

Shrub Establishment in the Presence of Cheatgrass: The Effect of Soil Microorganisms

Rosemary L. Pendleton¹

Burton K. Pendleton¹

Steven D. Warren²

Jeffrey R. Johansen³

Larry L. St. Clair⁴

Abstract: Invasive annual grasses, such as cheatgrass (*Bromus tectorum*), create changes in soil microorganism communities and severely limit shrub establishment, a situation that is of considerable importance to land managers. We examined the effects of biological crust-forming algae and arbuscular mycorrhizal fungi on growth and survival of *Ephedra viridis* (EPVI), *Coleogyne ramosissima* (CORA), *Artemisia filifolia* (ARFI), *Chrysothamnus nauseosus* ssp. *hololeucus* (CHNA), and *Artemisia nova* (ARNO), with and without competition from cheatgrass, in a controlled laboratory pot experiment. Shrub survival declined as soil fertility increased. Few shrubs were able to survive in competition with cheatgrass in fertilized growth medium. Under low nutrient conditions, the addition of mycorrhizal inoculum intensified competition with cheatgrass, reducing shrub shoot biomass over that of the control treatment in all species except ARFI. However, shoot growth of cheatgrass in the mycorrhizal treatment was reduced to an even greater extent in all cases except when grown with ARNO. Algal inoculation increased shrub survival and appeared to beneficially affect the growth of ARFI and EPVI at the expense of cheatgrass. Our findings suggest that soil microorganisms can, to some extent, improve shrub establishment in the presence of cheatgrass.

Introduction

Restoring disturbed rangelands infested with invasive annual grasses is a complex and difficult process. Once established, invasive annual grasses, such as cheatgrass (*Bromus tectorum*), create changes in soil moisture regimes, decomposition cycles, nutrient availability, and soil microorganism communities, as well as changes in fire frequency and frequency of native plant recruitment (Whisenant 1990; Belnap and Phillips 2001; Norton and others 2004). Competition from cheatgrass for limiting soil resources severely reduces shrub establishment (Stevens and Monsen 2004). Reversing these changes and reestablishing a functional native plant community is a difficult and often unsuccessful process. The role that soil organisms might play in such restoration efforts has yet to be explored.

Biological soil crusts of arid and semiarid lands contribute to a variety of ecological functions, including soil stabilization, nitrogen (N) fixation, nutrient availability, and vascular plant establishment (Belnap and others 2001). Considerable evidence also indicates that mycorrhizal fungi play an important role in plant uptake of N, including atmospheric N fixed by soil crust organisms, as well as that of more immobile elements such as phosphorus (Ibijbjen and others 1996; Hawkes 2003).

This study examines the effects of mass-produced, crust-forming soil algae and arbuscular mycorrhizal fungi on growth and survival of shrub seedlings grown with and without competition from cheatgrass under controlled greenhouse conditions. The ultimate goal of this research is to provide information on microorganism interactions that will aid revegetation efforts of disturbed arid lands.

Methods

Seeds of five shrub species—*Ephedra viridis* (EPVI), *Coleogyne ramosissima* (CORA), *Artemisia filifolia* (ARFI), *Chrysothamnus nauseosus* ssp. *hololeucus* (CHNA), and *Artemisia nova* (ARNO), representing both cold and mixed desert communities—were obtained from native populations in Utah. Cheatgrass (BRTE) seed was collected from the Whiterocks area in northern Utah.

Algal inoculum of the genus *Schizothrix* was produced in concentrated slurry form, pelletized, dried, and ground to a powder according to the procedures described in Buttars and others (1998). Mycorrhizal inoculum was produced from soil collected from beneath shrubs growing near Toquerville, UT. Shrubs growing at this location included *Coleogyne ramosissima*, *Artemisia filifolia*, *Artemisia tridentata*, and one species of *Ephedra*. We assumed that spores collected from this site, which had many of the same genera as were used in our experiment, would provide a compatible source of mycorrhizal inoculum. Spores were extracted from the soil by wet-sieving and decanting, followed by sucrose centrifugation (Daniels and Skipper 1982; Walker and others 1982). Freshly extracted spores were suspended in water and added to pots using a pipette. Replicate aliquots were decanted onto filter paper and spore counts made. Non-mycorrhizal treatments received an equal volume of killed (autoclaved) spore suspension combined with microbial-containing washings that had passed a 25-micron sieve.

