AN ABSTRACT OF THE DISSERTATION OF

Nicole M. DeCrappeo for the degree of Doctor of Philosophy in Soil Science presented on June 8, 2010.

Title: Soil Community Dynamics in Sagebrush and Cheatgrass-invaded Ecosystems of the Northern Great Basin.

Abstract approved:

_____

Peter J. Bottomley David D. Myrold

Sagebrush steppe ecosystems in the Great Basin have become increasingly threatened by the proliferation of cheatgrass (*Bromus tectorum* L.), an invasive annual grass. Diverse sagebrush and perennial bunchgrass landscapes can be converted to homogenous cheatgrass grasslands mainly through the effects of fire. Although the consequences of this conversion are well understood in the context of plant community dynamics, information on changes to soil communities has not been well documented. I characterized soil surface, microbial, and nematode community dynamics in sagebrush steppe and cheatgrass-invaded areas across the northern Great Basin. I also examined how restoration treatments, such as seeding with a low impact rangeland drill and applying herbicide or sugar to plots, affected soil communities. Soil community functional diversity and structure were alike at sites where soil pH and percent bare ground were similar. Rangeland drill seeding and associated human trampling decreased biological soil crust cover at sites with high proportions of cyanobacteria. Herbicide treatments had little effect on soil communities, but addition of sugar to plots increased carbohydrate utilization and fungal biomass of cheatgrass-

invaded soils. In studying paired intact and cheatgrass-invaded sagebrush plots, I found that microbial functional diversity and community composition were different in sagebrush, bunchgrass, cheatgrass, and interspace soils. Fungal biomass and species richness were highest under sagebrush and decreased under cheatgrass. To examine how soil community shifts might affect ecosystem processes, I investigated the contribution of fungi to inorganic nitrogen (N) mineralization in sagebrush and cheatgrass rhizospheres. Results from a ^{15}N pool dilution experiment modified with the fungal protein synthesis inhibitor cycloheximide showed that gross and net N cycling rates did not differ between control sagebrush and cheatgrass soils and that fungi were important for gross NH_4^+ production and consumption in both soil types. However, net nitrification increased in sagebrush soils after 24 h, suggesting that when organic matter decomposition by fungi ceased bacteria became carbon limited and could no longer assimilate NH_4^+. These studies demonstrate that cheatgrass invasion into sagebrush steppe ecosystems can bring about significant changes to soil communities and that these changes may have repercussions for ecosystem functioning in the northern Great Basin.

Soil Community Dynamics in Sagebrush and Cheatgrass-invaded Ecosystems of the Northern Great Basin

by
Nicole M. DeCrappeo

A DISSERTATION

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Presented June 8, 2010
Commencement June 2011

Doctor of Philosophy dissertation of Nicole M. DeCrappeo presented on June 8, 2010.

APPROVED:

Co-Major Professor, representing Soil Science

Co-Major Professor, representing Soil Science

Head of the Department of Crop and Soil Science

Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Nicole M. DeCrappeo, Author

ACKNOWLEDGEMENTS

So many people have contributed to the work contained in this dissertation, and they have waited a long time for a proper thank you. First and foremost, thank you to my boss and committee member, David Pyke, for hiring me to work for the U.S. Geological Survey. The opportunities he has given me have been, to put it mildly, life changing, and I am indebted to him for having unwavering confidence in me even when I did not place that same confidence in myself. My major advisor, Peter Bottomley, has been the perfect advisor for me. His no-nonsense attitude provided me with structure and discipline, and more than anyone he has pushed me to become a better experimental scientist. His commitment to student mentoring is easy to see: most of our meetings lasted a minimum of two hours, and I never felt that he was in a rush to move on to more important things (unless he had a lunchtime racquetball match). I only hope that working with the "federales" hasn't been too painful of an experience for him.

Dave Myrold was always generous with his time and feedback. Our Tuesday morning journal club meetings continue to be a source of wonder to me as Dr. Myrold's intellectual wizardry are on full display. The isotope dilution experiment could not and would not have been done without his expertise. Jayne Belnap is a hero and role model to me in this world, and anyone who hints that I could be "the next Jayne Belnap" is paying me the highest compliment. I don't understand how she does all that she does, but I'm extremely appreciative to have her on my team.

The members of the Pyke lab have been my constant source of support, amusement, and good-natured harassment. Many pages of acknowledgement could be written for Liz DeLorenze alone. Everyone is well aware that this dissertation and my degree belong as much to her as they do to me. Everyone is also well aware of what a quality employee Liz is, which I know because people are always trying to hire her away from me. As I have told her many times before, if I could have Liz surgically attached to me, I would do it in a second. But beyond her work in the lab, Liz has been my closest confidante for many years and is always my biggest cheerleader.

Kevin Knutson and Scott Shaff also deserve special recognition for enduring work, and in Kevin's case, home life with me all these long years. Troy Wirth added to the entertainment value of our clique in FSL 189, and while I might have finished my PhD sooner had we not all shared an office, I would have a lot fewer stories to reminisce about now. Upekala Wijayratne is a trusted colleague and friend, and I hope we have opportunities to work together in the future. Other current and past members of the lab have been invaluable in helping with fieldwork, lab work, statistical analyses, and general scientific discourse: Jessi Brunson, Dana Witwicki, Vijay Satyal, Steven Bekedam, Kara Hempy-Mayer, Kristen Harrison, Cindy Salo, Andrew Lindgren, Todd Wojtowicz, Ceder Hesse, Audrey Larimer, Jessica Boyd, Joe Fontaine, Tim Lair, and Leah Goldstein.

The Myrold/Bottomley lab group significantly broadened my scientific world. Elizabeth Brewer is probably the most generous person I know; she spends countless hours helping the rest of us figure out how to do what we need to do. Stephanie Yarwood is an amazing scientist with an amazing laugh, and Rockie Yarwood is able to conjure up impossible amounts of data from his analytical equipment—data that are always delivered in the nick of time. Laurel Kluber's help was most appreciated in learning the ways of TRFLP analysis and figuring out how to make GeneMapper Software work for us.

Many others have helped along the way. Members of the IFAFS research project, especially Paul Doescher, Bob Blank, and Tye Morgan (USDA-ARS), gave me a great introduction to the Great Basin. Roger Rosentreter (BLM) trained me to identify biological soil crust species, and Bruce McCune (Oregon State University) taught me how to analyze soil crust and microbial community dynamics. Chris Catricala (USGS-FRESC) has helped the Pyke lab with just about everything for the past seven years. Jane Smith and Doni McKay (USDA Forest Service) generously allowed us to use their genetics lab and equipment as if it were our own, and Doni and Tara Jennings spent many hours showing us techniques and answering questions. A very special thank you goes to Terri Young (USGS), who made the administrative side of working for the government as painless as possible.

Finally, to my parents, Nancy and Tony, and sister, Megan: you have always believed in me no matter what the circumstances. Your love and support mean more than words can express. And to Mike, my husband: for a guy who hates academics, you sure picked a weird person to marry. But you continue to provide me with everything I need to be happy in life, and for that I am very grateful.

CONTRIBUTION OF AUTHORS

Dr. David A. Pyke contributed to experimental design, data interpretation, and manuscript preparation for Chapters 2, 3, and 4. Elizabeth J. DeLorenze performed field and lab work and helped with data management and figure preparations for Chapters 3 and 4. Dana L. Witwicki contributed to experimental design and provided data for Chapter 3. Elizabeth A. Brewer and Stephanie A. Yarwood contributed to experimental design, lab work, data calculations, and interpretation for Chapter 4.

TABLE OF CONTENTS

Page

General Introduction 1

Soil community patterns in *Bromus tectorum*-invaded ecosystems of the northern Great Basin 9
- Abstract 10
- Introduction 11
- Materials and Methods 13
- Results 22
- Discussion 25
- Acknowledgements 30
- References 30

Soil community responses to *Bromus tectorum* invasion and nutrient manipulations in sagebrush ecosystems: implications for restoration 55
- Abstract 56
- Introduction 57
- Materials and Methods 59
- Results 67
- Discussion 70
- Acknowledgements 75
- References 75

The contribution of soil fungi to inorganic nitrogen cycling in sagebrush and cheatgrass-influenced soils 93
- Abstract 94
- Introduction 95
- Materials and Methods 97
- Results 101
- Discussion 102
- Acknowledgements 107
- References 107

General Conclusions 119

Bibliography 123

Appendix 136

LIST OF FIGURES

Figure Page

2.1 Eight site locations (red circles) across the northern Great Basin where restoration experiments, soil community sampling, and biological soil crust surveys were carried out. 44

2.2 Plot designs for the Transition Species and Functional Groups experiments. 45

2.3 Examples of soil surface cover at select sites in the northern Great Basin. 46

2.4 Nonmetric multidimensional scaling (NMS) ordinations for (A) biological soil crust surveys, (B) community level physiological profiles (CLPP), (C) phospholipid fatty acid (PLFA) analysis, and (D) soil nematode trophic group structure, Transition Species experiment, March 2004. 47

2.5 Proportion of soil surface cover at eight study sites in the northern Great Basin. 49

2.6 Effects of herbicide treatment and rangeland drill seeding on biological soil crust components at Cinder Cone Butte, ID (A & B), Izzenhood Ranch, NV (C & D), and Simpson Springs, UT (E & F). 50

2.7 Nonmetric multidimensional scaling (NMS) ordinations of soil samples analyzed for (A) community level physiological profiles (CLPP), (B) phospholipid fatty acid (PLFA) analysis, and (C) nematode trophic group structure, Functional Groups experiment, March 2004. 51

2.8 Phospholipid fatty acid (PLFA) bacteria and fungi biomass and bacterial- and fungal-feeding nematode abundances from control and sugar-treated plots, Functional Groups experiment, May 2004. 53

2.9 Conceptual diagram of latent and composite variables affecting soil community structure in cheatgrass-invaded sagebrush ecosystems of the northern Great Basin. 54

3.1 Nonmetric multidimensional scaling (NMS) ordination of soil samples collected in fall 2003 and spring 2004 for PLFA analysis. 85

3.2 Nonmetric multidimensional scaling (NMS) ordinations of soil samples collected in spring 2004 for community level physiological profiling (CLPP) and phospholipid fatty acid (PLFA) analysis. 86

LIST OF FIGURES (Continued)

Figure Page

3.3 Nonmetric multidimensional scaling (NMS) ordinations of soil samples collected in spring 2004 for bacterial (left panel) and fungal (right panel) terminal restriction fragment length polymorphism (TRFLP). 87

3.4 Comparison of CLPP guilds from soil samples collected in spring 2004 from all six study sites. 88

3.5 Comparison of PLFA biomarker taxa from soil samples collected in spring 2004 from all six study sites. 89

3.6 Comparison of fatty acid fungal to bacterial ratios from sagebrush, bunchgrass, interspace, and cheatgrass soils from treated and control plots sampled in fall 2003 (top) and spring 2004 (bottom). 90

3.7 Comparison of bacterial (top) and fungal (bottom) terminal restriction fragments (TRFs) recovered from sagebrush, bunchgrass, interspace, and cheatgrass soils from treated and control plots sampled in spring 2004..... 91

3.8 NMS ordination of soil samples collected in spring 2004 for nematode community analysis. 92

4.1 Gross and net N cycling rates from control and cycloheximide-treated sagebrush and cheatgrass rhizosphere soils collected at six Snake River Plain sites. 113

4.2 Changes in gross and net N cycling rates from control and cycloheximide-treated sagebrush and cheatgrass rhizosphere soils collected at six Snake River Plain sites. 115

4.3 Soil microbial biomass (MB) C, N, and C:N estimates from a 48 h isotope dilution experiment using control and cycloheximide-treated sagebrush and cheatgrass soils. 117

LIST OF TABLES

Table Page

2.1 Locations and soil characteristics for eight study sites in the northern Great Basin. 37

2.2 Plant species and accessions for the Transition Species (TS) experiment under which soil samples were collected for community level physiological profiling (CLPP), phospholipid fatty acid (PLFA) analysis, and nematode abundance and trophic group composition. 38

2.3 Plant species monocultures and mixes for the Functional Groups (FG) experiment under which soil samples were collected for community level physiological profiling (CLPP), phospholipid fatty acid (PLFA) analysis, and nematode abundance and trophic group composition. 38

2.4 Means (SE) for abiotic soil parameters measured at eight sites in the northern Great Basin in March and May 2004. 39

2.5 Soil chemical properties and enzyme activies from herbicide- (Transition Species experiment) or sugar- (Functional Groups experiment) treated and control plots at study sites across the northern Great Basin. 40

2.6 Multi-response permutation procedures (MRPP) *A*-statistics and *p*-values for biological soil crust community surveys for each hierarchical level in the Transitional Species (TS) experiment. 41

2.7 Multi-response permutation procedures (MRPP) *A*-statistics (*p*-values) for soil community analyses for each hierarchical level in the Transitional Species (TS) and Functional Groups (FG) experiments. 42

2.8 T-test *p*-values for treatment effects on total nematode numbers at each site for the Transitional Species (TS) and Functional Groups (FG) experiments. 43

3.1 Summary of site characteristics for three sites each in eastern Oregon and southwestern Idaho sampled in fall 2003 and spring 2004. 81

3.2 Estimates of soil chemical properties by date and cover type. 82

3.3 Multi-response permutation procedures (MRPP) *A*-statistics (*P*-values) for soil community analyses for each hierarchical level in the study. 83

3.4 Two-way factorial PerMANOVA results from soil community analyses, spring 2004. 83

LIST OF TABLES (Continued)

Table		Page
3.5	Indicator species analysis results for soil fungi as determined by terminal restriction fragment length polymorphism (TRFLP), spring 2004	84
4.1	Physical, chemical, and biological properties of sagebrush and cheatgrass rhizosphere soils collected from paired native and annual plots at six Snake River Plain study sites.	111
4.2	Soil C and N concentrations and microbial biomass estimates from sagebrush and cheatgrass rhizosphere soils collected at paired native and annual plots at six Snake River Plain study sites.	112
4.3	Tests for the significance of site, the random effect in the mixed model ANOVA, for gross and net N cycling rates in soils from six sites in the northern Great Basin.	112

DEDICATION

For Amos Moses.

Hopefully, you will be the only one who rips this dissertation to shreds.

Chapter 1

General Introduction

Soil community dynamics in sagebrush and cheatgrass-invaded ecosystems of the northern Great Basin

Invasion of the sagebrush steppe

The Intermountain West of the United States is covered by what has been called the "sagebrush sea." A patchy landscape of sagebrush shrubs and perennial bunchgrasses, this semi-arid desert biome comprises the largest grassland region in North America, totaling over 64 million ha (Bolton et al. 1993). It is also an ecosystem severely threatened by both natural and human-induced changes. Livestock grazing, minerals extraction, energy development, urban expansion, climate change, and exotic plant species invasions are examples of activities and processes that jeopardize the health and long-term sustainability of the sagebrush steppe (Knapp 1996, McArthur and Goodrich 2004).

Invasion by the exotic annual grass cheatgrass (*Bromus tectorum* L.) has been one of the more compelling stories of the last 100 years in the Intermountain West. Cheatgrass was introduced from Eurasia to the Great Basin in 1894 and was considered common in sagebrush grasslands by the end of the 1920s (Mack 1981). It now covers 20,000 km^2 (7%) of land in the Great Basin (Bradley and Mustard 2005). Cheatgrass is successful in the Intermountain West in part because it can germinate in the fall and winter when native perennial bunchgrasses are dormant (Stewart and Hull 1949, Mack and Pyke 1984). When cheatgrass shoot growth begins the following spring, the well-developed roots reduce soil water availability for native plants (Melgoza et al. 1990). Cheatgrass is fast growing and a prolific seed producer (Hempy-Mayer and Pyke 2008). The litter has higher carbon (C)-to-nitrogen (N) and lignin-to-N ratios than other native plants (Evans et al. 2001, Monaco et al. 2003) and builds up over time on the soil surface. This produces highly flammable and continuous fuel for fires, which kill shrubs and perennial bunchgrasses and leave virtual monocultures of cheatgrass in their place (D'Antonio and Vitousek 1992).

