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The response of mycorrhizal colonization to elevated CO_2 and climate change in *Pascopyrum smithii* and *Bouteloua gracilis*

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Abstract

Large intact soil cores of nearly pure stands of *Pascopyrum smithii* (western wheatgrass, C_3) and *Bouteloua gracilis* (blue grama, C_4) were extracted from the Central Plains Experimental Range in northeastern Colorado, USA and transferred to controlled environment chambers. Cores were exposed to a variety of water, temperature and CO_2 regimes for a total of four annual growth cycles. Root subsamples were harvested after the completion of the second and fourth growth cycles at a time corresponding to late winter, and were examined microscopically for the presence of mycorrhizae. After two growth cycles in the growth chambers, 54% of the root length was colonized in *P. smithii*, compared to 35% in blue grama. Field control plants had significantly lower colonization. Elevation of CO_2 increased mycorrhizal colonization in *B. gracilis* by 46% but had no effect in *P. smithii*. Temperatures 4°C higher than normal decreased colonization in *P. smithii* by 15%. Increased annual precipitation decreased colonization in both species. Simulated climate change conditions of elevated CO_2, elevated temperature and lowered precipitation decreased colonization in *P. smithii* but had less effect on *B. gracilis*. After four growth cycles in *P. smithii*, trends of treatments remained similar, but overall colonization rate decreased.

Introduction

Extensive research using crop species has shown a number of plant responses to elevated CO_2, with increases in photosynthetic rate and growth being common (Allen, 1990; Bazzaz, 1990). These responses are species specific, are dependent on environmental factors, and have been studied primarily in systems in which plant resources are not limiting (Bazzaz, 1990). Currently, there is great interest in examining the effects of elevated CO_2 and predicted climate change on native systems in which water and nutrients are more limiting. Mechanisms may be present that would enable native species to respond to elevated CO_2 in spite of these limiting conditions (Luxmore, 1981). Strain and Bazzaz (1983) suggest that the high availability of photosynthate resulting from elevated CO_2 increases root growth and root exudation. In turn, plant nutrition is affected by increasing root density and mycorrhizal colonization. In recent experimental work, total biomass, root biomass, and ectomycorrhizal colonization of *Pinus echinata* and *Quercus alba* seedlings increased under elevated CO_2 in unfertilized forest soil (O'Neill et al., 1987).

The objective of this study was to investigate the response of vesicular-arbuscular mycorrhiza (VAM) formation in a C_3 and a C_4 native perennial grass subjected to elevated CO_2 and simulated climate change. We know of no reports of the VAM response to atmospheric CO_2 in intact native grasses. In addition, examination of the interacting and possibly offsetting effects of CO_2, temperature and precipitation is important in predicting the response to potential climate change.

Materials and methods

Plant material

Intact soil cores, 24 cm (w) × 45 cm (h), were collected from the Central Plains Experimental Range (CPER), approximately 12 km north of Nunn, Colorado, USA (40°42' north, 104°42' west). Two soil-plant associations were sampled in late May 1989; one a sandy clay loam dominated by *Pascopyrum smithii* (western wheatgrass, C_3) and the other a sandy loam dominated by *Bouteloua gracilis* (blue grama, C_4). The two associations were located on the same site approximately 150 m apart within an ungrazed enclosure. Soil cores were obtained by driving steel irrigation pipe (25.4 cm × 45.7 cm) into the sod with a backhoe and then removing the entire intact soil core in the pipe. Sods were retained within the irrigation pipe throughout the experiment.

Experimental design

The field design consisted of 8 field blocks with 24 cores each for each soil-plant association. A total of 456 cores were removed and transported by refrigerated truck to the Duke University Phytotron (Durham, NC, USA). All cores were maintained at 2–5°C during transit and arrived at the Phytotron with minimal disturbance. Cores were randomly assigned to treatment combinations within the 8 replicate blocks, in a complete factorial design consisting of the two plant species, two CO_2 levels (350 and 700 μL L^{-1}), two temperature regimes (field average and 4°C elevated), three precipitation regimes (15 cm, 25 cm and 36 cm per year), and two destructive harvest dates (after 2 and 4 growth cycles) totaling forty-eight treatment combinations. In the field, one control cylinder was driven and left in place and one non-cylinder control plot was designated in each block for each of the two destructive harvest dates.