One-liter Durapots (Hummert Int.) were filled with a steam-sterilized bank sand that had a pH of 8.3, conductivity of 0.58 mmhos/cm, and plant-available nutrient concentrations of 4.96, 2.48, and 41.6 ppm nitrate-N, phosphorus,

In: Sosebee, R.E.; Wester, D.B.; Britton, C.M.; McArthur, E.D.; Kitchen, S.G., comp. 2007. Proceedings: Shrubland dynamics—fire and water; 2004 August 10-12; Lubbock, TX. Proceedings RMRS-P-47. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 173 p.

and potassium, respectively. Following steaming, approximately one-third of the sand was amended using Osmocote 17-7-12 NPK (5 oz per cubic foot) formulated for 12 to 14 months continuous fertilization to produce a medium soil fertility level. The low fertility level had no additional fertilizer added.

Plants were grown using one of four inoculation treatments: algal crust inoculum applied to the soil surface at a rate of 100 g/m²; arbuscular mycorrhizal inoculum consisting of 50 to 100 spores added to pots at a depth of 1 to 2 inches; dual inoculation; and a non-inoculated control. Seeds of CORA and EPVI were pre-germinated on moistened filter paper before planting. All other shrub species were seeded into a sterile, sand-filled flat then transplanted to the treatment pots following emergence of the first true leaves. Half of the low fertility treatment pots were subsequently planted with one seed of BRTE. BRTE was not planted in the medium fertility pots because previous trials found that few shrubs could survive competition with BRTE in fertilized soils. Ten pots were used for each fertility/treatment/competition combination for a total of 120 pots per species.

Plants were grown for 5 months in a greenhouse that had been cleaned, sprayed with a biocide, and equipped with new evaporative cooling pads. Additional lighting was provided for 12 hours per day using mercury vapor lamps. Pots were randomized within the greenhouse bench using a computer-generated randomization procedure and watered as needed using a hose with a fine spray head. Shrub planting/transplanting was carried out from late November to mid-December 1996. Harvesting was accomplished throughout May. At harvest, shoots were excised at ground level, dried at 65°C, and weighed. Roots were washed free of sand, dried, and weighed.

Statistical analyses were accomplished using SAS version 6.11 for the personal computer (SAS Institute Inc. 1989). Survival data were analyzed using a logistic regression procedure. Growth effects were analyzed separately for each shrub species using the GLM procedure, with mycorrhizal inoculation, algal inoculation, and either soil fertility or competition with cheatgrass as main effects. These analyses were further subdivided depending on significance of the interaction terms. Treatment effects were also examined using GLM by first coding the four inoculation treatment combinations 1 to 4. Mean separations were determined using Tukey and GT2 procedures.

Results

The initial model used in the logistic regression for shrub survival included soil fertility level, shrub species, mycorrhizal inoculation, and algal inoculation, along with all two-way interactions. Stepwise elimination resulted in a final model containing soil fertility ($P = 0.0001$), shrub species ($P = 0.0001$), and algal inoculation ($P = 0.0014$) as significant effects in predicting shrub survival. There was no significant mycorrhizal effect for shrub survival. Survival of the two

sagebrush species (ARFI and ARNO) was high (> 95 percent at both soil fertility levels). Survival of the other three shrub species was reduced at the medium fertility level (35 percent for CORA, 53 percent for CHNA and EPVI). Algal inoculation increased shrub survival from 95 percent to 99 percent at low fertility and from 62 percent to 72 percent at medium fertility. Algal inoculation has consistently increased plant survival in our other greenhouse experiments (R. Pendleton, data on file, Rocky Mountain Research Station, Albuquerque, NM). Whether this effect is due to the presence of the algae themselves (possible precluding colonization by facultative pathogens) or to the alginate carrier is not known.