The consequences of cheatgrass invasion on plant community dynamics have been well documented, but the effects on soil physical, chemical, and biological properties are less well understood. The changes in root morphology, litter quality and

quantity, and fire disturbance regime that accompany cheatgrass invasion can lead to soil conditions that are highly altered from their original state. Norton et al. (2004) found that A horizons in cheatgrass soils had significantly more very fine roots and fine tubular pores and lower bulk density than paired native soils. At lower depths, soil organic carbon concentration decreased sharply in cheatgrass soils, while in surface horizons total N and NO_3^- concentrations were higher in cheatgrass vs. native soils. Other researchers have found that gross and net N mineralization rates are faster in cheatgrass soils (Booth et al. 2003). The soil morphological and organic matter storage changes induced by cheatgrass have been likened to the effects of cultivation of grassland soils (Norton et al. 2004). It has been suggested that cheatgrass-invaded sites have crossed an "ecological threshold" and reached new stable states that require an enormous amount of energy to reverse (Laycock 1991, Briske et al. 2005).

With such drastic modifications to the soil environment, we may expect to see concomitant shifts in biological components of the soil ecosystem. Soil bacteria, fungi, mosses, lichens, and micro- and macroinvertebrates all contribute and respond to soil conditions. Cheatgrass invasion and increased fire frequency can have strong impacts on the composition and structure of soil communities. Biological soil crust diversity declines with cheatgrass-promoted fire (Hilty et al. 2004), arbuscular mycorrhizal fungi species richness can increase with exotic grass invasion (Hawkes et al. 2006), and fungal and invertebrate abundance and species richness can decrease in cheatgrass-invaded soils (Belnap and Phillips 2001). Whether these changes affect ecosystem processes, such as decomposition or plant productivity, remains open to debate (Belnap et al. 2005). Also unclear is whether cheatgrass soil communities facilitate or promote continued cheatgrass dominance, or conversely, impede the restoration of native bunchgrasses and shrubs. Rowe and Brown (2008) reported that soil communities did not contribute to cheatgrass invasion success or inhibit native plant reestablishment at high elevation sites in Rocky Mountain National Park, but more studies are needed to confirm this in other cheatgrass-invaded systems.

Much of our knowledge about the effects of cheatgrass invasion on soil systems comes from studies conducted in the Colorado Plateau (Belnap and Phillips 2001, Evans et al. 2001, Miller et al. 2001, Hawkes et al. 2006, Sperry et al. 2006), at calcareous sites in Utah (Booth et al. 2003, Saetre and Stark 2005, Hooker et al. 2008), or at higher elevation sites where precipitation and vegetation patterns are quite different from those in the northern Great Basin (Rowe et al. 2007, Rowe and Brown 2008). Although there are excellent studies on patterns of nutrient availability and microbial biomass in Wyoming sagebrush systems of the Columbia Plateau (Bolton et al. 1990, Bolton et al. 1993, Halvorson et al. 1994, Smith et al. 1994, Halvorson et al. 1997), most do not include cheatgrass or examine soil community function, structure, or composition.

The studies presented here describe soil patterns and processes in the northern Great Basin with particular emphasis on loam and silt loam sites within the Snake River Plain. The broad objective was to assess the effects of cheatgrass invasion on sagebrush steppe soil communities at various scales within this geographic region. In Chapter 2, I asked whether site variability, restoration treatment, or seeded plant species are most important for structuring biological soil crust, microbial, and nematode community composition and function at cheatgrass-invaded sites in eastern Oregon, southwestern Idaho, northern Utah, and northern Nevada. Chapter 3 focuses on plant species effects on soil communities at paired sagebrush/cheatgrass sites in the Snake River Plain. I characterized microbial functional diversity, structure, and composition differences in Wyoming big sagebrush (*Artemisia tridentata* Nutt. ssp. *wyomingensis* Beetle & Young), perennial bunchgrass, cheatgrass, and interspace soils. In Chapter 4, the objective was to determine if sagebrush and cheatgrass soil fungal communities differentially affect nitrogen (N) cycling processes and rates. After water, N is the limiting resources in sagebrush ecosystems, and it has been suggested that cheatgrass promotes conversion from conservative N cycling to one where N is readily lost from the system (Norton et al. 2007). I tested whether N

cycling rates were different in sagebrush vs. cheatgrass soils and discerned what role soil fungi played in the process.

These studies provide valuable information on the status of cheatgrass-invaded sagebrush steppe soils that may be used to help inform restoration efforts in the northern Great Basin. There are many compelling ecological and financial reasons for broadening our knowledge of how cheatgrass affects sagebrush ecosystems. Significant changes in plant community composition, nutrient cycling, and SOM pools in this large region can have profound effects on U.S. estimates of carbon storage (Bradley et al. 2006). The sagebrush biome is home to many sagebrush obligate species such as sage grouse, sage thrashers, pygmy rabbits, and pronghorn antelope, many of which are now threatened due to habitat loss. In addition, there are huge monetary costs associated with invasive species containment and native plant restoration projects in the United States (Pimentel et al. 2005), and the loss of productive and well-managed cattle rangelands is economically damaging to ranching communities in the Intermountain West. If the goal is restoring sagebrush ecosystems, then achieving it may require more than one-time treatments and seeding with perennial bunchgrasses. Reclamation of cheatgrass-invaded soils may be necessary before attempts are made at re-seeding native plant communities. It is important to have a full understanding of soil community dynamics in Wyoming big sagebrush steppe ecosystems in order to give native plants every advantage in their competitive interactions with cheatgrass.

References

Belnap, J. and S. L. Phillips. 2001. Soil biota in an ungrazed grassland: response to annual grass (*Bromus tectorum*) invasion. Ecological Applications **11**:1261-1275.

Belnap, J., S. L. Phillips, S. K. Sherrod, and A. Moldenke. 2005. Soil biota can change after exotic plant invasion: does this affect ecosystem processes? Ecology **86**:3007-3017.

Bolton, H., Jr., J. L. Smith, and S. O. Link. 1993. Soil microbial biomass and activity of a disturbed and undisturbed shrub-steppe ecosystem. Soil Biology & Biochemistry **25**:545-552.

Bolton, H., Jr., J. L. Smith, and R. E. Wildung. 1990. Nitrogen mineralization potentials of shrub-steppe soils with different disturbance histories. Soil Science Society of America Journal **54**:887-891.

Booth, M. S., J. M. Stark, and M. M. Caldwell. 2003. Inorganic N turnover and availability in annual- and perennial-dominated soils in a northern Utah shrub-steppe ecosystem. Biogeochemistry **66**:311-330.

Bradley, B. A., R. A. Houghton, J. F. Mustard, and S. P. Hamburg. 2006. Invasive grass reduces aboveground carbon stocks in shrublands of the Western US. Global Change Biology **12**:1815-1822.

Bradley, B. A. and J. F. Mustard. 2005. Identifying land cover variability distinct from land cover change: cheatgrass in the Great Basin. Remote Sensing of Environment **94**:204-213.

Briske, D. D., S. D. Fuhlendorf, and F. E. Smeins. 2005. State-and-transition models, thresholds, and rangeland health: a synthesis of ecological concepts and perspectives. Rangeland Ecology and Management **58**:1-10.

D'Antonio, C. M. and P. M. Vitousek. 1992. Biological invasions by exotic grasses, the grass/fire cycle, and global change. Annual Review of Ecology and Systematics **23**:63-87.

Evans, R. D., R. Rimer, L. Sperry, and J. Belnap. 2001. Exotic plant invasion alters nitrogen dynamics in an arid grassland. Ecological Applications **11**:1301-1310.

Halvorson, J. J., H. Bolton, and J. L. Smith. 1997. The pattern of soil variables related to *Artemisia tridentata* in a burned shrub-steppe site. Soil Science Society of America Journal **61**:287-294.

Halvorson, J. J., H. Bolton, J. L. Smith, and R. E. Rossi. 1994. Geostatistical analysis of resource islands under *Artemisia tridentata* in the shrub-steppe. Great Basin Naturalist **54**:313-328.

Hawkes, C. V., J. Belnap, C. D'Antonio, and M. K. Firestone. 2006. Arbuscular mycorrhizal assemblages in native plant roots change in the presence of invasive exotic grasses. Plant and Soil **281**:369-380.

Hempy-Mayer, K. and D. A. Pyke. 2008. Defoliation effects on *Bromus tectorum* seed production: implications for grazing. Rangeland Ecology & Management **61**:116-123.

Hilty, J., D. J. Eldridge, R. Rosentreter, M. Wicklow-Howard, and M. Pellant. 2004. Recovery of biological soil crusts following wildfire in Idaho. Journal of Range Management **57**:89-96.

Hooker, T. D., J. M. Stark, U. Norton, A. J. Leffler, M. Peek, and R. Ryel. 2008. Distribution of ecosystem C and N within contrasting vegetation types in a semiarid rangeland in the Great Basin, USA. Biogeochemistry **90**:291-308.

Knapp, P. A. 1996. Cheatgrass (*Bromus tectorum L*) dominance in the Great Basin desert. Global Environmental Change **6**:37-52.

Laycock, W. A. 1991. Stable states and thresholds of range condition on North American rangelands: a viewpoint. Journal of Range Management **44**:427-433.

Mack, R. N. 1981. Invasion of *Bromus tectorum* L. into Western North America: an ecological chronicle. Agro-Ecosystems **7**:145-165.

Mack, R. N. and D. A. Pyke. 1984. The demography of Bromus tectorum: the role of microclimate, grazing and disease. Journal of Ecology **72**:731-748.

McArthur, E. D. and S. K. Goodrich. 2004. The Intermountain setting. Pages 7-14 *in* S. B. Monsen, R. Stevens, and N. L. Shaw, editors. Restoring western ranges and wildlands, vol. 1. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fort Collins, CO.

Melgoza, G., R. S. Nowak, and R. J. Tausch. 1990. Soil water exploitation after fire: competition between *Bromus tectorum* (cheatgrass) and two native species. Oecologia **83**:7-13.

Miller, M., J. Belnap, S. Beatty, and B. Webb. 2001. Components of spatial and temporal soil variation at Canyonlands National Park: implications for P dynamics and cheatgrass (*Bromus tectorum)* performance. Pages 154-162 *in* Shrubland ecosystem genetics and biodiversity. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Provo, UT.

Monaco, T. A., D. A. Johnson, J. M. Norton, T. A. Jones, K. J. Connors, J. B. Norton, and M. B. Redinbaugh. 2003. Contrasting responses of Intermountain West grasses to soil nitrogen. Journal of Range Management **56**:282-290.

Norton, J. B., T. A. Monaco, and U. Norton. 2007. Mediterranean annual grasses in western North America: kids in a candy store. Plant and Soil **298**:1-5.

Norton, J. B., T. A. Monaco, J. M. Norton, D. A. Johnson, and T. A. Jones. 2004. Soil morphology and organic matter dynamics under cheatgrass and sagebrush-steppe plant communities. Journal of Arid Environments **57**:445-466.

Pimentel, D., R. Zuniga, and D. Morrison. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecological Economics **52**:273-288.

Rowe, H. I. and C. S. Brown. 2008. Native plant growth and seedling establishment in soils influenced by *Bromus tectorum*. Rangeland Ecology and Management **61**:630-639.

Rowe, H. I., C. S. Brown, and V. P. Claassen. 2007. Comparisons of mycorrhizal responsiveness with field soil and commercial inoculum for six native montane species and *Bromus tectorum*. Restoration Ecology **15**:44-52.

Saetre, P. and J. M. Stark. 2005. Microbial dynamics and carbon and nitrogen cycling following re-wetting of soils beneath two semi-arid plant species. Oecologia **142**:247-260.

Smith, J. L., J. J. Halvorson, and H. Bolton, Jr. 1994. Spatial relationships of soil microbial biomass and C and N mineralization in a semi-arid shrub-steppe ecosystem. Soil Biology & Biochemistry **26**:1151-1159.

Sperry, L. J., J. Belnap, and R. D. Evans. 2006. *Bromus tectorum* invasion alters nitrogen dynamics in an undisturbed arid grassland ecosystem. Ecology **87**:603-615.

Stewart, G. and A. C. Hull. 1949. Cheatgrass (*Bromus tectorum* L.)--an ecologic intruder in southern Idaho. Ecology **30**:58-74.

Chapter 2

Soil community patterns in *Bromus tectorum*-invaded ecosystems of the northern Great Basin

Nicole M. DeCrappeo and David A. Pyke

Abstract

Non-native invasive plants affect not only the aboveground vegetative components of ecosystems, but also soil surface, microbial, and invertebrate communities. At eight *Bromus tectorum* L. (cheatgrass)-invaded sites across the northern Great Basin, we examined whether geographic distance or local edaphic factors were more important in structuring soil communities. We also tested how restoration strategies, such as drill seeding, herbicide application, and nutrient manipulation, affected community function and composition. Biological soil crusts, which are integral parts of healthy sagebrush systems, were well developed with cyanobacteria, moss, or lichens at only two of eight sites. Cheatgrass litter dominated the soil surface at three of the high precipitation sites, and bare ground was predominate at three sites with high soil pH. Soil microbial function and structure, as measured by community level physiological profiles (CLPP), phospholipid fatty acids (PLFA), and nematode trophic group composition, grouped strongly by site; restoration treatment and seeded plant species had very little effect on community similarity when all sites were analyzed together. Soil communities that were most alike came from sites with similar edaphic factors, specifically high soil pH and percent bare ground. At the individual site level, we found that restoration treatments had varying effects on different components of the soil community. Cyanobacterial soil crust cover decreased at low precipitation sites after plant seeding using a low-impact rangeland drill, and carbohydrate utilization and bacterial and fungal biomass increased in plots treated with sugar. We propose using belowground community characteristics as indicators of areas that may benefit from restoration strategies.

Keywords: Biological soil crusts, soil microbial communities, CLPP, PLFA, soil nematodes, invasive plant species, *Bromus tectorum*, cheatgrass, *Artemisia tridentata*, Wyoming big sagebrush, sagebrush steppe ecosystems, Great Basin.

Introduction

Non-native invasive plant species potentially alter ecosystems by replacing native plant communities, varying the frequency or severity of disturbance regimes, altering above- and belowground food web dynamics, changing nutrient inputs and cycling, and potentially degrading economically important ecosystems (Chen and Stark 2000, Hilty et al. 2001). Although much work has focused on aboveground components of invaded systems, researchers have only recently begun to investigate potential impacts to the soil biotic community, including soil microbes, invertebrates, and biological soil crusts (Belnap and Phillips 2001, Kourtev et al. 2002, Hawkes et al. 2006). Soil organisms play vital roles in carrying out ecosystem processes and changes in their abundance, activity, or diversity should be understood in the context of broader landscape-level dynamics. The invasion of cheatgrass (*Bromus tectorum* L.) into Wyoming big sagebrush (*Artemisia tridentata* Nutt. ssp. *wyomingensis* Beetle & Young) communities in the northern Great Basin has resulted in increased fire frequency, loss of plant species richness, changes in the quantity and quality of litter inputs, decreased soil organic matter storage, and alteration of rhizosphere conditions (Stewart and Hull 1949, Bolton et al. 1993, Norton et al. 2004). These relatively new soil conditions may have a measurable effect on the abundance, diversity, and activity of certain groups of soil organisms. Further, plant community homogeneity may result in a more homogenous belowground community, and any loss in soil biological complexity may potentially affect ecosystem functioning (Wall 1999). Processes such as decomposition, primary productivity, nitrogen fixation and nutrient cycling are dependent upon activities of microbes and organisms that graze upon them, such as protozoa, nematodes and microarthropods (Wall and Moore 1999). As more species are lost or displaced from the soil matrix, we may see pronounced changes in the functioning of ecosystems.

In Wyoming big sagebrush ecosystems, a patchy distribution of shrubs, perennial bunchgrasses, and interspaces covered with biological soil crusts creates

islands of fertility and resource heterogeneity across the landscape (Bolton et al. 1993, Halvorson et al. 1994, Halvorson et al. 1997). Soil organisms and communities simultaneously respond to and help maintain this heterogeneity. Biological soil crusts, which are assemblies of lichens, mosses, cyanobacteria, fungi, and bacteria, play important ecological roles in arid and semiarid systems (Belnap et al. 2001). Great Basin soils tend to be silty or loamy and lack large accumulations of organic matter that stabilize soils in more productive ecosystems. Biological soil crusts, with their dense networks of cyanobacterial filaments and fungal hyphae, promote soil aggregate stability (Belnap and Gillette 1998, Rosentreter and Eldridge 2003), and contribute a significant amount of nitrogen to soil via the presence of free-living and lichen-associated nitrogen-fixing cyanobacteria (Belnap 2002). Disturbances can affect biological soil crusts directly or indirectly. Repeated grazing by large ungulates can physically crush crusts and decrease species richness (Rogers and Lange 1971, Anderson et al. 1982), while fire can kill mosses and lichens and change the amount of space available for crust colonization by altering plant community composition and litter quantity (Johansen 2001, Hilty et al. 2004).