This design occupied 4 large environmental chambers, each regulated for CO_2 and temperature regime with an average light intensity of 550 μE m^{-2} s^{-1} at core-top level. Precipitation regimes approximated seasonal patterns, with applications of distilled water in 2.5 cm events. The 25 cm precipitation treatment was selected to approximate field production levels. Total seasonal precipitation was controlled by the number of events. Growing season temperature regime and photoperiod closely followed field seasonal and diurnal patterns and were adjusted weekly to correspond to weekly field averages. Temperature maximum of each season was reached on simulated week 29 with highs of 31°C and lows of 13°C (field average treatment) while the photoperiod maximum of 15 h 8 min. was reached on simulated week 25. Late fall and winter seasons were shortened by thirteen weeks, compared with the field, with a temperature minimum of 3°C (no diurnal fluctuation in winter) and a photoperiod minimum of 9 h 13 min. The positions of all cores within chambers were rerandomized three times per season.

Sampling for mycorrhizae

After two and four growth cycles, three small soil cores (2.0 × 10.0 cm) were taken along a random transect within each of the larger cores. Field samples for both cylinder and non-cylinder control plots were taken after two seasons only. Soil was then passed through a 2 mm sieve, with the majority of the plant material (mainly roots with minimal litter) being retained on the sieve. Subsamples of fine roots ($\leq$ 0.5 mm diameter) were handpicked from the material retained on the sieve for mycorrhizal counts. Any obvious weed and senescent roots were eliminated. Roots were placed in a formalin acetic acid preservative solution (1 part glacial acetic acid, 6 parts 40% formalin, 20 parts 95% Et OH, and 40 parts DI H_2O, by volume) until staining. Root cytoplasm was cleared using a 10% KOH solution at 90°C for 90 minutes, and mycorrhizae were stained with 0.01% trypan blue. Mycorrhizal colonization was assayed with the gridline intercept method (Giovanetti and Mosse, 1980) under a dissecting microscope at 40–63×. Occasional verification of particular intercepts was made with a Zeiss compound scope at 200×. Intercept points were categorized as to the relative degree of colonization (heavy or light) and senescent roots (without cortexes) were counted to correct percent colonization to a live root basis. Vesicles and arbuscules were noted where present.

Statistical analysis was performed with Systat software (Wilkenson, 1989). Bartlet's test for homogeneity of group variances was employed throughout and arcsine transformations and Kruskal-Wallis nonparametric tests were utilized to supplement original ANOVA results where applicable.

Results

After two growth cycles under controlled conditions, both species showed high VAM colonization: 54%

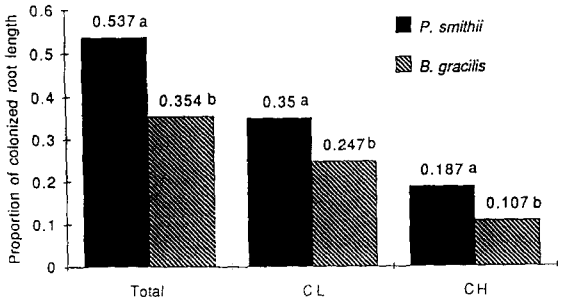

Fig. 1. Comparison of the proportion of the root length colonized by mycorrhizal fungi after two growth cycles in *Pascopyrum smithii* and *Bouteloua gracilis*. Categories are total colonization, lightly colonized (CL), and heavily colonized (CH). Values are means across all treatments. Within infection categories, means followed by the same letters are not significantly different at $p < 0.05$.

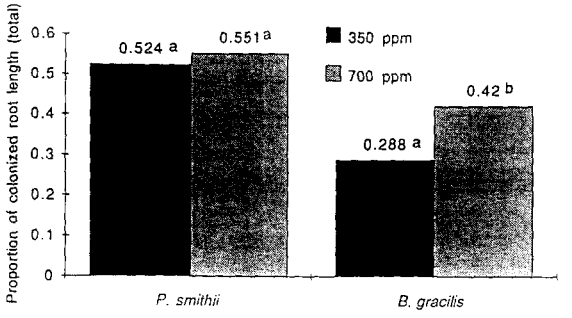

Fig. 2. Effect of atmospheric CO_2 on the proportion of the root length colonized by mycorrhizal fungi in *P. smithii* and *B. gracilis* after two growth cycles. Values are means. Within species, means followed by the same letters are not significantly different at $p < 0.05$.