Soil fertility level significantly affected root and shoot biomass of all shrubs ($P \leq 0.0138$), except EPVI. Fertilization increased shoot biomass of the faster growing species (ARFI, ARNO, and CHNA) 20- to 40-fold (fig. 1a). CORA also responded to a lesser degree, increasing shoot growth five-fold. Soil fertility significantly altered root/shoot ratios of all shrub species ($P < 0.0001$) indicating proportionately less investment in root biomass at higher soil nutrient levels (fig. 1b). All species invested more actual biomass in roots than shoots at low fertility, but at medium fertility, the reverse was true.

Statistical differences in growth of individual species due to inoculation treatment were few and varied with soil fertility level. However, a comprehensive examination at growth results by fertility level allows some meaningful generalizations to be made. At medium fertility, the faster growing ARFI, ARNO, and CHNA produced the greatest shoot and total growth with no microorganism additions (significantly so for ARNO and CHNA; fig. 2a). Shoot growth of CORA grown at medium fertility was suppressed in the algal inoculation treatment ($P = 0.0299$), whereas EPVI grew best in the presence of the algae ($P = 0.0133$). Under low nutrient conditions, only CORA showed a significant difference with inoculation treatment, producing the greatest shoot growth in the mycorrhizal treatment (fig. 2b). However, in no case did control plants exhibit the greatest growth at low soil fertility, and most shrub species appeared to respond positively to one or more inoculation treatments: ARNO and CORA to mycorrhizal inoculum, CHNA and EPVI to the algal inoculum.

Competition with BRTE significantly affected all aspects of shrub growth ($P \leq 0.0033$), reducing biomass production of both roots (data not shown) and shoots (fig. 3). The effect of inoculation treatment on competitive interactions differed among shrubs. ARFI showed no significant treatment differences in shoot growth whether grown alone or in competition with BRTE (fig. 2b, 4a). It is interesting to note, however, that when ARFI and BRTE were grown together, the control treatment had the least average ARFI biomass and the most BRTE biomass (fig. 4). The mycorrhizal treatment averaged a 30 percent higher shoot biomass of ARFI over that of control plants, whereas shoot biomass of BRTE in the mycorrhizal treatment was 8 percent lower than that of control plants.

CHNA likewise showed no significant treatment differences at low fertility, either with or without competition from BRTE. BRTE, however, showed a significant treatment effect

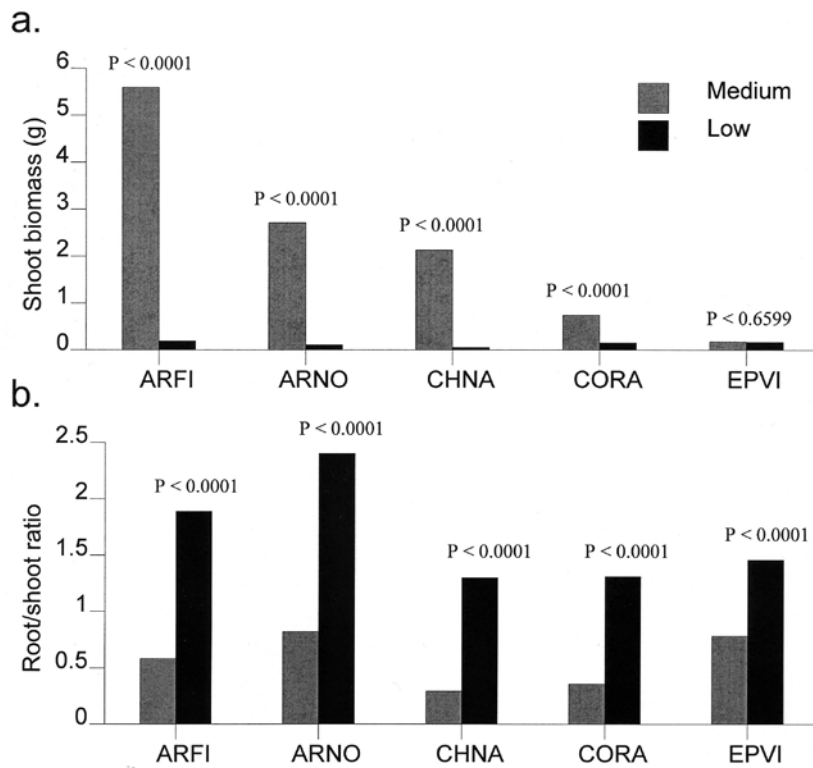


Figure 1. Effect of soil fertility level on (a) shoot biomass and (b) root/shoot ratio of five Intermountain shrub species. Shrub species are indicated by four letter codes. See text for an explanation of codes.