In the soil profile, free-living bacteria and fungi play roles in decomposition, nutrient cycling, and the formation of soil organic matter and stable soil aggregates. Soil nematodes occupy important positions as primary and secondary consumers in belowground food webs (Hunt et al. 1987), affect nutrient cycling dynamics and plant community structure (Yeates 1979, Wardle et al. 2004), and can be used as bioindicators of soil ecosystem status (Bongers 1990, Bongers and Ferris 1999, Porazinska et al. 1999). The importance of soil communities in biogeochemical cycling and plant-soil relations are relatively well understood, however researchers are just beginning to construct paradigms concerning microbial biogeography and factors that affect their patterns of biomass, community structure, and functional diversity (Fierer and Jackson 2006, Green and Bohannan 2006, Martiny et al. 2006, Fierer et al. 2009).

As land managers attempt to slow the spread of cheatgrass and re-establish native shrubs and perennial bunchgrasses, there is a unique opportunity to examine the potential impacts of restoration treatments on soil communities. Techniques for restoring plant communities commonly include the use of chemicals, prescribed burns, and physical techniques to prepare and seed lands (Monsen et al. 2004). However, little is known about how these techniques may impact soil communities in the northern Great Basin. The aim of this study was to characterize biological soil crust cover and diversity, soil microbial function and community composition, and soil nematode trophic structure in conjunction with two restoration experiments conducted at eight cheatgrass-dominated sites across the northern Great Basin and the Snake River Plain, USA. We had three objectives: 1) Determine whether geographic distance or local edaphic factors were more important in structuring soil communities; 2) Test how restoration techniques (drill seeding, herbicide application, and nutrient manipulation) affect soil community function and composition; 3) Assess the influence of individual plant species on belowground communities. Our work is the first comprehensive look at soil community dynamics in cheatgrass-dominated areas of the Wyoming big sagebrush biome.

Materials and Methods

Site descriptions

Soil community sampling and biological soil crust surveys were conducted in conjunction with restoration experiments at eight sites in the Snake River Plain (eastern Oregon and southwestern Idaho) and the northern Great Basin (northwestern Utah and northern Nevada), hereafter referred to as the northern Great Basin (Table 2.1, Fig. 2.1). All sites were on lands managed by the Bureau of Land Management (BLM) and classified as Wyoming big sagebrush/perennial bunchgrass ecological sites by the Natural Resources Conservation Service (USDA NRCS). Ecological site designations take into account climate, soil, and hydrologic conditions in addition to

potential plant community composition and productivity. Within each state, one low- and one high-precipitation (mean annual precipitation: 20-25 cm and 25-30 cm, respectively) site was established to encompass the typical precipitation range of Wyoming big sagebrush rangelands. Vegetation at all study sites was dominated by cheatgrass with relative cover of native perennials being < 20%. Two restoration experiments with separate aboveground plant objectives began in October 2003 and were maintained and monitored until June 2005.

Transition Species experiment

The Transition Species experiment was established to assess the competitiveness of 25 different plant accessions against cheatgrass (Allcock et al. 2006). Following principals from state-and-transition ecological models (Westoby et al. 1989, Briske et al. 2008), researchers wished to identify plant species that could be used as a transition stage during restoration from a cheatgrass-dominated stable state to sagebrush/bunchgrass reference plant community state. At each site, 25 subplots (3-m x 6-m) were assigned within six plots (21-m x 36.5-m) in a randomized split-plot design (Fig. 2.2A). Three plots were treated with the herbicide glyphosate (Roundup® or Rodeo® with a surfactant at 0.7 kg ai ha^{-1}) in spring 2003 to kill cheatgrass prior to experimental seeding. The remaining three plots were untreated, and cheatgrass was allowed to grow and compete with seeded accessions (Fig. 2.2A). In October 2003, 25 different plant accessions were seeded into the subplots using a low-impact rangeland drill (Truax Rough Rider®).

We conducted three soil surface cover surveys to assess if biological soil crust cover and composition changed with restoration treatments. The first survey occurred in October 2003 before subplots were seeded but after herbicide was applied. Follow-up surveys were conducted in March 2004 and 2005. We were interested in the effects of both the herbicide and the drill treatment on biological soil crust organisms. We randomly established four, 15-m transects in each plot. Two “drill” transects crossed

rows of five drill subplots, while two "control" transects were located outside the subplot grid but within the herbicide-treated boundaries of the plot. At Cinder Cone Butte, ID, we set-up three drill transects within each plot due to very high crust cover and diversity. We used a point-intercept frame with a 25- x 25-cm frame divided into a 5- x 5-cm grid to assess soil surface cover and biological soil crust composition (Fig. 2.3A). A nail was placed in the ground every 2 m (8 nails per transect), and the frame was placed on the right side of the measuring tape with the lower right corner touching the nail. The sampling frame was placed over the nail in the same position at each of the three survey dates. A pinflag pin (1.5-mm diameter) was lowered in the lower left corner of each of the frame's 20 grid points; we recorded information for 320 points per treatment x drill combination per plot (20 points per frame x 8 frames x 2 transects) at each site (except Cinder Cone Butte, where we surveyed 480 points in the drill transects). We recorded surface cover (bare ground, cyanobacteria, lichen, moss, cow patty, physical crust, rock, litter, or vascular plant) and specific information about the biological soil crust organism if one was present.

Soil aggregate stability, which is a qualitative measure of soil texture, organic matter content, microbial activity, and permeability, was determined using the slake test method (Herrick et al. 2001). Surface soils were collected from a total of 18 samples per plot per site. Stability was rated according to the time required for a small (~6 mm) ped to disintegrate during a 5-min immersion in water. Soils were categorized based on the proportion of soil fragment remaining after five extraction-immersion cycles. The higher the stability class, the more stable the soil surface (Table 2.1).

Soil samples for microbial and nematode community analyses were collected in March 2004, when plants were beginning to break dormancy, and again in May 2004 at the height of the growing season. We collected soils from 14 of the 25 accession subplots (Table 2.2), focusing mostly on native plant species that are found in intact sagebrush steppe communities. The exception was a hybrid crested

wheatgrass (*Agropyron cristatum* [L.] Gaertn x *desertorum* [Fisch. ex Link] Schult.), a non-native perennial that is seeded in rangelands as livestock forage or for post-fire soil stabilization or rehabilitation. Soils collected from accessions of the same species were pooled into a single sample. For example, we bulked two randomly collected cores from each of the four Sandburg bluegrass accession subplots, for a total of eight cores, to be used as one Sandberg bluegrass sample. Soil cores were collected using a 2 cm diameter soil corer to a depth of 10-12 cm, stored in coolers with icepacks, and placed in a 4°C refrigerator upon return to the laboratory. Within five days of collection, soils were homogenized, passed through a 2 mm sieve, and processed for pH_{water}, gravimetric soil moisture (oven-dried at 105°C for 24 h), water potential (filter paper method, Kaya and Stock 1997), and the microbial and nematode community analyses described below. Separate soil samples were collected from herbicide-treated and control plots and sent to B. Blank (USDA-ARS, Reno, NV) for soil texture analysis and quantification of NH_4^+-N, NO_3^--N, Mn, and Fe. Soil temperature was also measured in the field when soil cores were collected. We used a digital soil thermometer (Fisher Scientific, Pittsburgh, PA) to measure temperature in the top 0-10 cm of soil at three randomly selected points in each plot.

Functional Groups experiment

The Functional Groups experiment was established to determine if the depletion of soil nitrogen and other soil resources affects the competitive interactions between cheatgrass and native species. Two techniques were used to reduce soil resources: 1) application of carbon (C) to promote microbial immobilization of soil nitrogen (N) (McLendon and Redente 1992, Blumenthal et al. 2003), and 2) seeding with a mix of native species that differed in growth form, rooting characteristics, and phenology. At each site, six plots (15.5-m x 23-m) with 18 subplots (1.5-m x 2.5-m) were established in a randomized split-plot design (Fig. 2.2B). Three plots were treated with granulated sucrose (1500 kg C ha^{-1}) and the three remaining plots were left untreated. Each

subplot was hand-seeded (broadcasted and raked lightly into the soil) with either a monoculture or mixture of species. We randomly collected soils from within six of the 18 subplots (Table 2.3), focusing on perennial species mixes and their interactions with cheatgrass. Soils were collected at the same time and handled and processed in the same manner as those in the Transition Species experiment. We did not perform biological soil crust surveys or determine soil aggregate stability in the Functional Groups experiment plots.

Community level physiological profiles (CLPPs)

Community level physiological profiling (CLPP) was performed using Biolog EcoPlates™ (Biolog Inc., Hayward, CA, USA) to qualitatively determine soil microbial functional diversity (Sinsabaugh et al. 1999). Each EcoPlate consists of a 96-well microtiter plate filled with three replicates of 31 different carbon substrates and one water control. Each well is also filled with tetrazolium violet dye that is reduced to an insoluble purple formazan derivative as the inoculated microbes utilize the carbon source. The amount of well color development over five days represents the microbial community's ability to effectively use a substrate; the underlying assumption is that highly diverse microbial communities will utilize more substrates more completely (Sinsabaugh et al. 1999). Functional diversity cannot be correlated with taxonomic diversity because more than one microbial taxon will generally be able to use each substrate (Staddon et al. 1997).

For the Transition Species experiment, we collected soils from subplots seeded with plant species listed in Table 2.2 in both March and May 2004. To reduce CLPP processing time and equipment costs, we pooled soil samples from plots with the same treatment for each plant species. Thus we processed 14 EcoPlates samples (7 plant species x 2 treatments) per site per sampling date. For the Functional Groups experiment, we collected soils from subplots seeded with each of the six species mixtures listed in Table 2.3. As in the Transition Species experiment, we pooled soil

samples from the same treatment plots for each species mixture. A total of 12 EcoPlate samples (6 species mixtures x 2 treatments) per site were processed per sampling date.

In the laboratory, 1 g of soil was placed in 99 mL phosphate buffer with $MgCl_2$ (Fisher Scientific, Pittsburgh, PA, pH = 7.2) and placed in a refrigerator overnight. The following day samples were shaken for 20 min at 160 rpm on a clinical rotator, resulting in a well-mixed soil slurry. For each sample, 100 μL of the slurry was pipetted under a laminar flow hood into an EcoPlate. Plates were incubated at room temperature and color development was determined using a PowerWave X 340 spectrophotometer at a wavelength of 596 nm. The absorbance values, which represent the microbial community's ability to utilize a particular substrate, were recorded at 24-h intervals for 5 consecutive days. The average well color development (AWCD) method was used to standardize for differences in initial inoculum density. We subtracted the average optical density value of the three water wells from the 93 remaining wells and changed all resulting negative values to zero. We then calculated the AWCD for each plate, divided the data by the AWCD, and averaged the three substrate replicates (Kohler et al. 2005). We used values from the day when the AWCD $\geq$ 0.2 (Garland 1996, Grayston et al. 1998), thereby creating a matrix of data obtained from 48, 72, 96, and 120 h readings. The 31 carbon substrates were categorized into five biochemical guilds (amino acids, amines/amides, carbohydrates, carboxylic acids, and polymers) (Kohler et al. 2005) for community ordinations and statistical analyses. Because CLPPs provide only a qualitative assessment of functional diversity and, with few exceptions, every substrate was used to some degree in every sample, we did not use the absorbance values to calculate ecological diversity indices.

Lipid analysis

Fatty acids are found in cell membranes of microbes, and certain groups have "signature" lipids that serve as identifiers. These signature lipids are used to create a

taxonomic fingerprint of the microbial community, which includes gram-positive and gram-negative bacteria, actinomycetes, fungi, and protozoa. Fatty acid analysis is also used to generate estimates of microbial biomass, relative taxa abundances, and fungal to bacterial ratios (Bailey et al. 2002).

Fatty acid samples were processed for all plant species monocultures and mixtures in both experiments (Tables 2.2 and 2.3). Unlike the CLPP analysis, we did not pool soils based on treatments, but rather kept samples from each of the six plots separate. However, due to high processing costs we only extracted fatty acids from one control plot and one treated plot at each site. For the Transitional Species experiment, there were 14 samples (7 plant species x 1 plot x 2 treatments) per site per sampling date. For the Functional Groups experiment, there were 12 samples (6 species mixtures x 1 plot x 2 treatments) per site per sampling date.

Subsamples were freeze-dried and stored in a 0°C freezer in vacuum-sealed packages in sealed containers until further processing. We used a hybrid phospholipid fatty acid (PLFA) and fatty-acid methyl ester (FAME) technique (Smithwick et al. 2005) based on a modified Bligh and Dyer (1959) method. Samples were homogenized using a mortar and pestle, and 4 g subsamples were weighed into hexane-rinsed Teflon™ centrifuge tubes. Whole fatty acids were extracted from soils using a chloroform-methanol extractant in phosphate buffer (4 mL $CHCl_3$, 8 mL MeOH, 3.6 mL 0.1 M phosphate buffer [pH = 7.0]). Samples were covered, shaken for 1 h, and centrifuged for 10 min at 2500 rpm. The supernatant liquid was decanted into hexane-rinsed glass test tubes, and 3.6 mL phosphate buffer and 4 mL $CHCl_3$ were added to each sample. Test tubes were shaken by hand for 1 min and stored at room temperature in the dark overnight to allow for phase separation. After aspirating off the top layer, the chloroform phase was dried using N_2 gas. Saponification and methylation followed the Microbial ID Inc. (Hayward, CA) protocol. For saponification, we added 1 mL of a NaOH-MeOH solution to test tubes and placed the tubes in a 95°C water bath for 30 min. For methylation, we added 2 mL of a 1:1.2

MeOH-6 N HCl solution to tubes and heated them in an 80°C water bath for 10 min. Extraction was performed by adding 1.25 mL of a 1:1 MTBE-hexane solution. The resulting FAMEs were analyzed on an Agilent 6890 gas chromatograph (Agilent Technologies, Santa Clara, CA) with an HP Ultra™-2 (5% phenylmethylpolysiloxane) column and a flame ionization detector. Chromatograph peaks were aligned using GC ChemStation software version A.08 (Agilent Technologies, Santa Clara, CA).

The nomenclature used for fatty acids is as follows: the number before the colon is the total number of carbon atoms, the number following the colon is the number of double bonds, and 'ω' indicates the location of the double bond from the methyl end of the molecule. The prefixes '*i*' and '*a*' refer to iso and anteiso-branched fatty acids, while '*cy*', 'Me', and 'OH' indicate cyclopropane, methyl, and hydroxy groups, respectively. Total bacterial biomass was estimated by summing abundances of gram-positive bacterial biomarkers *i*14:0, *i*15:0, *a*15:0, *i*16:0, *i*17:0 and gram-negative bacterial biomarkers *cy*17:0, 16:1ω9c, 17:1ω9c, 18:1ω 7c, *cy*19:0. Actinobacteria were identified as 10Me16:0, 10Me17:0, and 10Me18:0. Saprotrophic and arbuscular mycorrhizal (AM) fungal biomass were estimated using fatty acids18:2ω6,9c and 16:1ω5c, respectively (Balser et al. 2004). Protozoan biomarkers were 20:4ω6c and 20:2ω6,9c. Total microbial biomass was estimated using the aforementioned markers in addition to the following non-specific fatty acids: 11Me18:1ω7c, 14:0, 15:0, 15:0 3OH, *a*15:1, i15:1, 16:0, 16:1 2OH, 16:1 3OH, 16:1ω7c, 17:0, 17:1ω8c, 18:0, 18:1ω5c, 18:1ω9c, 18:3ω6,9,12c, 19:0, 19:0 2OH, 19:1ω8t, 20:0, 21:0, and 22:0. For community analysis, the relative proportions (mol %) of all 41 biomarkers were calculated and used to create data matrices for each sampling date for both experiments.