Table 1. The effects of CO_2, precipitation, temperature, and climate change on mycorrhizal colonization in *Pascopyrum smithii* after two and four growth cycles. Values are means of the total proportion of the root length colonized. Within harvest date and treatment factor, means followed by the same letter are not significantly different at $p < 0.05$. For the climate change comparison, current ambient = 350 μL L^{-1} CO_2, field average temperature, and 25 cm yr^{-1} precipitation; climate change = 700 μL L^{-1} CO_2, 4°C elevated temperature, and 15 cm yr^{-1} precipitation

Treatment	Harvest date	
	2 years	4 years
CO_2		
350 μL L^{-1}	0.524a	0.259a
700 μL L^{-1}	0.551a	0.230a
Temperature		
Field average	0.698a	0.273a
4°C elevated	0.517b	0.216b
Precipitation (cm)		
15	0.558a	0.197b
25	0.623a	0.294a
36	0.392b	0.244a
Climate change comparison		
Current ambient	0.734a	0.330a
Climate change	0.512b	0.150b

of the subsampled root length was colonized in *P. smithii* and 35% in *B. gracilis* (Fig. 1). Species differences remained similar regardless of the degree of colonization (i.e., heavy or light). Field samples were less colonized than the 'current ambient' conditions simulated in the chambers, with colonization at 12% in *B. gracilis* and 9% in *P. smithii* in the field, and 39% in *B. gracilis* and 74% in *P. smithii* in the chambers. Hyphae were the predominant infective structure observed, with vesicles being rare and mature arbuscules being absent regardless of treatment and harvest date. ANOVA indicated a significant stimulation of colonization by elevated CO_2 in *B. gracilis* after two growth cycles ($p < 0.05$, species × CO_2 interaction $p = 0.077$, Fig. 2) but no effect on *P. smithii* after two or four years (Table 1, Fig. 2).

After two growth cycles, plants grown under the high precipitation treatment had significantly lower colonization than the plants grown under normal and low precipitation treatments (Fig. 3a). Here, the species × precipitation interaction was not significant ($p = 0.55$) and therefore means were averaged across species. Lower than normal precipitation had little effect on colonization of either species. After four growth cycles in *P. smithii*, however, this trend was not consistent, with the low precipitation treatment having significantly lower colonization (Table 1).

Higher temperatures had no effect on VAM colonization in *B. gracilis* but decreased colonization in *P. smithii* after both two and four growth cycles (Fig. 3b, Table 1). The climate change scenario of elevated CO_2, elevated temperature, and decreased precipitation resulted in a significant decrease in colonization in *P. smithii* after two and four growth cycles (Fig. 4, Table 1). In contrast, climate change led to increased colonization in *B. gracilis* (Fig. 4). The effects of climate change are compatible with the main effects, since colonization in *B. gracilis* increased with elevated CO_2 and did not respond to temperature or reduction