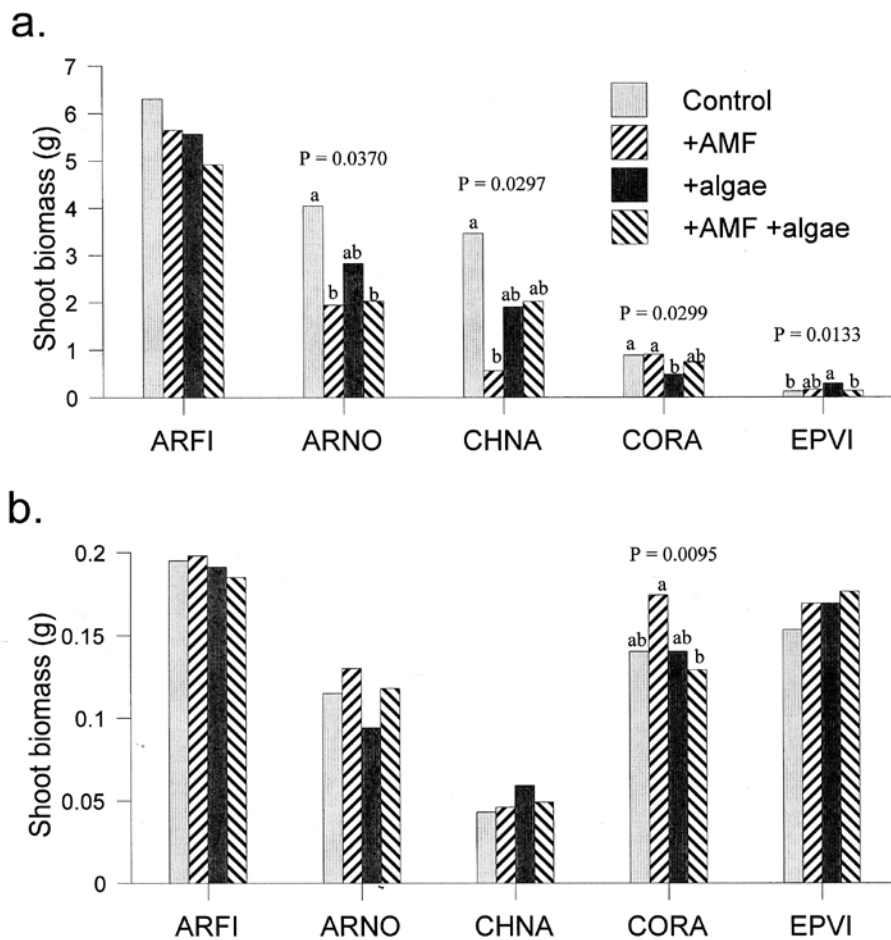


Figure 2. Effect of inoculation treatment on shoot growth of five Intermountain shrub species growing at (a) medium and (b) low soil fertility. Letters denote significant differences at $P < 0.05$ using the Tukey test for multiple comparisons.

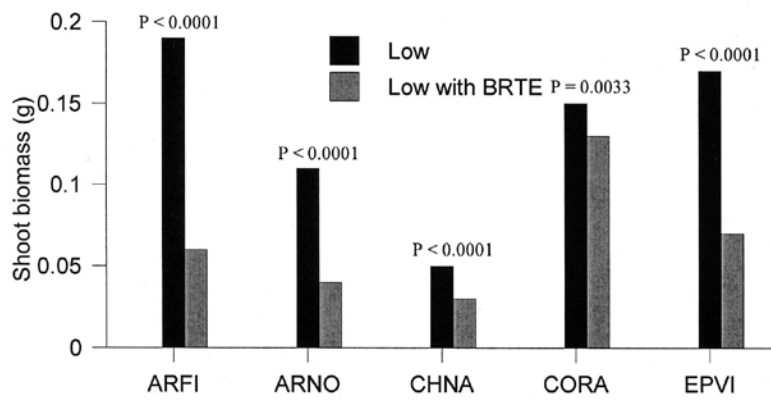


Figure 3. Effect of competition with cheatgrass on shoot biomass of five Intermountain shrub species. P values indicate level of significance when comparing shrubs grown alone versus those grown in competition with cheatgrass.