Soil nematodes

For both experiments, nematode samples were processed for all plant species monocultures and mixtures listed in Tables 2.2 and 2.3. We did not extract nematodes

from Lincoln Bench, OR, soils due to prohibitively high soil moisture levels at the time of collection. For the Transitional Species experiment, there were a total of 42 samples (7 plant species x 3 plots x 2 treatments) per site per sampling date. For the Functional Groups experiment, there were 36 samples (6 species mixtures x 3 plots x 2 treatments) per site per sampling date. Soil nematodes were extracted using the sugar centrifugation technique (Kaya and Stock 1997), which separates live and dead adults, juveniles, and eggs from the soil matrix using a series of sieves in conjunction with density-dependent flotation. A subsample of 100 g of soil was mixed in 800 mL of water and stirred in a figure-8 pattern for 30 sec to 1 min. The mixture was allowed to settle for 1 min and then poured over two stacked sieves with 250 μm and 38 μm openings. The silt and nematodes remaining on the bottom sieve were poured into a 50 mL centrifuge tube. Samples were centrifuged at 3000 rpm for 3 min, and after pouring off half of the water, the tube was filled with a 1M sucrose solution and centrifuged again at 3000 rpm for 3 min. Nematodes remaining in the supernatant were poured onto a small sieve, rinsed thoroughly with tap water, and transferred to a glass vial. Samples were refrigerated at 4°C until identifications could be completed. Nematode counts and trophic group identifications were performed on a Leica inverted interference contrast compound microscope. Nematodes were categorized into one of the following six trophic groups based on their feeding structures: bacterial feeders, fungal feeders, root associates, plant parasites, omnivores, and predators (Porazinska et al. 2003).

Statistical analyses

Multivariate statistical analyses on soil biological community data were performed using PC-Ord version 5.11 beta (McCune and Mefford 1999). Nonparametric multidimensional scaling (NMS) with the Sørensen distance measure was used to assess soil community patterns at the sampling date, site, treatment, and plant species levels. NMS is an iterative ordination technique that attempts to find a stable solution

with minimum stress, or departure from monotonicity in reduced ordination space (McCune and Grace 2002). The medium setting of 'autopilot' was used with random starting configurations and 50 runs with real data. Monte Carlo randomization tests were performed against real data to evaluate the significance of the *k*-dimensional solution. Prior to analysis, CLPP data were square root transformed, PLFA and surface cover proportion data were arcsine square root transformed, and nematode data were relativized by the maximum value in each sample. Outliers were identified as sample units with an average Sørensen distance greater than 2.5 standard deviations from the mean that exhibited undue influence on ordination results; these were deleted from the dataset. Phospholipid fatty acid data from Canyon Creek were not used in any analyses because all values were outliers and, when used, forced 1-dimensional ordination solutions. Statistical significance and strength of *a priori* within-in group membership were tested using one-way multi-response permutation procedures (MRPP) with Sørensen distance, which provides a *p*-value and effect size, *A*, that is independent of sample size. Treatment effects on total nematode abundances at each site were analyzed with two-sample t-tests in SAS 9.2 (SAS Institute, Cary, NC, USA).

Results

Abiotic soil factors

Abiotic soil factors (soil moisture, water potential, soil temperature, and pH) varied across sampling dates and sites (Table 2.4). Percent gravimetric soil moisture was always higher in March than in May, with Idaho and Oregon sites having the highest soil moisture in March. Water potential, which represents soil water available to plants and soil organisms, was most negative in May for all sites. However soil moisture at Succor Creek, the site with the highest clay content (Table 2.1), remained high in May compared to the other sites. Soil temperature was higher in May than March at all sites (Table 2.4). There were large differences in soil pH among sites, ranging from 7.05 at

Lincoln Bench (high precipitation site with high percentage of litter and organic matter) to 8.68 at Simpson Springs (low precipitation site with high percentage of bare ground and little organic matter) (Table 2.5). Vernon Hills control soils had high pH, NO_3^--N, and total N and the lowest levels of Mn, and Fe (Table 2.5).

Transition Species experiment

Site was the most important factor structuring soil surface community patterns in the Transition Species experiment (Table 2.6, Fig. 2.4A). There were no significant groupings by survey date, herbicide treatment, or drill treatment when all sites were analyzed together (Table 2.6). Separation of sites in ordination space was related to soil pH and the proportion of cyanobacteria along axis 1 and the proportions of bare ground, litter, lichens, and mosses on axis 2 (Fig. 2.4A). Cinder Cone Butte (Fig. 2.3B) had the highest proportion of moss (19%) and lichen (2%) cover and Izzenhood Ranch had the highest cyanobacterial crust cover (40%) (Fig. 2.5). Most sites had a large proportion of litter, but Lincoln Bench and Canyon Creek were particularly high with thick thatch covering 95 and 87%, respectively, of the soil surface (Fig. 2.5). Simpson Springs (Fig. 2.3D) had the highest proportion (71%) of bare ground and physical soil crust (Fig. 2.5). Although survey date and drill treatment were not significant grouping variables across sites, we did find changes in soil surface community composition at some individual sites. There was a slight decrease in bare ground and increase in biological crust cover at Cinder Cone Butte from October 2003 to March 2004 (Figs. 2.6A and B). We found dramatic changes in soil surface community composition at Izzenhood Ranch and Simpson Springs (Figs. 2.6C - F), where the proportion of cyanobacterial cover declined and bare ground increased at both sites after the rangeland drill seeding event took place in November 2003.

Community level physiological profiles were strongly patterned by site (Table 2.7). CLPP patterns were similar in March and May 2004, and treatment and seeded plant species were not significant grouping variables when all sites were analyzed

together (Table 2.7). In March 2004, Nevada sites clustered closely together, and Lincoln Bench, Simpson Springs, and the Idaho sites were tightly clustered in the opposite corner of the ordination space (Fig. 2.4B). There were no CLPP guilds or environmental gradients that correlated with the ordination axes (Fig. 2.4B).

As with CLPP patterns, PLFA communities were strongly patterned by site at both sampling dates (Table 2.7). The high pH sites Simpson Springs and Vernon Hills were separated in ordination space from the other sites (Fig. 2.4C). Soil pH and moisture were correlated with axis 1 (r = -0.62 and 0.48, respectively) and bacteria and fungi were correlated with axis 3 (r = 0.69 and -0.47, respectively). Arbuscular mycorrhizal fungal abundance was correlated with both axes (r = -0.53, -0.52) and made up the largest proportion of the PLFA community at Simpson Springs and Vernon Hills (Fig. 2.4C).

As with the other soil community analyses, site was the most significant grouping variable for soil nematode trophic group structure at both sampling dates (Table 2.7). We found no evidence that treatment or seeded plant species were important for structuring the nematode community when all sites were analyzed together (Table 2.7). Similar to PLFA analysis, we found that the high pH sites (Succor Creek, Simpson Springs, and Vernon Hills) clustered together in ordination space (Fig. 2.4D). Nematode abundance was higher in May than March, with the highest numbers in control plots at Canyon Creek and Cinder Cone Butte (data not shown). In addition, there was a reduction in abundance with herbicide treatment at Canyon Creek in May (Table 2.8), but this trend was not found at any of the other sites.

Functional Groups experiment

In the Functional Groups experiment, site was again the most important grouping variable for CLPP, PLFA, and nematode communities (Table 2.7, Fig. 2.7). For CLPP communities, there was a great deal of overlap between sites in ordination space (Fig.

2.7A). Although treatment was not a significant grouping variable in March 2004 (A = 0.02, Table 2.7), sugar-treated plots from all sites except Vernon Hills and Simpson Springs separated from their respective control plots (Fig. 2.7A). Carbohydrate utilization increased in sugar-treated plots and was strongly correlated with axis 1 (r = 0.70).

We found a significant effect of the sugar treatment on PLFA community structure (Table 2.7). As in the Transition Species experiment, the high pH sites separated away from the other sites in ordination space, but there was a distinct shift from control plots (left side of graph) to sugar-treated plots (right side of graph) at all sites except Vernon Hills (Fig. 2.7B). The proportion of fungi in the PLFA community was highly correlated with axis 2 (r = 0.85) and clearly increased with the sugar treatment (Fig. 2.7B). Both bacterial and fungal biomass increased with sugar treatment at all sites (Figs. 2.8A and B). The proportion of AM fungi was highest at Simpson Springs and Vernon Hills (Fig. 2.7B).

As with the other soil community analyses, site was the most significant grouping variable for soil nematode trophic group structure at both sampling dates (Table 2.7). High pH sites again clustered together in ordination space, and these sites had a higher proportion of bacterial feeding nematodes (Fig. 2.7C). There was evidence of a slight shift in community structure with the sugar treatment at individual sites (Fig. 2.7C). Total nematode abundance increased in sugar-treated plots at five sites in May 2004 (Table 2.8). The number of bacterial feeding nematodes increased at all sites except Izzenhood Ranch (Fig. 2.8C), and fungal feeding nematode abundance increased at the two Oregon sites (Lincoln Bench and Succor Creek), but decreased at Izzenhood Ranch (Fig. 2.8D).

Discussion

Soil community composition varies due to a myriad of factors. Soil forming factors, such as climate, topography, parent material, vegetation, and time play important roles

at the landscape level, while local weather patterns, soil texture and aggregate structure, rhizosphere effects, soil nutrient status, and belowground food web dynamics work to create extremely heterogeneous habitats at the microsite level (Fig. 2.9). We were interested in characterizing soil community composition at both of these levels in conjunction with sagebrush steppe restoration experiments across the northern Great Basin. We found that soil communities were strongly patterned by site differences, and that those differences could be best explained by soil pH. Soil pH is an indirect and integrative measure of, among other things, precipitation, soil organic matter content, cation exchange capacity, and plant root and microorganism activity (Brady and Weil 2002, Kemmitt et al. 2006). Therefore, it is not surprising that pH emerged as an important abiotic factor in structuring microbial and nematode community patterns in these semi-arid ecosystems. Others have found that pH is important to microbial biogeography at large geographic scales (Fierer and Jackson 2006). In this study, there was some evidence that local edaphic factors were more important in shaping soil community structure than geographic distance. Succor Creek, the low precipitation and high pH site in Oregon, more consistently grouped with the high pH Utah sites than it did with the high precipitation Oregon site (Lincoln Bench) just 50 miles away. However, there was more variability in how soil communities at other sites were related to one another, with high and low productivity sites grouping in different patterns depending on the particular community measure. Cinder Cone Butte, the low precipitation site in Idaho with well-developed biological soil crusts, tended to group with the high precipitation sites and was usually the farthest away from the high pH sites in ordination space.

Restoration treatment effects were not detectable when all sites were analyzed together, but we did see some effects on soil community structure when sites were examined individually. In the Transition Species experiment, biological soil crust cover decreased after the rangeland drill seeding event in sites that initially had high proportions of cyanobacterial crust cover (Izzenhood Ranch and Simpson Springs,

Figs. 2.5B and C). We cannot attribute this effect to the drill itself, however, because crust cover also declined in control transects that were untouched by the drill. The decrease was actually the result of trampling by the many researchers and technicians who were walking around the site during the seeding events. It was our observation that the Izzenhood Ranch site was particularly disturbed, as the soil was loose and easily whipped into dust devils after seeding. Soil aggregate stability is low at this site (stability class = 2), and the cyanobacterial crust cover was probably important in reducing soil erosion by wind and water (Belnap et al. 2001). There was some recovery of cyanobacterial crust at Izzenhood Ranch in March 2005 (data not shown), but our data show that it is important for land managers and researchers to consider how the implementation of restoration treatments may affect the success or failure of the treatment itself. The benefits of a low impact rangeland drill may be lost on sites like Izzenhood Ranch when the soil is so easily disturbed by human trampling. Cinder Cone Butte, however, showed more resilience to the drill and trampling disturbance (Fig. 2.5A). Soil aggregate stability was higher (stability class = 4) and the dominant crust components were mosses and lichens. Sites with stable soils like Cinder Cone Butte may be more appropriate for this type of restoration tool.

In the Functional Groups experiment, the sugar treatment increased CLPP carbohydrate utilization, PLFA bacterial and fungal biomass, and bacterial-feeding nematode abundance at all sites (except nematode abundance at Izzenhood Ranch). Sugar stimulates opportunistic members of the soil community by providing a high-energy and easily degradable carbon source (Jonasson et al. 1996, Jones and Murphy 2007). The increase in fungal biomass was of particular interest in light of other work showing that fungal abundance and diversity decrease when the dominant plant species changes from sagebrush to cheatgrass (Chapter 3, this thesis). As a restoration tool, applying sugar may have the added benefit of not just limiting the nitrogen supply for cheatgrass, but helping to re-establish the fungal component of the soil ecosystem. However, it is important to note that we did not identify the specific fungal

groups that responded to the sugar, and it is possible that fast-growing zygomycetes were principally responsible for the increase in fungal growth. At Izzenhood Ranch, fungal-feeding nematodes were abundant in control plots but dropped in sugar-treated plots despite the increase in soil fungal abundance. Perhaps sugar stimulated the growth and activity of nematophagous fungi, which can trap and digest nematode prey using specialized hyphae or become endoparasites of nematodes (Morton 1998). Food web dynamics were not specifically studied, however we can presume that sugar-induced changes in the structure of soil communities affected shifts in food supply and belowground predator/prey relations.

We observed similar treatment differences in our March and May samplings, indicating that the effect of the sugar treatment lasted at least throughout the growing season. Witwicki et al. (submitted) found that sugar effects on C and N levels were evident in paired sagebrush/cheatgrass plots eight months after application. However, Brunson et al. (2010), working at the Canyon Creek and Lincoln Bench sites in 2005, found that the effects of varying dosages of sugar on microbial biomass did not last until the end of the growing season. This is an important factor to consider: if the goal of sugar treatment is to provide a competitive edge to perennial bunchgrasses over annual grasses, then longer-lasting sources of C or multiple C applications should be considered (Brunson et al. 2010). Additional work is needed to understand how sugar treatments and fungi may work together in promoting native plant growth.

We saw no plant species effects on microbial or nematode community patterns at any sites. It is well documented that plant species can alter soil communities (Grayston et al. 1998, Kuske et al. 2002, Batten et al. 2006, Callaway et al. 2008), but when we sampled in spring 2004, plants were still very small and rhizosphere effects were probably negligible. In a 2009 follow-up survey of the Oregon and Idaho sites, we found that a number of the seeded sagebrush and perennial bunchgrass plants had survived and grown into large individuals (N. DeCrappeo and D. Pyke, personal observation). These plants, however, are surrounded by acres of cheatgrass. Re-

sampling these plots now would allow us to assess whether soil communities are responding to the local sagebrush/bunchgrass rhizosphere environment or if they are still functionally and taxonomically similar to the general cheatgrass soil community.

Restoring cheatgrass-invaded areas is extremely difficult because of the myriad changes brought about by the grass (Westoby et al. 1989). Land managers must work to overcome changes in plant community structure, litter quantity and quality, soil nutrient distribution and availability, and fire frequency (D'Antonio and Vitousek 1992, Knapp 1996, Evans et al. 2001). Single herbicide or sugar treatments followed by seeding of native species are likely insufficient to restore perennial bunchgrass communities. In light of this, it would be prudent to choose sites with a high probability of restoration success, and the status of soil physical, chemical, and biological factors may be useful in guiding site selection. In our study, for example, Simpson Springs had low precipitation, high percent sand (46%), an extremely high proportion of bare ground, high soil pH, low levels of phosphorus (P) (B. Blank, unpublished data) and large proportions of AM fungi. Although it might seem that this site was an unlikely candidate for successful restoration, perennial bunchgrass establishment and persistence in the Transition Species experiment were much higher here than at any of the other sites studied (T. Monaco, T. Jones, J. Landmesser, and R. Nowak, unpublished data). Soil P complexes with carbonate and bicarbonate species such as $CaCO_3$ and $Ca(HCO_3)_2$ in high pH semi-arid soils, and it is likely that mycorrhizal associations are critical for plant acquisition of P under these conditions (Goodwin 1992). Seeded plants may have benefited from the high proportion of AM fungi at Simpson Springs. In contrast, the high productivity Idaho and Oregon sites had very low establishment and persistence of any seeded plant species, despite having higher precipitation, higher percent clay, more neutral pH, and higher levels of P and manganese (Mn) (B. Blank, unpublished data). Although seeded species at high productivity sites may have had more favorable soil conditions, they also had to compete with cheatgrass and medusahead plants growing at the field sites.

The development of soil quality indices or soil biological indicators (Bowker et al. 2008, Blecker et al. 2010) for sagebrush ecosystems may also help in identifying areas with a high probability of restoration success. This information, combined with other biotic and environmental factors, may help determine whether a site is a good candidate for native plant restoration.