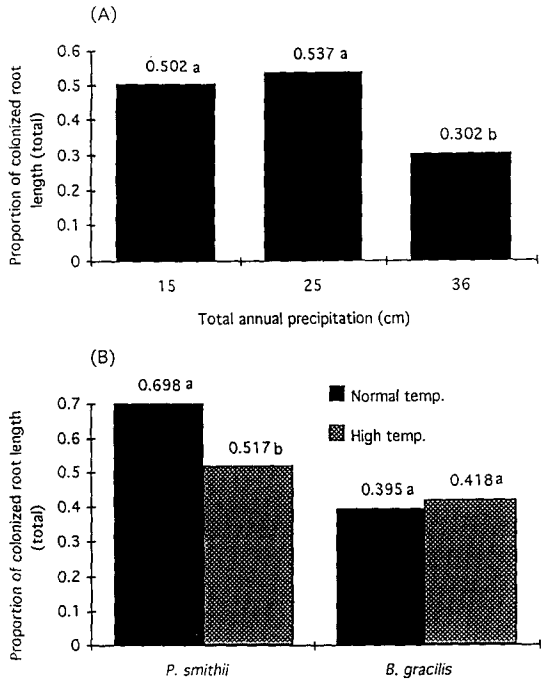

Fig. 3. Effects of annual precipitation and seasonal temperature regime on the proportion of root length colonized by mycorrhizal fungi after two growth cycles. Annual precipitation (**A**) is averaged across both species while seasonal temperature regime (**B**) is by species. Values are means. Within species and between precipitation levels, means followed by the same letter are not significantly different at $p < 0.05$.

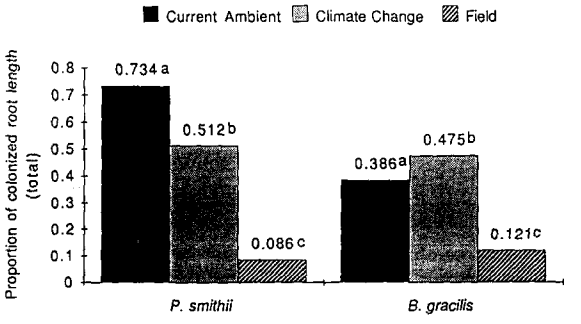

Fig. 4. Comparison of possible climate change effects on mycorrhizal colonization in *P. smithii* and *B. gracilis* after two growth cycles. 'Current ambient' = growth chamber conditions designed to mimic field levels of CO_2, temperature and precipitation; 'Climate Change' = Elevated CO_2, elevated temperature, and reduced precipitation; 'Field' = samples obtained from field control plots. Within species, means followed by the same letters are not significantly different at $p < 0.05$.

of precipitation from 25 to 15 cm, whereas colonization in *P. smithii* either decreased or did not respond to these same factors.

Discussion

C_4 grasses are usually considered more likely to be mycorrhizal dependent than C_3 grasses (Hetrick et al., 1990). However, greater colonization in *P. smithii* (C_3) in chamber-grown plants may indicate greater mycorrhizal dependence than in *B. gracilis* (C_4) (Graham et al., 1991). Although *B. gracilis* had a fairly high colonization rate, there is some evidence that mycorrhizae are parasitic rather than mutualistic in this C_4 species (Hayes et al., 1982). In field samples, *B. gracilis* exhibited slightly higher colonization than *P. smithii*, and overall colonization was significantly lower than in the chamber grown plants. These field levels appear lower than have been previously reported for these species (Allen et al., 1984; Davidson and Christensen, 1977) but direct comparisons are difficult due to differences in time of sampling and enumeration methodology.

Despite C_4 photosynthetic physiology, *B. gracilis* has been shown to respond to elevated CO_2 with enhanced photosynthesis (Morgan et al., 1991) and growth (Hunt et al., 1990; Reichers and Strain, 1988). In this species, increased carbon availability from the host plant may play a significant role in stimulating mycorrhizal colonization. Primary production of both species apparently responded to elevated CO_2, with *B. gracilis* yielding 19% and *P. smithii* 14% greater plant size after two seasons under elevated CO_2 (Hunt et al., 1992). *P. smithii* is also photosynthetically responsive to elevated CO_2 (Morgan et al., 1991) but mycorrhizal colonization was not significantly affected.

Mycorrhizae have been shown to play a role in the nitrogen uptake of some plants (Ames et al., 1983; Jakobsen and Jensen, 1992; Raven et al., 1978; Van Kessel et al., 1985) and N nutrition may be a more important factor in this system which appears to be limited by nitrogen and not phosphorus (Clark et al., 1980). After two growth cycles, colonization was negatively correlated with nitrogen concentration of the shoot ($r = -0.494$, $p < 0.001$) across all treatments, which may indicate the formation of additional mycorrhizae under nitrogen limited conditions.

