications of an increased hyphal density are not clear. Also, it is unlikely that all fungal species are equally efficient in nutrient acquisition and transport (Jakobsen 1995; Ravnskov & Jakobsen 1995). Since there may have been changes in the mycorrhizal fungal community (see Fig. 4), hyphal length alone cannot be directly extrapolated to changes in symbiotic nutrient translocation. However, it is likely that an increase in plant nutrition did result, given the very clear shift in fungal functional groups in the soil.

Quantitative and qualitative shifts in fungal biomass can alter soil food-web dynamics. Various soil animals are mycophagous and feed on fast-growing saprophytic and pathogenic fungi (Visser 1985). In this study collembolan populations were stimulated under combined elevated CO_2 and high soil N. Non-mycorrhizal fungal hyphal length was more abundant under this treatment also, and it is this fungal group that has been previously shown to be more palatable and nutritious, compared to arbuscular mycorrhizal fungi (Klironomos & Kendrick 1995; Klironomos & Kendrick 1996) Mite populations were not found to be affected in this study, but it is possible that populations were affected at the species level, or at higher resolution functional groups, but were not detected at the resolution used here.

In conclusion, this study presents field evidence for a strong interaction between atmospheric CO_2 concentration and soil N availability on mycorrhizal standing crop and on the fungal-based soil food web. The trends are similar to those presented by Klironomos *et al*. (1996), even though that was a pot-study and involved organisms adapted to a semi-arid, mediterranean-type ecosystem. Under N-poor soil conditions, elevated CO_2 stimulated the mycorrhizal-based soil food web, whereas at high N availability it was the opportunistic-saprobic/pathogenic fungus-based food web which was stimulated.

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