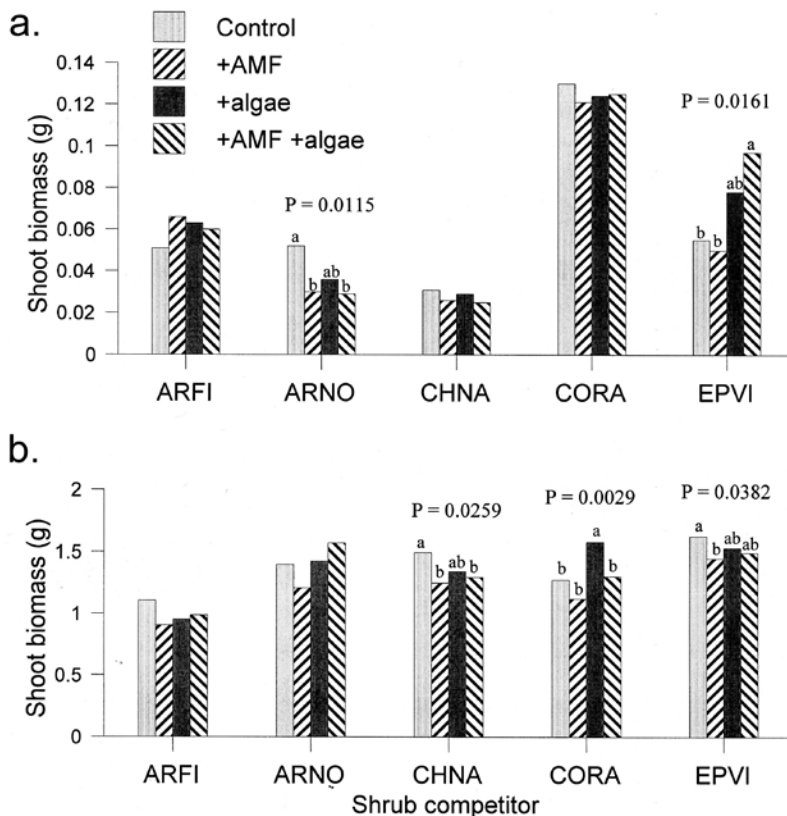


Figure 4. Shoot biomass of (a) shrubs and (b) cheatgrass grown together in a low fertility soil. Letters denote significant differences at $P < 0.05$ using the Tukey test for multiple comparisons.

for mycorrhizal inoculation ($P = 0.0259$). Shoot biomass of BRTE was reduced 17 percent (fig. 4) and root biomass was reduced 22 percent in the presence of mycorrhizae (root data not shown).

When grown alone at low soil fertility, CORA grew best in the presence of mycorrhizae (fig. 2b.). This difference disappeared when grown in competition with BRTE (fig. 4a). Shoot biomass of mycorrhizal CORA plants growing with BRTE averaged 7 percent lower (nonsignificant) than that of controls. Above-ground biomass of BRTE, however, was significantly affected by inoculation treatment. Shoot growth of BRTE was reduced by 12 percent in the presence of mycorrhizae ($P = 0.0112$), but increased 24 percent in the presence of the algal inoculum ($P = 0.0046$).

In the absence of competition, EPVI showed a slight, though not significant, increase in shoot growth in response to algal inoculation (fig. 2b). When grown with BRTE, algal inoculation made a much greater difference in EPVI shoot growth ($P = 0.0161$). The two algal treatments averaged a 58 percent increase in EPVI shoot growth (fig. 4a) and a 75 percent increase in root growth (data not shown) over that of control plants. Mycorrhizae alone did not affect shoot growth of the shrub, but significantly decreased shoot biomass of BRTE ($P = 0.0382$; fig. 4b).