Hunt et al. (1992) reported a significant increase in root nitrogen with elevated temperature in *P. smithii* but not in *B. gracilis*, a difference that may be related to photosynthetic temperature optima in *P. smithii*, a cool season grass and *B. gracilis*, a warm season grass (Monson et al., 1986). In *B. gracilis*, both plant growth and N mineralization increased with temperature, with little net change in degree of N limitation (Hunt et

al., 1992). In *P. smithii*, growth showed no response to temperature, perhaps because of a lower optimum temperature for photosynthesis, while mineralization still increased. Therefore, in *P. smithii*, the adverse direct effect of elevated temperature may have been offset by reduced N limitation, and mycorrhizal colonization could be responding to this lack of a nitrogen stress.

Elevated soil water has been shown to decrease mycorrhizal colonization in some plants such as sorghum (Sieverding, 1981) perhaps due to the limitation of oxygen diffusion to the rhizosphere. In addition, simulation modeling of temperate grassland systems (Hunt et al., 1991) has suggested decreases in colonization with increased precipitation. Reid (1984) suggested this is possibly due to a decrease in the advantage of mycorrhizae over roots in nutrient uptake in wetter soil. On the other hand, N limitation would be expected to be greater with greater precipitation, as evidenced by lower plant N concentration in the high water treatment (Hunt et al., in prep), which should lead to greater VAM colonization. Our observations are inconsistent in this regard with elevated precipitation yielding lower colonization after two years in both species and decreased precipitation yielding lower colonization after 4 years in *P. smithii*.

Colonization rates declined after four growth cycles in *P. smithii* in all treatments. This may have resulted from a pulse of decomposition and subsequent release of nitrogen stress in the system, an hypothesis that we can test with data forthcoming.

Our chamber grown plants exhibited higher colonization than found in the field. Compared to the field, the chambers had lower light intensity, less extreme temperatures with no freezing, and higher relative humidity. Total plant growth in the field was comparable with the reduced precipitation treatment in the chambers, indicating a decrease in water stress in the chambers. These conditions somehow favored VAM development, perhaps by increasing nutrient stress or carbon availability from the host plant.

Currently, there is little information on the ecophysiology of the mycorrhizal symbiosis in these species. There is some evidence that mycorrhizae can be parasitic in *B. gracilis* (Hayes, 1982), but there are no data in this regard for *P. smithii*. Consequently, it is difficult to infer specific response mechanisms from these data. In addition, we know of no published data on the response of a parasitic symbiosis to elevated CO_2.

In conclusion, our results indicate several important mycorrhizal responses to elevated CO_2 and climate change in these grasses: colonization in intact native sods of *B. gracilis* was stimulated by elevated CO_2, alterations of annual precipitation yielded inconclusive results, and elevation of temperature yielded lower colonization in *P. smithii*. We plan to undertake a thorough analysis of the nutrient and production dynamics of this system with data forthcoming in an attempt to further interpret all of these responses. In addition, further work on the nature of the symbiosis in these grasses (i.e., parasitism or mutualism?) may assist in interpreting these results mechanistically.

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References

Allen L H 1990 Plant responses to rising carbon dioxide and potential interactions with air pollutants. J. Environ. Qual. 19, 15–34.

Allen M F, Allen E B and Stahl P D 1984 Differential niche response of *Bouteloua gracilis* and *Pascopyrum smithii* to VA mycorrhizae. Bull. Torrey Bot. Club 111, 361–365.

Ames R N, Reid C P P, Porter L K and Cambardella C 1983 Hyphal uptake and transport of nitrogen from 2 ^{15}N-labeled sources by *Glomus mosseae*, a vesicular-arbuscular mycorrhizal fungus. New Phytol. 95, 381–396.

Davidson D E and Christensen M 1977 Root-microfungal and mycorrhizal associations in a shortgrass prairie. *In* The Belowground Ecosystem: A Synthesis of Plant-associated Processes. Ed. J K Marshall. pp 279–287. Range Science Department Series No. 26. Colorado State University, Fort Collins.

Bazzaz F A 1990 The response of natural systems to the rising of global CO_2. Annu. Rev. Ecol. Syst. 21, 167–196.

Clark F E, Cole C V and Bowman R A 1980 Nutrient Cycling. *In* Grasslands, Systems Analysis and Man. Eds. A J Breymeyer and G M Van Dyne. pp 659–712. International Biological Programme, Cambridge University Press, Cambridge.