Acknowledgements

We would like to thank E. DeLorenze, J. Boyd, C. Hesse, T. Wojtowicz, A. Larimer, L. Goldstein, and T. Lair for field and laboratory assistance. B. Blank and T. Morgan provided soil texture and nutrient data. This study was funded by the U.S. Geological Survey Forest and Rangeland Ecosystem Science Center's Coordinated Intermountain Restoration Project and the U.S. Department of Agriculture Initiative for Future Agriculture and Food Systems program (CREES Agreement No. 2001-52103-11322). Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

References

Allcock, K., R. Nowak, B. Blank, T. Jones, T. Monaco, J. Chambers, R. Tausch, P. Doescher, V. Satyal, T. J., D. Ogle, L. St. John, M. Pellant, D. Pyke, E. Schupp, and C. Call. 2006. Integrating weed management and restoration on western rangelands. Ecological Restoration **24**:199-300.

Anderson, D. C., K. T. Harper, and S. R. Rushforth. 1982. Recovery of cryptogamic soil crusts from grazing in Utah winter ranges. Journal of Range Management **35**:355-359.

Bailey, V. L., A. D. Peacock, J. L. Smith, and H. Bolton, Jr. 2002a. Relationships between soil microbial biomass determined by chloroform fumigation-extraction, substrate-induced respiration, and phospholipid fatty acid analysis. Soil Biology & Biochemistry **34**:1385-1389.

Bailey, V. L., J. L. Smith, and H. Bolton. 2002b. Fungal-to-bacterial ratios in soils investigated for enhanced C sequestration. Soil Biology & Biochemistry **34**:997-1007.

Balser, T. C., K. K. Treseder, and M. Ekenler. 2004. Using lipid analysis and hyphal length to quantify AM and saprotrophic fungal abundance along a soil chronosequence. Soil Biology & Biochemistry **37**:601-604.

Batten, K. M., K. M. Scow, K. F. Davies, and S. P. Harrison. 2006. Two invasive plants alter soil microbial community composition in serpentine grasslands. Biological Invasions **8**:217-230.

Belnap, J. 2002. Nitrogen fixation in biological soil crusts from southeast Utah, USA. Biology and Fertility of Soils **35**:128-135.

Belnap, J. and D. A. Gillette. 1998. Vulnerability of desert soil surfaces to wind erosion: impacts of soil texture and distrubance. Journal of Arid Environments **39**:133-142.

Belnap, J. and S. L. Phillips. 2001. Soil biota in an ungrazed grassland: response to annual grass (*Bromus tectorum*) invasion. Ecological Applications **11**:1261-1275.

Belnap, J., R. Rosentreter, S. Leonard, J. H. Kaltenecker, J. Williams, and D. Eldridge. 2001. Biological soil crusts: ecology and management. United States Department of the Interior, Bureau of Land Management, National Science and Technology Center Information and Communications Group, Denver, CO.

Blecker, S. W., L. L. Stillings, M. C. Amacher, J. A. Ippolito, and N. M. DeCrappeo. 2010. Ecosystem health in mineralized terrane; data from podiform chromite (Chinese Camp mining district, California), quartz alunite (Castle Peak and Masonic mining districts, Nevada/California), and Mo/Cu porphyry (Battle Mountain mining district, Nevada) deposits. U.S. Geological Survey Open-File Report 2010-1040.

Bligh, E. G. and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemical Physiology **37**:911-917.

Blumenthal, D. M., N. R. Jordan, and M. P. Russelle. 2003. Soil carbon addition controls weeds and facilitates prairie restoration. Ecological Applications **13**:605-615.

Bolton, H., Jr., J. L. Smith, and S. O. Link. 1993. Soil microbial biomass and activity of a disturbed and undisturbed shrub-steppe ecosystem. Soil Biology & Biochemistry **25**:545-552.

Bongers, T. 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia **83**:14-19.

Bongers, T. and H. Ferris. 1999. Nematode community structure as a bioindicator in environmental monitoring. Trends in Ecology and Evolution **14**:224-228.

Bowker, M. A., M. E. Miller, J. Belnap, T. D. Sisk, and N. C. Johnson. 2008. Prioritizing conservation effort through the use of biological soil crusts as ecosystem function indicators in an arid region. Conservation Biology **22**:1533-1543.

Brady, N. C. and R. R. Weil. 2002. The nature and property of soils. Prentice Hall, Upper Saddle River, NJ, USA.

Briske, D. D., B. T. Bestelmeyer, T. K. Stringham, and P. L. Shaver. 2008. Recommendations for development of resilience-based state-and-transition models. Rangeland Ecology & Management **61**:359-367.

Brunson, J. L., D. A. Pyke, and S. S. Perakis. 2010. Yield responses of ruderal plants to sucrose in invasive-dominated sagebrush steppe of the northern Great Basin. Restoration Ecology:doi: 10.1111/j.1526-1100X.2009.00644.x.

Callaway, R. M., D. Cipollini, K. Barto, G. C. Thelen, S. G. Hallett, D. Prati, K. Stinson, and J. Klironomos. 2008. Novel weapons: invasive plant suppresses fungal mutualists in America but not in its native Europe. Ecology **89**:1043-1055.

Chen, J. and J. M. Stark. 2000. Plant species effects and carbon and nitrogen cycling in a sagebrush-crested wheatgrass soil. Soil Biology & Biochemistry **32**:47-57.

D'Antonio, C. M. and P. M. Vitousek. 1992. Biological invasions by exotic grasses, the grass/fire cycle, and global change. Annual Review of Ecology and Systematics **23**:63-87.

Evans, R. D., R. Rimer, L. Sperry, and J. Belnap. 2001. Exotic plant invasion alters nitrogen dynamics in an arid grassland. Ecological Applications **11**:1301-1310.

Fierer, N. and R. B. Jackson. 2006. The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Sciences of the United States of America **103**:626-631.

Garland, J. L. 1996. Analytical approaches to the characterization of samples of microbial communities using patterns of potential C source utilization. Soil Biology & Biochemistry **28**:213-221.

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.

Grayston, S. J., S. Q. Wang, C. D. Campbell, and A. C. Edwards. 1998. Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biology & Biochemistry **30**:369-378.

Green, J. and B. J. M. Bohannan. 2006. Spatial scaling of microbial biodiversity. Trends in Ecology & Evolution **21**:501-507.

Halvorson, J. J., H. Bolton, and J. L. Smith. 1997. The pattern of soil variables related to *Artemisia tridentata* in a burned shrub-steppe site. Soil Science Society of America Journal **61**:287-294.

Halvorson, J. J., H. Bolton, J. L. Smith, and R. E. Rossi. 1994. Geostatistical analysis of resource islands under *Artemisia tridentata* in the shrub-steppe. Great Basin Naturalist **54**:313-328.

Hawkes, C. V., J. Belnap, C. D'Antonio, and M. K. Firestone. 2006. Arbuscular mycorrhizal assemblages in native plant roots change in the presence of invasive exotic grasses. Plant and Soil **281**:369-380.

Herrick, J. E., W. G. Whitford, A. G. de Soyza, J. W. Van Zee, K. M. Havstad, C. A. Seybold, and M. Walton. 2001. Field soil aggregate stability kit for soil quality and rangeland health evaluations. Catena **44**:27-35.

Hilty, J., L. Bond, M. Wicklow-Howard, and R. Rychert. 2001. Functional diversity of soil microbial communities: effects of exotic plant invasion and implications for restoration of semi-arid rangelands. Submitted to Bureau of Land Management, Idaho State Office and Lower Snake River District, Biology Department, Boise State University.

Hilty, J., D. J. Eldridge, R. Rosentreter, M. Wicklow-Howard, and M. Pellant. 2004. Recovery of biological soil crusts following wildfire in Idaho. Journal of Range Management **57**:89-96.

Hunt, H. W., D. C. Coleman, E. R. Ingham, R. E. Ingham, E. T. Elliott, J. C. Moore, S. L. Rose, C. P. P. Reid, and C. R. Morley. 1987. The detrital food web in a shortgrass prairie. Biology and Fertility of Soils **3**:57-68.

Johansen, J. R. 2001. Impacts of fire on biological soil crusts. Pages 385-397 *in* J. Belnap and O. Lange, editors. Biological soil crusts: structure, function, and management. Springer-Verlag, Berlin.

Jonasson, S., A. Michelsen, I. K. Schmidt, E. V. Nielsen, and T. V. Callaghan. 1996. Microbial biomass C, N and P in two arctic soils and responses to addition of NPK fertilizer and sugar: implications for plant nutrient uptake. Oecologia **106**:507-515.

Jones, D. L. and D. V. Murphy. 2007. Microbial response time to sugar and amino acid additions to soil. Soil Biology & Biochemistry **39**:2178-2182.

Kaya, H. K. and S. P. Stock. 1997. Techniques in insect nematology. Pages 281-324 *in* L. Lacey, editor. Manual of techniques in insect pathology. Academic Press, San Diego, CA.

Kemmitt, S. J., D. Wright, K. W. T. Goulding, and D. L. Jones. 2006. pH regulation of carbon and nitrogen dynamics in two agricultural soils. Soil Biology & Biochemistry **38**:898-911.

Knapp, P. A. 1996. Cheatgrass (*Bromus tectorum L*) dominance in the Great Basin desert. Global Environmental Change **6**:37-52.

Kohler, F., J. Hamelin, F. Gillet, J. Gobat, -M., and A. Buttler. 2005. Soil microbial community changes in wooded mountain pastures due to simulated effects of cattle grazing. Plant and Soil **278**:327-340.

Kourtev, P. S., J. G. Ehrenfeld, and M. Haggblom. 2002. Exotic plant species alter the microbial community structure and function in the soil. Ecology **83**:3152-3166.

Kuske, C. R., L. O. Ticknor, M. E. Miller, J. M. Dunbar, J. A. Davis, S. M. Barns, and J. Belnap. 2002. Comparison of soil bacterial communities in rhizospheres of three plant species and the interspaces in an arid grassland. Applied and Environmental Microbiology **68**:1854-1863.

Martiny, J. B. H., B. J. M. Bohannan, J. H. Brown, R. K. Colwell, J. A. Fuhrman, J. L. Green, M. C. Horner-Devine, M. Kane, J. A. Krumins, C. R. Kuske, P. J. Morin, S. Naeem, L. Ovreas, A.-L. Reysenbach, V. H. Smith, and J. T. Staley. 2006. Microbial biogeography: putting microorganisms on the map. Nature Reviews Microbiology **4**:102-112.

McCune, B. and J. B. Grace. 2002. Analysis of ecological communities. MjM Software Design, Gleneden Beach, OR, USA.

McCune, B. and M. J. Mefford. 1999. PC-ORD. Multivariate analysis of ecological data. MjM Software, Gleneden Beach, OR, USA.

McLendon, T. and E. F. Redente. 1992. Effects of nitrogen limitation on species replacement dynamics during early secondary succession on a semiarid sagebrush site. Oecologia **91**:312-317.

Monsen, S. B., R. Stevens, and N. L. Shaw. 2004. Restoring western ranges and wildlands, vol. 1. Gen. Tech. Rep. RMRS-GTR-136-vol-1, U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fort Collins, CO.

Morton, J. B. 1998. Fungi. Pages 72-93 *in* D. M. Sylvia, J. J. Fuhrmann, P. G. Hartel, and D. A. Zuberer, editors. Principals and applications of soil microbiology. Prentice Hall, Upper Saddle River, NJ, USA.

Norton, J. B., T. A. Monaco, J. M. Norton, D. A. Johnson, and T. A. Jones. 2004. Soil morphology and organic matter dynamics under cheatgrass and sagebrush-steppe plant communities. Journal of Arid Environments **57**:445-466.

Porazinska, D. L., R. D. Bardgett, M. B. Blaauw, H. W. Hunt, A. N. Parsons, T. R. Seastedt, and D. H. Wall. 2003. Relationships at the aboveground-belowground interface: plants, soil biota, and soil processes. Ecological Monographs **73**:377-395.

Porazinska, D. L., L. W. Duncan, R. McSorley, and J. H. Graham. 1999. Nematode communities as indicators of status and processes of a soil ecosystem influenced by agricultural management practices. Applied Soil Ecology **13**:69-86.

Rogers, R. W. and R. T. Lange. 1971. Lichen populations on arid soil crusts around sheep watering places in South Australia. Oikos **22**:93-100.

Rosentreter, R. and D. J. Eldridge. 2003. Monitoring rangeland health: using a biological soil crust stability index. Pages 74-76 *in* Seed and soil dynamics in shrubland ecosystems. U.S. Dept. of Agriculture, Forest Service, Rocky Mountain Research Station, Laramie, WY.

Sinsabaugh, R. L., M. J. Klug, H. P. Collins, P. E. Yeager, and S. O. Petersen. 1999. Characterizing soil microbial communities. Pages 318-348 *in* G. P. Robertson, D. C. Coleman, C. S. Bledsoe, and P. Sollins, editors. Standard soil methods for long-term ecological research. Oxford University Press, New York, NY.

Smithwick, E. A. H., M. G. Turner, K. L. Metzger, and T. C. Balser. 2005. Variation in NH_4^+ mineralization and microbial communities with stand age in lodgepole pine (*Pinus contorta*) forests, Yellowstone National Park (USA). Soil Biology & Biochemistry **37**:1546-1559.

Staddon, W. J., L. C. Duchesne, and J. T. Trevors. 1997. Microbial diversity and community structure of postdisturbance forest soils as determined by sole-carbon-source utilization patterns. Microbial Ecology **34**:125-130.

Stewart, G. and A. C. Hull. 1949. Cheatgrass (*Bromus tectorum* L.)--an ecologic intruder in southern Idaho. Ecology **30**:58-74.

Wall, D. H. 1999. Biodiversity and ecosystem functioning. BioScience **49**:107-108.

Wall, D. H. and J. C. Moore. 1999. Interactions underground: soil biodiversity, mutualism, and ecosystem processes. BioScience **49**:109-117.

Wardle, D. A., R. D. Bardgett, J. N. Klironomos, H. Setälä, W. H. van der Putten, and D. H. Wall. 2004. Ecological linkages between aboveground and belowground biota. Science **304**:1629-1633.

Westoby, M., B. Walker, and I. Noy-Meir. 1989. Opportunistic management for rangelands not at equilibrium. Journal of Range Management **42**:266-274.

Witwicki, D. L., P. S. Doescher, D. A. Pyke, S. S. Perakis, and N. M. DeCrappeo. submitted. Growth and ^{15}N retention of Wyoming big sagebrush communities and exotic annual grasslands following nitrogen and sugar additions. Oecologia.

Yeates, G. W. 1979. Soil nematodes in terrestrial ecosystems. Journal of Nematology **11**:213-229.

Table 2.1 Locations and soil characteristics for eight study sites in the northern Great Basin.

Site	Latitude Longitude	Precip. Zone[1]	Soil map unit[2]	Taxonomic class	Sand (%)	Clay (%)	Stability class[3]
Canyon Creek, ID	43˚17'36"N 115˚44'49"W	High	Lanktree-Chilcott loams, 0 to 12 percent slopes	Xeric Haplargids	28.3	26.4	4
Cinder Cone Butte, ID	43˚14'49"N 115˚54'9"W	Low	Lankbush-Jenness association, 0 to 4 percent slopes	Xeric Haplargids	37.6	19.0	4
Eden Valley, NV	41˚14'1"N 117˚23'56"W	High	Hunnton-Zevadez-Enko association	Xeric Argidurids	36.6	21.1	2
Izzenhood Ranch, NV	40˚58'10"N 116˚58'39"W	Low	Enko fine sandy loam, 2 to 8 percent slopes	Durinodic Xeric Haplocambids	40.0	13.3	2
Lincoln Bench, OR	43˚53'51"N 117˚7'59"W	High	Not mapped		21.9	20.3	4
Succor Creek, OR	43˚36'3"N 117˚7'22"W	Low	Not mapped		24.0	33.8	2
Vernon Hills, UT	40˚3'49"N 112˚18'37"W	High	Taylorsflat loam, 1 to 5 percent slopes	Xeric Haplocalcids	35.9	22.0	2
Simpson Springs, UT	40˚8'49"N 112˚52'12"W	Low	Timpie silt loam, saline, 0 to 4 percent slopes	Typic Torriorthents	46.0	16.0	2

[1]Mean annual precipitation: low = 20-25 cm; high = 25-30 cm

[2]From USDA-NRCS Web Soil Survey (http://websoilsurvey.nrcs.usda.gov)

[3]Soil aggregate stability determined using the slake test method (after Herrick et al. 2001). Stability class 2 = 50% of structural integrity lost 5-30 sec. after insertion in water; stability class 4 = 10-25% of soil remaining on sieve after 5 dipping cycles.