ARNO also showed a small, though not significant, increase in shoot growth in response to mycorrhizal inoculation when growing alone at low soil fertility (fig. 2b). In competition with BRTE, however, control treatment shrubs produced the

greatest shoot growth ($P = 0.0115$; fig. 4a). Growth of BRTE was not significantly affected by treatment (fig. 4b). It therefore appears that, for ARNO, soil microorganisms did not provide a benefit for shrubs growing in competition with BRTE.

Discussion

Associations with arbuscular mycorrhizal fungi have been reported for many aridland plant species, including many shrubs (for example, see Bethlenfalvay and others 1984; Lindsey 1984). Benefits to the plant include enhanced uptake of minerals, improved water relations, and reduced susceptibility to pathogens (Miller and Jastrow 1994; Newsham and others 1995; Mathur and Vyas 2000). The presence or absence of mycorrhizal fungi can also affect plant community composition and diversity (Grime and others 1987; van der Heijden and others 1998; Hartnett and Wilson 1999). The role that mycorrhizal fungi might play in regulating competitive interactions between native vegetation and exotic annual grasses, such as cheatgrass, is not well understood. In monoculture, cheatgrass shows no positive response to mycorrhizal fungi, although colonization levels can be quite high (Allen 1984; Benjamin and Allen 1987; Schwab and Loomis 1987). Goodwin (1992) hypothesized that competition between native and exotic grasses would be little changed by mycorrhizal fungi. Experiments between cheatgrass and shrubs, however, had not previously been done.

Biological soil crusts have been shown to improve growth and establishment of many aridland plant species, including CORA (Harper and Pendleton 1993; Belnap and others 2003; Pendleton and others 2004). Unfortunately, manipulative studies looking at effects of soil crusts on interspecific competition have not been done. Larsen (1995) reported that density of cheatgrass was lower on intact crusts as compared to uncrusted soils, whereas density of native *Stipa* was unaffected. Biological crust-forming organisms interact with a variety of other organisms, including small invertebrates, rhizobia, and mycorrhizal fungi (Harper and Pendleton 1993; Belnap 2003). Furthermore, mycorrhizae may enhance a plant's ability to utilize nitrogen fixed by crust-forming cyanobacteria and lichens (Ibijbjen and others 1996; Hawkes 2003). These studies suggest that soil microorganisms may be important regulators of competition in arid shrubland ecosystems.

In this experiment, the addition of mycorrhizae appeared to intensify competition between shrub seedlings and cheatgrass. At low fertility, shrubs with mycorrhizal additions were equal to or slightly larger than controls. When grown with cheatgrass, however, mycorrhizal treatment shrubs tended to be smaller than control plants for all shrub species except ARFI. However, in four of five cases, shoot growth of cheatgrass growing with shrubs was reduced to a greater extent, suggesting that the presence of mycorrhizae may positively affect the shrub's ability to compete with cheatgrass.

Results for the algal inoculation were mixed. Algal inoculation increased growth of EPVI (and possibly ARFI) grown in competition with cheatgrass. In contrast, the algal inoculum appeared to benefit cheatgrass when grown in competition with ARNO and CORA. Competition for soil nutrients likely occurs between algae and vascular plants during initial crust formation. Results may have been different had the crust been allowed to establish for a period, or if mature crusts had been used.

The finding that soil microorganisms may affect survival, growth, and competitive interactions of native shrubs with exotic annuals, such as cheatgrass, is of considerable importance to current land management practices. Shrubs comprise the dominant form of plant life throughout much of the western United States and are important components of many other vegetation types. Shrubs contribute to vegetation structure, biodiversity, and provide food and shelter to a variety of wildlife. Reestablishing shrub cover is a priority for many management plans; however, seedling establishment in the presence of annual grasses is unlikely to succeed without some kind of weed control (Stevens and Monsen 2004). Our results indicate that soil microorganisms, including crust-forming algae and arbuscular mycorrhizal fungi, may enhance seedling establishment in the presence of cheatgrass. Additional field studies are warranted.