Giovanetti M and Mosse B 1980 An evaluation of the techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol. 84, 489–500.

Graham J H, Eissenstat D M and Drouillard D L 1991 On the relationship between plant mycorrhizal dependency and rate of vesicular-arbuscular mycorrhizal colonization. Functional Ecol. 5, 773–779.

Hayes R, Reid C P P, St. John T V and Coleman D C 1982 Effects of nitrogen and phosphorus on blue grama growth and mycorrhizal infection. Oecologia 54, 260–265.

Hetrick B A D, Wilson G W T and Todd T C 1990 Differential responses of C_3 and C_4 grasses to mycorrhizal symbiosis, phosphorus fertilization, and soil microorganisms. Can. J. Bot. 68, 461–464.

Hunt H W, Detling J K, Elliott E T, Monz C A and Strain B R 1990 The effects of elevated CO_2 and climate change on grasslands. I. Response of aboveground primary production in intact sods of native shortgrass prairie. Bull. Ecol. Soc. Am. 71,196.

Hunt H W, Trlica M J, Redente E F, Moore J C, Detling J K, Kittel T G F, Walter D E, Fowler M C, Klein D A and Elliott E T 1991 Simulation model for the effects of climate change on temperate grassland ecosystems. Ecol. Model. 53, 205–246.

Hunt H W, Elliott E T, Detling J K, Monz C A and Reuss D E 1992 Plant size and shoot to root ratio in intact sods of native shortgrass prairie exposed to elevated CO_2 for two growing seasons. Bull. Ecol. Soc. Am. 73, 254.

Jakobsen J I and Jensen E S 1992 Hyphal transport of ^{15}N-labeled nitrogen by a vesicular-arbuscular mycorrhizal fungus and its effect on depletion of inorganic soil N. New Phytol. 122, 281–288.

Luxmore R J 1981 CO_2 and phytomass. Bioscience 31, 626.

Monson R K, Sackschewsky M R and Williams III G J 1986 Field measurements of photosynthesis, water-use efficiency and growth in *Agropyron smithii* (C_3) and *Bouteloua gracilis* (C_4) in the Colorado shortgrass steppe. Oecologia 68, 400–409.

Morgan J, Hunt H W, Monz C A, LeCain D and Detling J K 1991 The effects of elevated CO_2 and climate change on the photosynthetic response of *Agropyron smithii* and *Bouteloua gracilis*. Bull. Ecol. Soc. Am. 72, 200.

O'Neill E G, Luxmore R J and Norby R J 1987 Increases in mycorrhizal colonization and seedling growth in *Pinus echinata* and *Quercus alba* in an enriched CO_2 atmosphere. Can. J. Forest Res. 17, 878–883.

Raven J A, Smith S E and Smith F A 1978 Ammonium assimilation and the role of mycorrhizas in climax communities in Scotland. Trans. Bot. Soc. Edinburgh 43, 27–35.

Reichers G H and Strain B R 1988 Growth of blue grama (*Bouteloua gracilis*) in response to atmospheric CO_2 enrichment. Can. J. Bot. 66, 1570–1573.

Reid C P P 1984 Mycorrhizae: A root-soil interface in plant nutrition. *In* Microbial-Plant Interactions. pp 29–50. Soil Science Society of America, Madison.

Sieverding E 1981 Influence of soil water regimes on VA-mycorrhiza. I. Effect on plant growth, water utilization, and development of mycorrhiza. J. Agron. Crop. Sci. 150, 400–411.

Strain B R and Bazzaz F A 1983 Terrestrial plant communities. *In* CO_2 and Plants: The Response of Plants to Rising Levels of Carbon Dioxide. Ed. E R Lemon. pp 177–222. Westview, Boulder.

Van Kessel C, Singleton P W and Hoben H J 1985 Enhanced N-transfer from a soybean to maize by vesicular arbuscular mycorrhizal (VAM) fungi. Plant Physiol. 79, 562–563.

Wilkenson L 1989 SYSTAT: The system for statistics. SYSTAT, Inc., Evanston. 638p.