Table 2.2 Plant species and accessions for the Transition Species (TS) experiment under which soil samples were collected for community level physiological profiling (CLPP), phospholipid fatty acid (PLFA) analysis, and nematode abundance and trophic group composition.

Common name	Accession	Scientific name
Crested wheatgrass	CD-II	*Agropyron cristatum* x *desertorum* (L.) Gaertn.
Big squirreltail	Sand Hollow	*Elymus elymoides* (Raf.) Swezey
Snake River wheatgrass	Secar	*Elymus wawawaiensis* J. Carlson & Barkworth
Snake River wheatgrass	SERDP	*E. wawawaiensis*
Basin wildrye	Magnar	*Leymus cinereus* (Scribn. & Merr.) A. Löve
Basin wildrye	Trailhead	*L. cinereus*
Sandberg bluegrass	Hanford Source	*Poa secunda* J. Presl
Sandberg bluegrass	High Plains	*P. secunda*
Sandberg bluegrass	Mountain Home	*P. secunda*
Sandberg bluegrass	Sherman	*P. secunda*
Bluebunch wheatgrass	Anatone	*Pseudoroegneria spicata* (Pursh) A. Löve
Bluebunch wheatgrass	Goldar	*P. spicata*
Bluebunch wheatgrass	P-12	*P. spicata*
Bluebunch wheatgrass	P-7	*P. spicata*

Table 2.3 Plant species monocultures and mixes for the Functional Groups (FG) experiment under which soil samples were collected for community level physiological profiling (CLPP), phospholipid fatty acid (PLFA) analysis, and nematode abundance and trophic group composition.

Common name(s)	Scientific name(s)
Cheatgrass	*Bromus tectorum* L.
Unseeded	--
Mix[a]	Mix
Mix + cheatgrass	Mix + *B. tectorum*
Vavilov Siberian wheatgrass	*Agropyron fragile* (Roth) P. Candargy
Vavilov Siberian wheatgrass + cheatgrass	*A. fragile* + *B. tectorum*

[a]'Mix' refers to a mixture of six native species: Wyoming big sagebrush, high plains Sandberg bluegrass, sand hollow big squirreltail, Anatone bluebunch wheatgrass, common yarrow (*Achillea millefolium* L.*)*, and globemallow (*Sphaeralcea ambigua* A. Gray)

Table 2.4 Means (SE) for abiotic soil parameters measured at eight sites in the northern Great Basin in March and May 2004.

Site	Sampling date	Gravimetric soil moisture (%)	Water potential (MPa)	Soil temperature (ºC)
Canyon Creek, ID	March	26.5 (0.5)	0.00 (0.00)	6.5 (0.1)
	May	10.4 (0.4)	-2.12 (0.32)	24.2 (0.3)
Cinder Cone Butte, ID	March	19.0 (0.4)	-0.01 (0.00)	10.8 (0.2)
	May	7.0 (0.3)	-4.66 (1.09)	15.7 (0.2)
Eden Valley, NV	March	15.9 (0.4)	-0.33 (0.05)	11.0 (0.2)
	May	11.9 (0.2)	-1.57 (0.21)	13.7 (0.4)
Izzenhood Ranch, NV	March	11.5 (0.3)	-0.07 (0.01)	14.1 (0.2)
	May	5.2 (0.2)	-10.45 (1.58)	23.0 (0.2)
Lincoln Bench, OR	March	28.5 (0.2)	0.00 (0.00)	7.8 (0.2)
	May	9.5 (0.2)	-4.61 (0.90)	22.2 (0.2)
Succor Creek, OR	March	26.8 (0.6)	-0.07 (0.01)	7.9 (0.1)
	May	19.8 (0.6)	-0.12 (0.00)	16.7 (0.2)
Vernon Hills, UT	March	15.0 (0.2)	-0.12 (-0.01)	11.3 (0.1)
	May	8.1 (0.3)	-17.71 (3.51)	21.8 (0.2)
Simpson Springs, UT	March	15.9 (0.4)	-0.05 (0.00)	13.9 (0.2)
	May	6.6 (0.2)	-23.68 (5.23)	25.1 (0.2)

Table 2.5 Soil chemical properties and enzyme activies from herbicide- (Transition Species experiment) or sugar- (Functional Groups experiment) treated and control plots at study sites across the northern Great Basin[1]. Yellow and gray highlights indicate the highest and lowest values, respectively, within a column for each experiment.

	pH		NH_4^+-N (µmol g^{-1} soil)[1]		NO_3^-N (µmol g^{-1} soil)[1]		Total N (µmol g^{-1} soil)[1]		Mn (µg g^{-1} soil)[2]		Fe (µg g^{-1} soil)[2]	
Transition Species	**Herb**	**Control**	**Herb**	**Control**	**Herb**	**Conttol**	**Herb**	**Control**	**Herb**	**Control**	**Herb**	**Control**
Canyon Creek, ID	7.02	7.14	0.074	0.061	0.210	0.201	0.284	0.261	15.681	11.784	10.045	8.855
Cinder Cone Butte, ID	7.48	7.36	0.066	0.013	0.498	0.194	0.564	0.207	9.655	10.513	10.565	8.524
Eden Valley, NV	7.59	7.48	0.195	0.014	0.136	0.724	0.331	0.738	3.664	4.510	4.352	5.137
Izzenhood Ranch, NV	7.47	7.49	0.026	0.048	0.908	0.828	0.934	0.877	4.478	3.175	4.191	3.440
Lincoln Bench, OR	6.97	7.05	0.016	0.071	0.621	0.547	0.636	0.617	6.697	6.757	11.123	10.320
Succor Creek, OR	8.52	8.36	0.023	0.052	0.334	0.331	0.358	0.383	4.067	4.160	3.016	2.681
Vernon Hills, UT	8.46	8.50	0.006	0.067	1.001	0.948	1.007	1.015	3.360	2.324	1.727	1.385
Simpson Springs, UT	8.68	8.66	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Functional Groups	**Sugar**	**Control**	**Sugar**	**Control**	**Sugar**	**Control**	**Sugar**	**Control**	**Sugar**	**Control**	**Sugar**	**Control**
Canyon Creek, ID	7.12	7.20	0.036	0.044	0.079	0.036	0.115	0.080	23.476	12.297	10.691	6.799
Cinder Cone Butte, ID	7.17	7.30	0.061	0.007	0.312	0.236	0.373	0.243	12.365	5.866	10.369	6.591
Eden Valley, NV	7.37	7.27	0.015	0.033	0.645	0.493	0.660	0.526	7.494	8.718	6.070	8.372
Izzenhood Ranch, NV	7.60	7.61	0.287	0.069	0.030	0.383	0.317	0.452	22.709	5.046	8.398	3.445
Lincoln Bench, OR	7.05	7.09	0.143	0.020	0.363	0.314	0.506	0.333	12.654	7.732	12.563	9.068
Succor Creek, OR	8.12	7.94	0.440	0.019	0.180	0.206	0.620	0.225	12.067	4.593	4.228	2.828
Vernon Hills, UT	8.54	8.49	0.149	0.048	0.417	0.573	0.565	0.621	2.515	2.368	1.397	1.492
Simpson Springs, UT	8.54	8.59	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

[1]KCl extractable
[2]DTPA extractable

Table 2.6 Multi-response permutation procedures (MRPP) *A*-statistics[1] and *p*-values for biological soil crust community surveys for each hierarchical level in the Transitional Species (TS) experiment. Values were generated using Sorensen distance measure. Shading indicates statistical significance[2].

	Date	***A*-statistic**	***p*-value**
Survey date	N/A	0.03	0.01
Site	October 2003	0.74	0.00
	March 2004	0.68	0.00
	March 2005	0.66	0.00
Treatment[3]	October 2003	-0.02	0.90
	March 2004	-0.01	0.48
	March 2005	0.00	0.43
Rangeland drill[4]	March 2004	-0.03	1.00
	March 2005	-0.03	1.00

[1] $A = 0$ when heterogeneity within groups equals expectation by chance; $A_{max} = 1$ when all items are identical within groups.
[2] *A*-statistic ≥ 0.10 accompanied by *p*-value < 0.05 is considered significant for ecological data (McCune and Grace 2002).
[3] Herbicide vs. control
[4] Rangeland drill seeding occurred after the October 2003 survey

Table 2.7 Multi-response permutation procedures (MRPP) *A*-statistics[1] (*p*-values) for soil community analyses for each hierarchical level in the Transitional Species (TS) and Functional Groups (FG) experiments. Values were generated using Sorensen distance measure with square root- (CLPP) or arcsine square root- (PLFA) transformed data. Shading indicates statistical significance[2].

		Transition Species			Functional Groups		
	Date	**CLPP**	**PLFA**	**Nematodes**	**CLPP**	**PLFA**	**Nematodes**
Sampling date	N/A	.03 (.00)	.01 (.00)	.02 (.00)	.01 (.00)	.00 (.04)	.00 (.01)
Site	March	.14 (.00)	.36 (.00)	.29 (.00)	.15 (.00)	.19 (.00)	.33 (.00)
	May	.12 (.00)	.36 (.00)	.40 (.00)	.10 (.00)	.19 (.00)	.30 (.00)
Treatment	March	.00 (.07)	.00 (.84)	.00 (.17)	.02 (.00)	.10 (.00)	.00 (.25)
	May	.00 (.59)	.00 (.13)	.00 (.25)	.01 (.02)	.14 (.00)	.01 (.03)
Plant species	March	-.01 (.93)	-.03 (.99)	-.01 (.92)	-.01 (.98)	-.03 (.99)	.00 (.66)
	May	-.01 (.93)	-.04 (.99)	-.01 (.93)	-.01 (.99)	-.03 (.99)	.00 (.80)

[1] $A = 0$ when heterogeneity within groups equals expectation by chance; $A_{max} = 1$ when all items are identical within groups.

[2] *A*-statistic ≥ 0.10 accompanied by *p*-value < 0.05 is considered significant for ecological data (McCune and Grace 2002).

Table 2.8 T-test *p*-values for treatment effects on total nematode numbers at each site for the Transitional Species (TS) and Functional Groups (FG) experiments. Transitional Species treatment = herbicide vs. control; Functional Groups treatment = sugar vs. control. Bold font indicates significance at the $p < 0.1$ level.

	Transitional Species				**Functional Groups**			
	March 2004		May 2004		March 2004		May 2004	
Site	*n*	*p*-value	*n*	*p*-value	*n*	*p*-value	*n*	*p*-value
Canyon Creek, ID	42	**0.01**	8	**0.03**	36	0.64	8	0.34
Cinder Cone Butte, ID	42	0.77	42	0.13	36	0.90	36	**0.07**
Eden Valley, NV	42	0.27	40	0.88	36	0.45	36	0.50
Izzenhood Ranch, NV	42	0.59	42	0.40	36	0.12	35	**<0.01**
Simpson Springs, UT	42	0.87	40	**0.02**	36	**0.02**	36	**<0.01**
Succor Creek, OR	42	0.38	42	0.99	36	**<0.01**	36	**<0.01**
Vernon Hills, UT	42	0.52	42	0.26	36	0.98	36	**0.06**

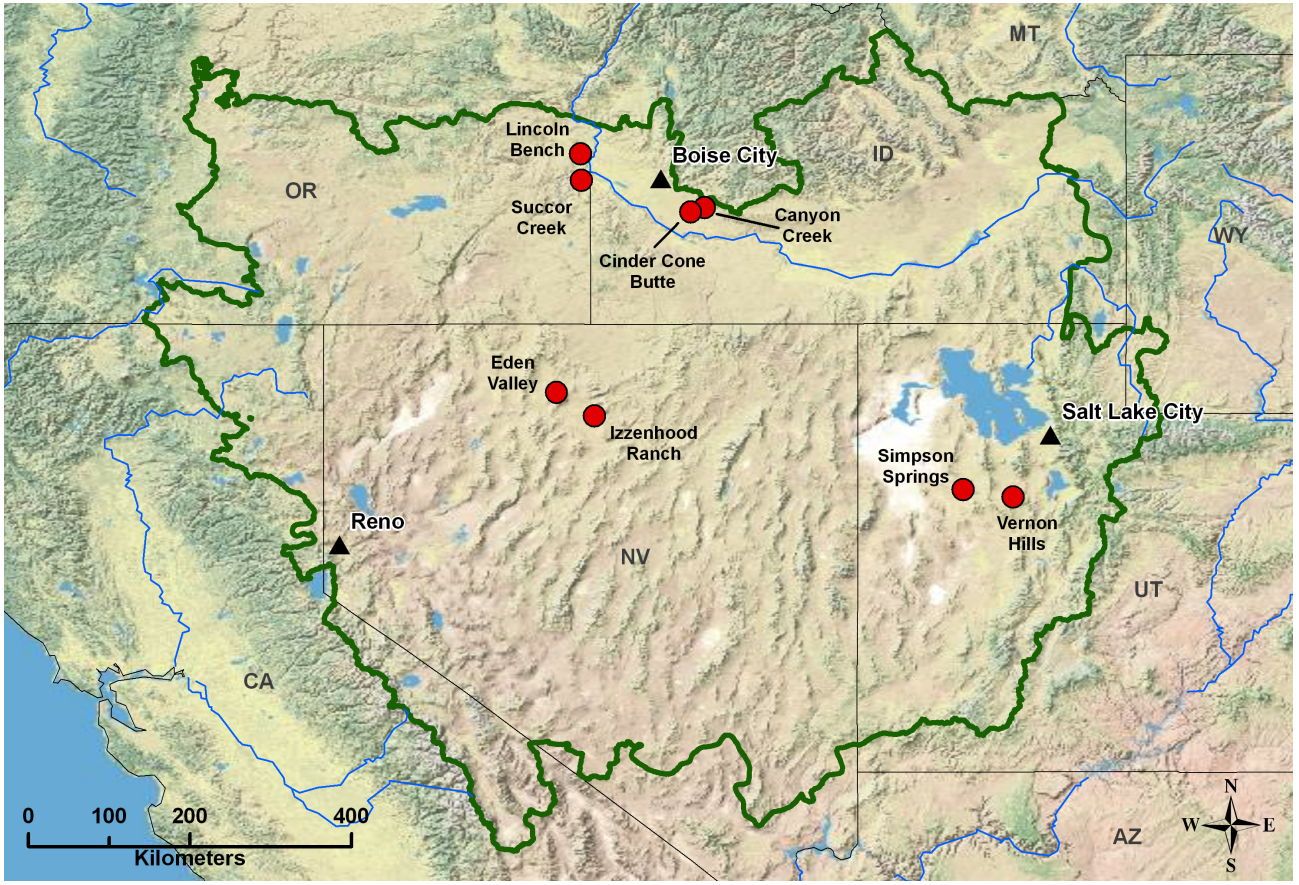

Figure 2.1 Eight site locations (red circles) across the northern Great Basin where restoration experiments, soil community sampling, and biological soil crust surveys were carried out.

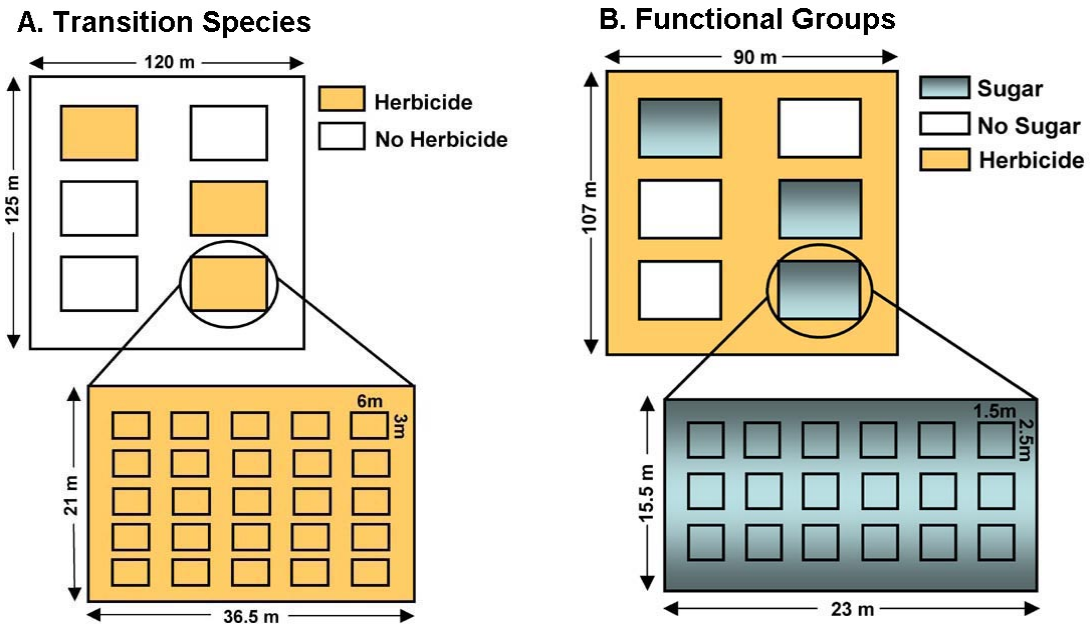

Figure 2.2 Plot designs for the Transition Species and Functional Groups experiments. **A) Transition Species** experimental plot configuration showing 6 plots (3 treated with herbicide, 3 untreated) at top and detail of 25 subplots at bottom. Each subplot was drill-seeded with a different plant accession to test its establishment ability with cheatgrass. **B) Functional Groups** experimental plot configuration showing 6 plots (3 treated with sugar, 3 untreated) at top and detail of 18 subplots at bottom. Each subplot was hand-seeded with either a monoculture or mixture of six different native species, cheatgrass, and Vavilov Siberian wheatgrass to test how the depletion of soil resources affects competitive interactions between cheatgrass and native species.