Literature Cited

- Allen, E. B. 1984. The role of mycorrhizae in mined land diversity. In: Munshower, F. F.; Fisher, S. E., Jr., eds. Third biennial symposium on surface coal mine reclamation on the Great Plains. Bozeman, MT: Montana State University: 273-295.
- Belnap, J. 2003. Microbes and microfauna associated with biological soil crusts. In: Baldwin, I. T.; Caldwell, M. M.; Heldmaier, G.; Lange, O. L.; Mooney, H. A.; Schulze, E. -D.; Sommer, U., eds. Biological soil crusts: structure, function, and management, 2nd edition, Ecological Studies 150. Berlin: Springer: 167-174.
- Belnap, J.; Prasse, R.; Harper, K. T. 2003. Influence of biological soil crusts on soil environments and vascular plants. In: Baldwin, I. T.; Caldwell, M. M.; Heldmaier, G.; Lange, O. L.; Mooney, H. A.; Schulze, E. D.; Sommer, U., eds. Biological soil crusts: structure, function, and management, 2nd edition, Ecological Studies 150. Berlin: Springer: 281-300.
- Belnap, J.; Kaltenecker, J. H.; Rosentreter, R.; Williams, J.; Leonard, S.; Eldridge, D. 2001. Biological soil crusts: ecology and management. BLM/ID/ST-01/001+1730, Technical Reference 130-2. Denver, CO: National Science and Technology Center, Bureau of Land management. 110 p.
- Belnap, J.; Phillips, S. L. 2001. Soil biota in an ungrazed grassland: response to annual grass (*Bromus tectorum*) invasion. Ecological Applications 11: 1261-1275.
- Benjamin, P. K.; Allen, E. B. 1987. The influence of VA mycorrhizal fungi on competition between plants of different successional stages in sagebrush grassland. In: Sylvia, D. M.; Hung, L. L.; Graham, J. H., eds. Mycorrhizae in the next decade, practical applications and research priorities. Gainesville, FL: University of Florida, Institute of Food and Agricultural Sciences: 144.