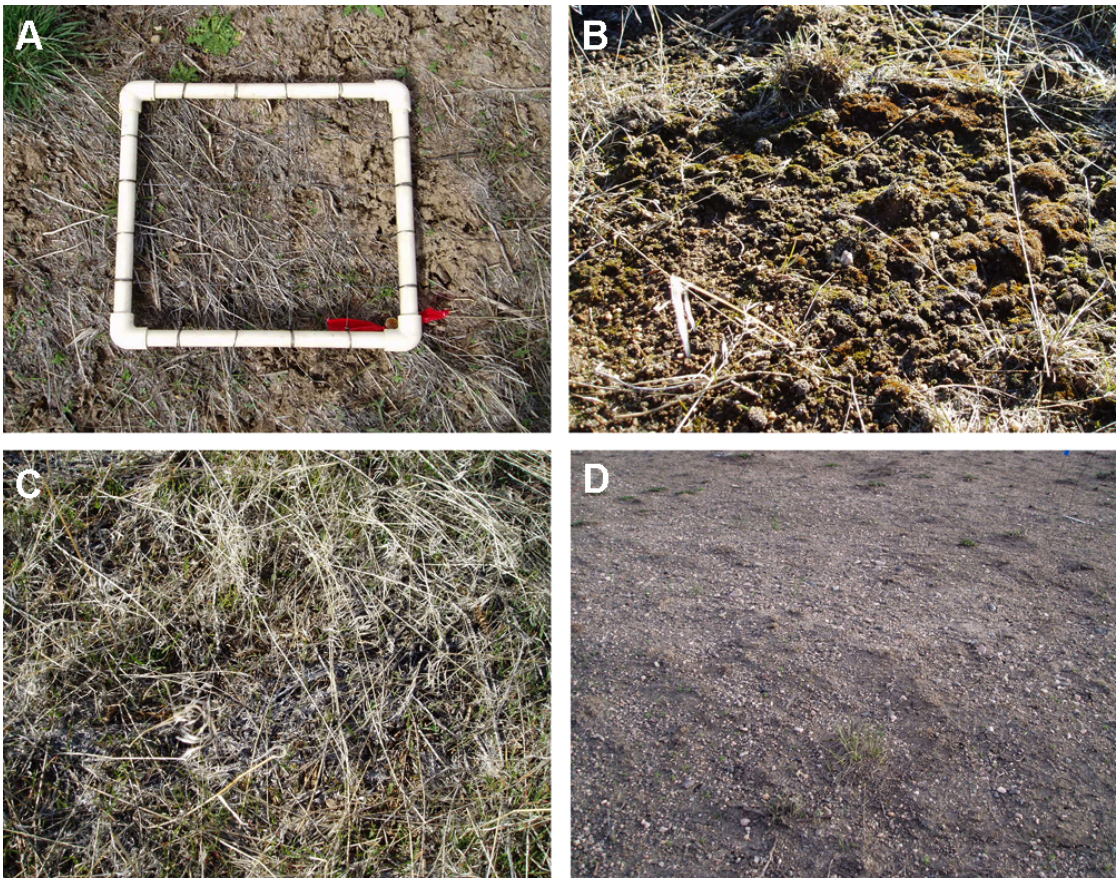

Figure 2.3 Examples of soil surface cover at select sites in the northern Great Basin. **A)** Soil surface survey frame. Red flagging mark a nail in the ground; the frame was placed over the nail in the same position at each of the three survey dates. **B)** Rolling moss and lichen crust cover at Cinder Cone Butte, ID (low precipitation site). **C)** Litter cover at Vernon Hills, UT (high precipitation site). **D)** Bare ground at Simpson Springs, UT (low precipitation site).

Figure 2.4 Nonmetric multidimensional scaling (NMS) ordinations for (A) biological soil crust surveys, (B) community level physiological profiles (CLPP), (C) phospholipid fatty acid (PLFA) analysis, and (D) soil nematode trophic group structure, Transition Species experiment, March 2004. Points represent mean axes scores and bars are standard error of the mean. All ordinations presented are 3-dimensional solutions; R^2 values given below are cumulative for the three axes. Vectors indicate the direction and magnitude of Pearson correlations (r) between ordination scores and environmental variables or taxonomic groups. CCK = Canyon Creek, ID; CCB = Cinder Cone Butte, ID; EV = Eden Valley, NV; IZ = Izzenhood Ranch, NV; LB = Lincoln Bench, OR; SC = Succor Creek, OR; VH = Vernon Hills, UT; SS = Simpson Springs, UT. **A) Biological soil crust transects (n = 4) in cover class space.** Final ordination stress = 5.17, R^2 = 0.97. Vectors: bare ground (r = 0.39, -0.87), cyanobacteria (r = -0.56, 0.38), lichens (r = -0.06, 0.71), litter (r = -0.50, 0.59), moss (r = -0.03, 0.75), and soil pH (r = 0.71, -0.50). **B) CLPP samples (n = 14) in carbon substrate space.** Final stress = 16.25, R^2 = 0.81. Vector: soil moisture (r = 0.14, -0.58). **C) PLFA samples (n = 14) in mol% space.** Final stress = 12.93, R^2 = 0.87. Vectors: actinobacteria (r = -0.34, 0.50), arbuscular mycorrhizal fungi (AMF) (r = -0.53, -0.52), bacteria (r = -0.19, 0.69), fungi (r = 0.27, -0.47), pH (r = -0.62, -0.08), soil moisture (r = 0.48, 0.02). **D) Soil nematode samples (n = 42) in trophic group space.** Final stress = 9.75, R^2 = 0.95. Vector: pH (r = 0.02, 0.56). Points represent central tendencies of nematode trophic groups.

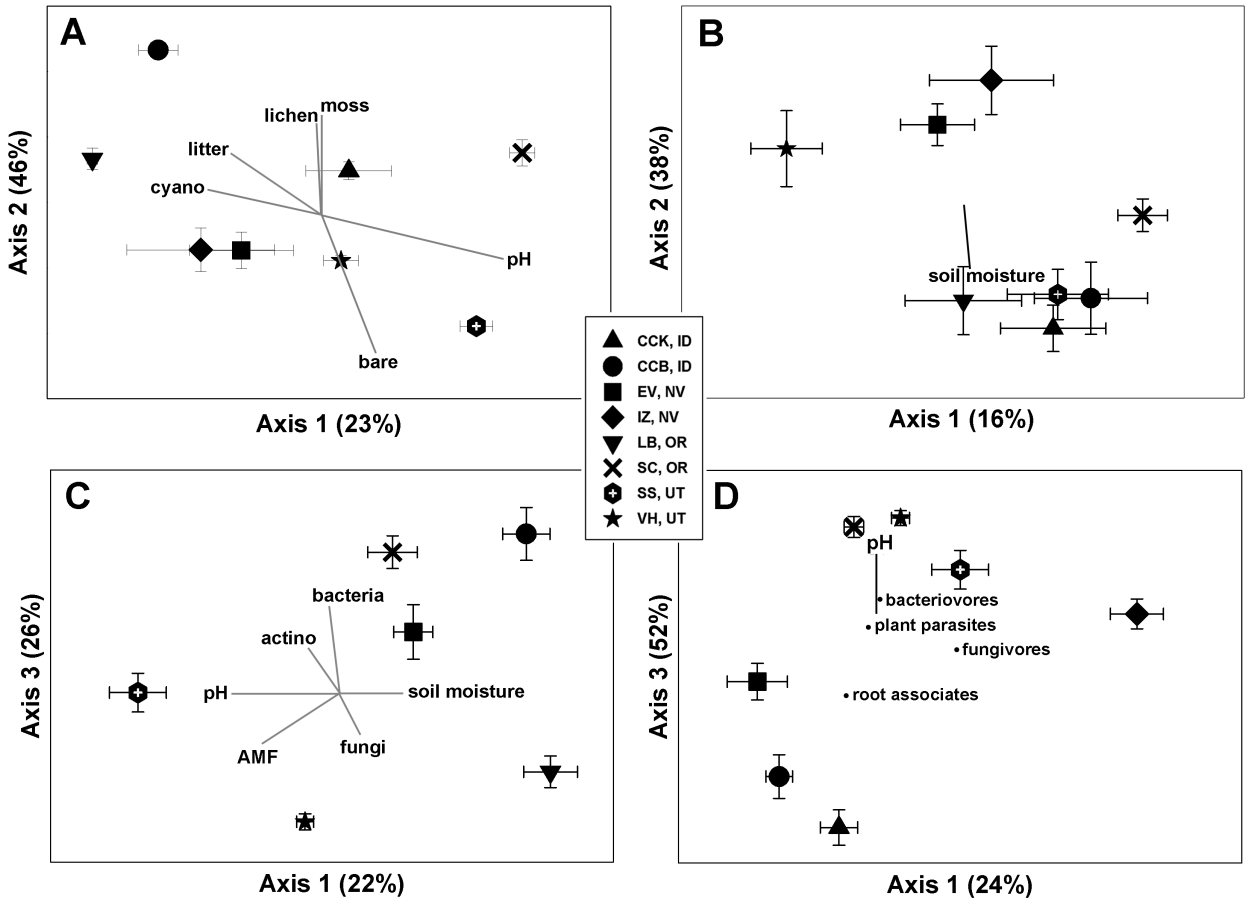

Figure 2.4 Nonmetric multidimensional scaling (NMS) ordinations for (A) biological soil crust surveys, (B) community level physiological profiles, (C) phospholipid fatty acid analysis, and (D) soil nematode trophic group structure, Transition Species experiment, March 2004.

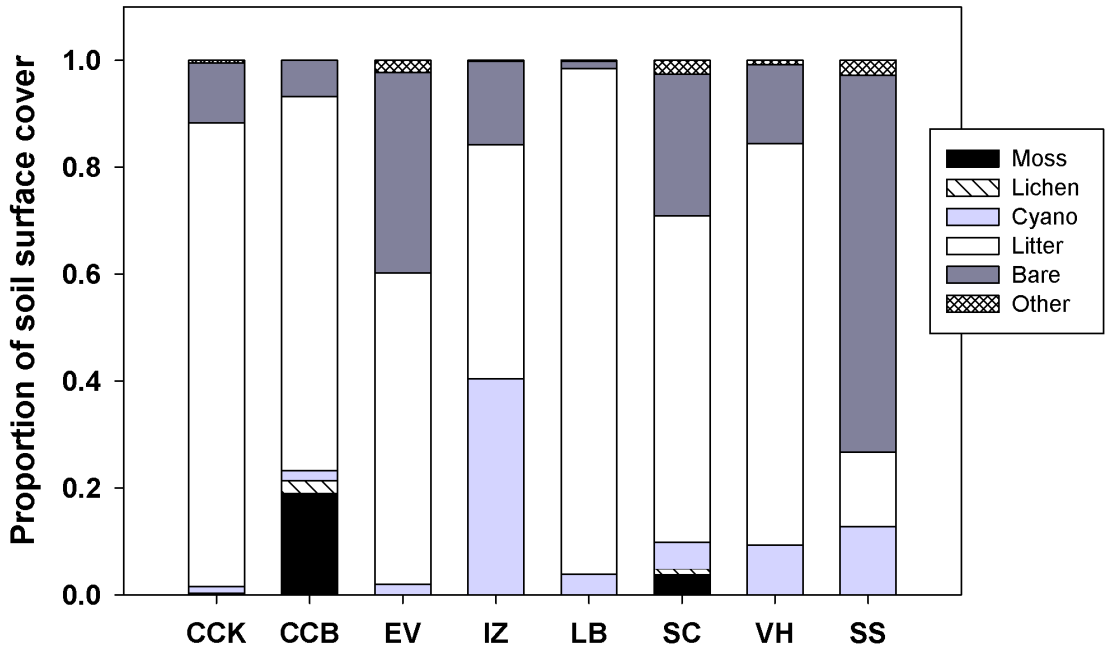

Figure 2.5 Proportion of soil surface cover at eight study sites in the northern Great Basin. Data are from control plots (n = 3) in the Transition Species experiment surveyed October 2003. "Bare" = bare ground or physical soil crust, "Cyano" = cyanobacterial crust cover. CCK = Canyon Creek, ID; CCB = Cinder Cone Butte, ID; EV = Eden Valley, NV; IZ = Izzenhood Ranch, NV; LB = Lincoln Bench, OR; SC = Succor Creek, OR; VH = Vernon Hills, UT; SS = Simpson Springs, UT.

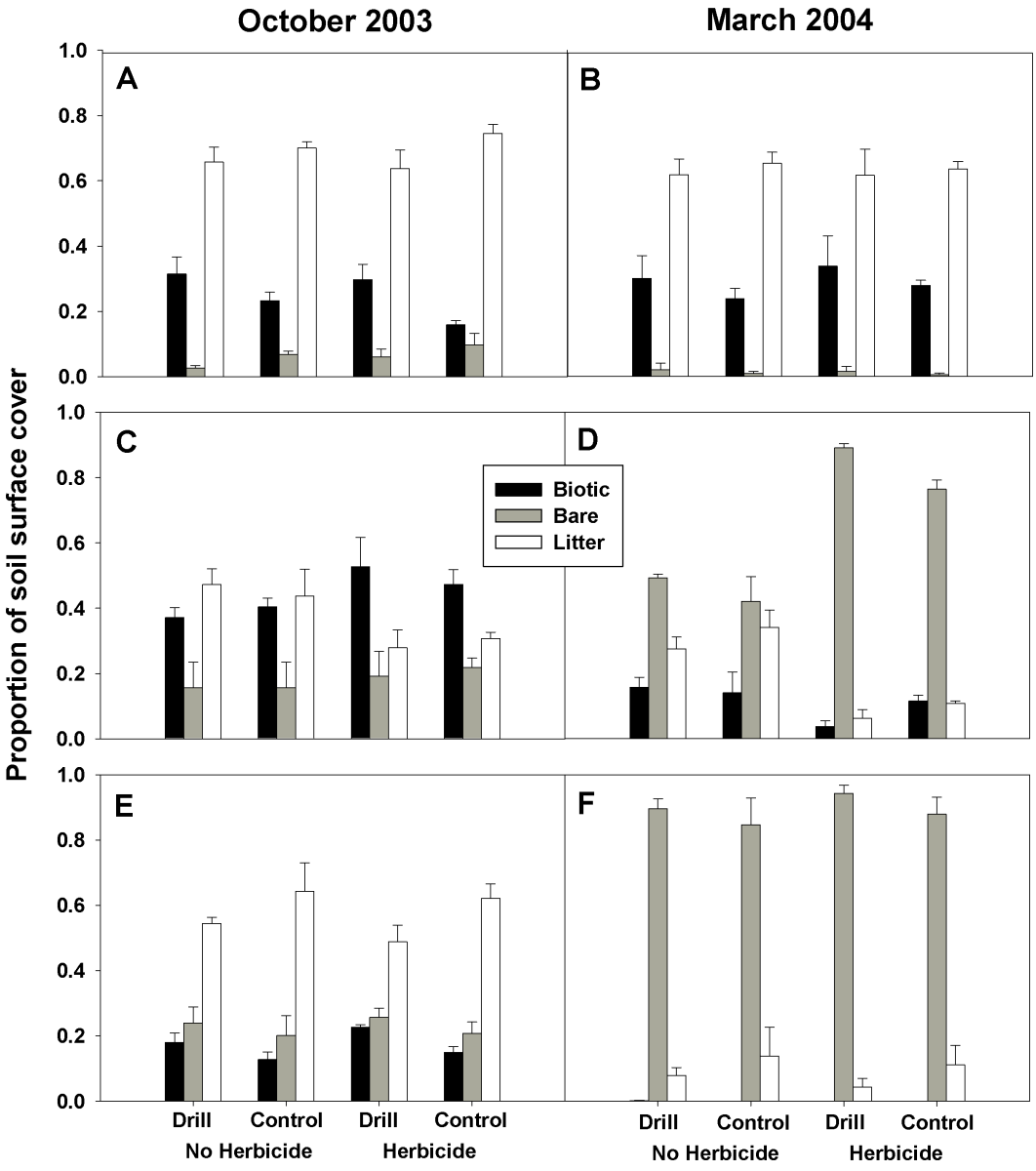

Figure 2.6 Effects of herbicide treatment and rangeland drill seeding on biological soil crust components at Cinder Cone Butte, ID (A & B), Izzenhood Ranch, NV (C & D), and Simpson Springs, UT (E & F).