- Bethlenfalvay, G. J.; Dakessian, S.; Pacovsky, R. S. 1984. Mycorrhizae in a southern California desert: ecological implications. *Canadian Journal of Botany* 62: 519-524.
- Buttars, S. M.; St. Clair, L. L.; Johansen, J. R.; Sray, J. C.; Payne, M. C.; Webb, B. L.; Terry, R. E.; Pendleton, B. K.; Warren, S. D. 1998. Pelletized cyanobacterial soil amendments: laboratory testing for survival, escapability, and nitrogen fixation. *Arid Soil Research and Rehabilitation* 12: 165-178.
- Daniels, B. A.; Skipper, H. D. 1982. Methods for the recovery and quantitative estimation of propagules from soil. In: Schenck, N. C., ed. *Methods and principles of mycorrhizal research*. St. Paul, MN: American Phytopathological Society: 29-36.
- Goodwin, J. 1992. The role of mycorrhizal fungi in competitive interactions among native bunchgrasses and alien weeds: a review and synthesis. *Northwest Science* 66: 251-260.
- Grime, J. P.; MacKey, J. M.; Hillier, S. H.; Read, D. J. 1987. Floristic diversity in a model system using experimental microcosms. *Nature* 328: 420-422.
- Harper, K. T.; Pendleton, R. L. 1993. Cyanobacteria and cyanolichens: can they enhance availability of essential minerals for higher plants? *Great Basin Naturalist* 53: 59-72.
- Hartnett, D. C.; Wilson, G. W. T. 1999. Mycorrhizae influence plant community structure and diversity in tallgrass prairie. *Ecology* 80: 1187-1195.
- Hawkes, C. V. 2003. Nitrogen cycling mediated by biological soil crusts and arbuscular mycorrhizal fungi. *Ecology* 84(6): 1553-1562.
- Ibijbjen, J.; Uriquiaga, S.; Ismaili, M.; Alves, B. J. R.; Boddey, R. M. 1996. Effect of arbuscular mycorrhizas on uptake of nitrogen by *Brachiaria arrecta* and *Sorghum vulgare* from soils labeled for several years with ¹⁵N. *New Phytologist* 134: 353-360.
- Larsen, K. D. 1995. Effects of microbiotic crusts on the germination and establishment of three range grasses. Masters Thesis. Boise, ID: Boise State University.
- Lindsey, D. L. 1984. The role of vesicular-arbuscular mycorrhizae in shrub establishment. In: Williams, Stephen E.; Allen, Michael F., eds. *VA mycorrhizae and reclamation of arid and semi-arid lands*. Scientific Report Number SA1261: proceedings; 1982 August 17-19; Dubois, WY. Laramie, WY: Wyoming Agricultural Experiment Station, University of Wyoming: 53-68.
- Mathur, N.; Vyas, A. 2000. Influence of arbuscular mycorrhizae on biomass production, nutrient uptake and physiological changes in *Ziziphus mauritiana* Lam. under water stress. *Journal of Arid Environments* 45: 191-195.
- Miller, R. M.; Jastrow, J. D. 1994. The application of VA mycorrhizae to ecosystem restoration and reclamation. In: Allen, M. F., ed. *Mycorrhizal functioning: an integrative plant-fungal process*. New York: Chapman and Hall: 438-467.
- Newsham, K. K.; Fitter, A. H.; Watkinson, A. R. 1995. Multi-functionality and biodiversity in arbuscular mycorrhizas. *Trends in Ecology and Evolution* 10: 407-411.
- Norton, J. B.; Monaco, T. A.; Norton, J. M.; Johnson, D. A.; Jones, T. A. 2004. Soil morphology and organic matter dynamics under cheatgrass and sagebrush-steppe plant communities. *Journal of Arid Environments* 57: 445-466.
- Pendleton, R. L.; Pendleton, B. K.; Howard, G. L.; Warren, S. D. 2004. Growth and nutrient content of herbaceous seedlings associated with biological soil crusts. *Arid Land Research and Management* 17: 271-281.
- SAS Institute Inc. 1989. *SAS/STAT User's Guide*, Vol. 2, version 6, fourth ed. Cary, N.C.: SAS Institute Inc. 1686 p.
- Schwab, S. M.; Loomis, P. A. 1987. VAM effects on growth of grasses in monocultures and mixtures. In: Sylvia, D. M.; Hung, L. L.; Graham, J. H., eds. *Proceedings of the 7th North American conference on mycorrhizae*, Gainesville, FL: University of Florida, Institute of Food and Agricultural Sciences: 7.
- Stevens, R.; Monsen, S. B. 2004. Guidelines for restoration and rehabilitation of principal plant communities. In: Monsen, S. B.; Stevens, R.; Shaw, N. L., comps. *Restoring western ranges and wildlands*. Gen. Tech. Rep. RMRS-GTR-136-vol-1. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station: 199-294.
- van der Heijden, M. G. A.; Klironomos, J. N.; Ursic, M.; Moutoglis, P.; Strietwolf-Engel, R.; Boller T.; Wiekman, A.; Sanders, I. R. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* 396: 69-72.
- Walker, C.; Mize, C. W.; McNabb, H. S., Jr. 1982. Populations of endogonaceous fungi at two locations in central Iowa. *Canadian Journal of Botany* 60: 2518-2529.
- Whisenant, S. G. 1990. Changing fire frequencies on Idaho's Snake River plains: ecological and management implications. In: McArthur, E. D.; Romney, E. M.; Smith, S. D.; Tueller, P. T., eds. *Proceedings: symposium on cheatgrass invasion, shrub die-off, and other aspects of shrub biology and management*. Gen. Tech. Rep. INT-276. Ogden, UT: U.S. Department of Agriculture, Forest Service, Intermountain Research Station: 4-10.

The Authors

- ¹Research Ecologists, USDA Forest Service, Rocky Mountain Research Station, Albuquerque, NM. rpendleton@fs.fed.us
- ²Research Scientist, Center for Environmental Management of Military Lands, Colorado State University, Fort Collins, CO.
- ³Professor, Department of Biology, John Carroll University, University Heights, OH.
- ⁴Professor, Department of Integrative Biology and Curator of Lichens, M.L. Bean Museum, Brigham Young University, Provo, UT.