Figure 2.7 Nonmetric multidimensional scaling (NMS) ordinations of soil samples analyzed for (A) community level physiological profiles (CLPP), (B) phospholipid fatty acid (PLFA) analysis, and (C) nematode trophic group structure, Functional Groups experiment, March 2004. Points represent mean axes scores and bars are standard error of the mean. Black symbols are plots treated with sugar; open symbols are control plots. All ordinations presented are 3-dimensional solutions; R^2 values given below are cumulative for the three axes. Vectors indicate the direction and magnitude of Pearson correlations (r) between ordination scores and environmental variables or taxonomic groups. CCK = Canyon Creek, ID; CCB = Cinder Cone Butte, ID; EV = Eden Valley, NV; IZ = Izzenhood Ranch, NV; LB = Lincoln Bench, OR; SC = Succor Creek, OR; VH = Vernon Hills, UT; SS = Simpson Springs, UT. **A) CLPP samples (n = 6) in carbon substrate space.** Final ordination stress = 14.03, R^2 = 0.86. Vectors: amino acids (r = -0.46, -0.30), carbohydrates (r = 0.70, -0.11), carboxylic acids (r = -0.45, -0.04), polymers (r = 0.02, 0.47). **B) PLFA samples (n = 6) in mol% space.** Final stress = 10.52, R^2 = 0.93. Vectors: actinobacteria (r = -0.57, 0.39), arbuscular mycorrhizal fungi (AMF) (r = -0.08, 0.53), fungi (r = 0.85, -0.25), gram+ bacteria (r = -0.18, -0.47), pH (r = -0.16, 0.80), stress (r = -0.20, 0.45). **C) Soil nematode samples (n = 21) in trophic group space.** Final stress = 8.71, R^2 = 0.96. Vector: pH (r = -0.60, 0.08). Points represent central tendencies of nematode trophic groups.

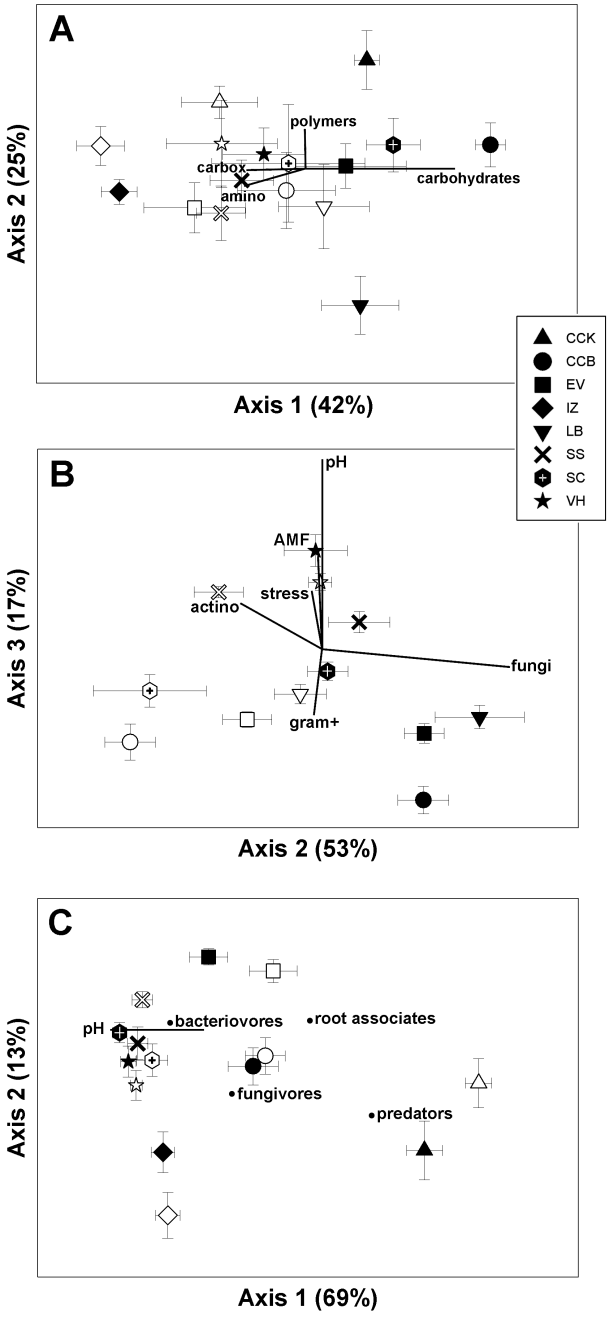

Figure 2.7 Nonmetric multidimensional scaling (NMS) ordinations of soil samples analyzed for (A) community level physiological profiles (CLPP), (B) phospholipid fatty acid (PLFA) analysis, and (C) nematode trophic group structure, Functional Groups experiment, March 2004.

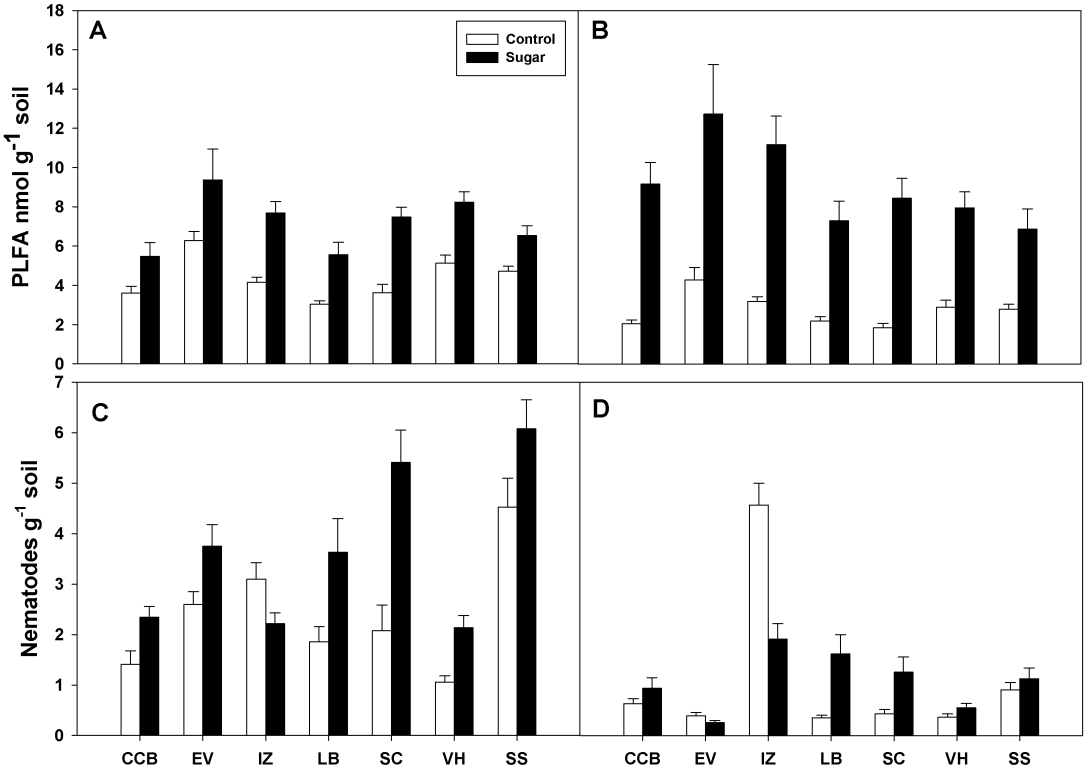

Figure 2.8 Phospholipid fatty acid (PLFA) bacteria and fungi biomass and bacterial- and fungal-feeding nematode abundances from control and sugar-treated plots, Functional Groups experiment, May 2004. Bars are means (SE), $n = 6$. **A)** PLFA bacteria; **B)** PLFA fungi (18:2 _6, 9); **C)** Bacterial-feeding nematodes; **D)** Fungal-feeding nematodes.

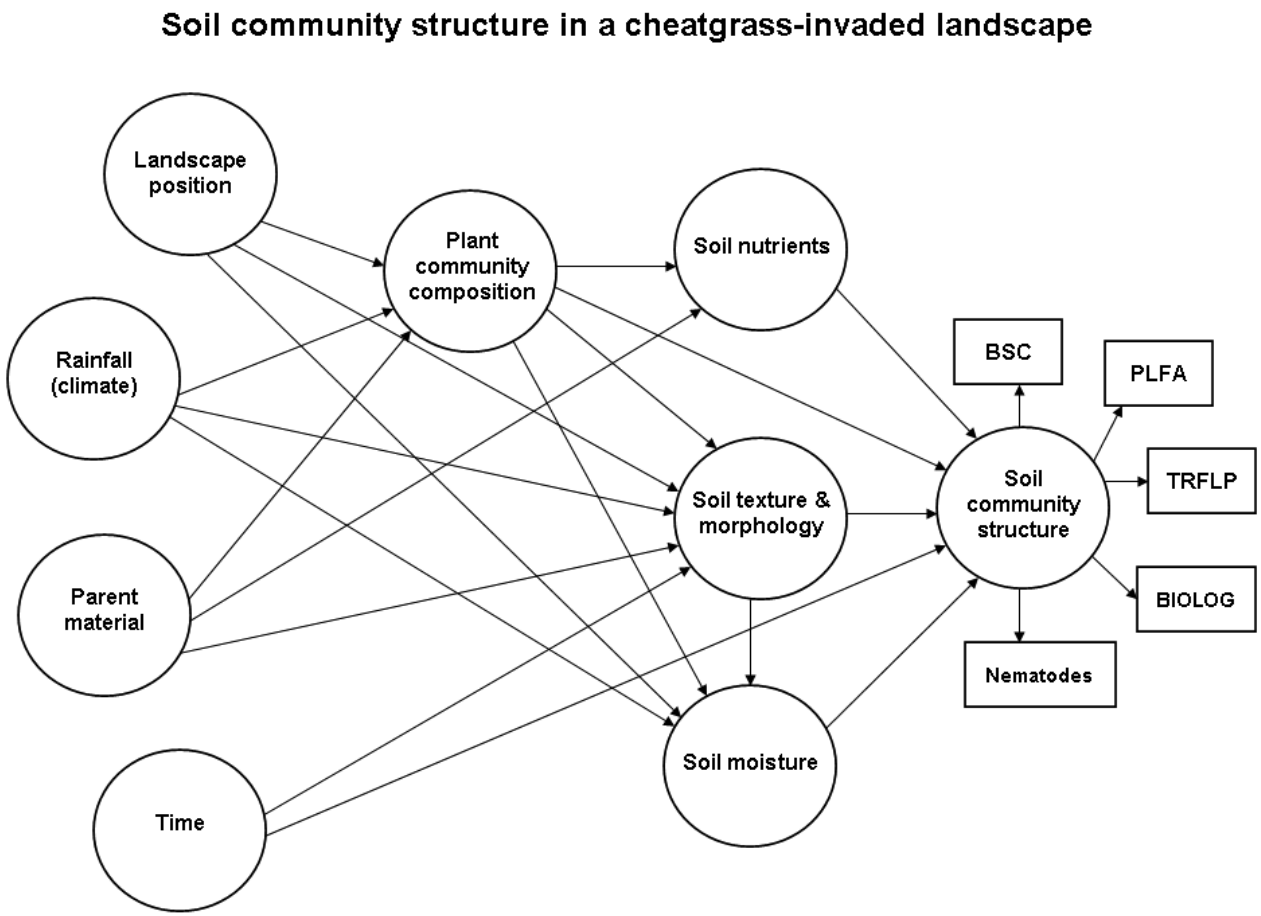

Figure 2.9 Conceptual diagram of latent and composite variables affecting soil community structure in cheatgrass-invaded sagebrush ecosystems of the northern Great Basin.

Chapter 3

Soil community responses to *Bromus tectorum* invasion and nutrient manipulations in sagebrush ecosystems: implications for restoration

Nicole M. DeCrappeo, Elizabeth J. DeLorenze, David A. Pyke, Peter J. Bottomley, and Dana L. Witwicki

Abstract

Cheatgrass *(Bromus tectorum* L.) invasion into the northern Great Basin has converted millions of acres of big sagebrush (*Artemisia tridentata* Nutt.) steppe to annual grasslands, a dramatic shift that has resulted in measurable effects on soil physical, chemical, and biological properties. This study focused on the effects of cheatgrass and soil nutrient levels on soil biological communities. Paired cheatgrass-invaded ("invaded") and uninvaded ("native") plots were established at six sites in eastern Oregon and southwestern Idaho. Subplots were treated with sugar to promote microbial N immobilization or ammonium-nitrate to increase N availability. In both fall 2003 and spring 2004, composite soil samples were collected from under cheatgrass plants in invaded plots and from under three different microsites within native plots: sagebrush, perennial bunchgrasses, and interspace. Soil community measures consisted of biological soil crust surveys, community level physiological profiles (CLPP), phospholipid fatty acid (PLFA) analysis, terminal restriction fragment length polymorphism (TRFLP), and nematode community composition. All analyses indicated that vegetation type was most important in structuring microbial community composition and structure, whereas N availability and site differences played minor roles. Sagebrush soil communities had higher fungal biomass and diversity and were able to utilize amino acids more effectively than soil communities under cheatgrass. Biomass of arbuscular mycorrhizal fungi decreased significantly in subplots treated with sugar. Our results suggest that conversion of sagebrush shrublands to cheatgrass grasslands can have important effects not only on the aboveground components of these ecosystems, but on belowground microbial community composition and activity as well.

Keywords: *Bromus tectorum*, cheatgrass, invasive species, Wyoming big sagebrush, Great Basin, soil microbial communities, CLPP, PLFA, TRFLP, soil nematodes, biological soil crusts.

Introduction

Non-native invasive plant species can become "ecosystem engineers" in their new environments, directly or indirectly controlling resource availability by modifying physical state factors (Crooks 2002). Cheatgrass (*Bromus tectorum* L.) is one such exotic grass that has transformed landscapes in the northern Great Basin from biologically diverse shrub/perennial bunchgrass ecosystems to homogenous annual grasslands (Knapp 1996). Cheatgrass invasion greatly increases fire frequency, reduces native plant diversity, alters nutrient inputs and cycling rates, and degrades economically important rangeland ecosystems (D'Antonio and Vitousek 1992, Chen and Stark 2000, Hilty et al. 2001). Cheatgrass invasion brings significant changes to soil physical and chemical properties (Norton et al. 2004) and effectively eliminates the patchy distribution of resources that is a fundamental feature of the sagebrush steppe (Smith et al. 1994, Halvorson et al. 1997, Chen and Stark 2000, Evans et al. 2001). Big sagebrush (*Artemisia tridentata* Nutt.) serve as "islands of fertility" in these low-productivity ecosystems: carbon (C) and nitrogen (N) availability, soil organic matter (SOM) accumulation, and microbial biomass are all greater under sagebrush than in non-vegetated interspaces that occur just centimeters away (Bolton et al. 1993, Belnap and Phillips 2001, Evans et al. 2001, Norton et al. 2004). In addition, sagebrush canopies mediate soil temperature and water availability and promote herbaceous understory growth (Davies et al. 2007). When cheatgrass invades and dominates a landscape, spatial variability of resources disappears and environmental dynamics can be modified to the point where a new ecological stable state is attained (Westoby et al. 1989, Laycock 1991, Briske et al. 2005). Re-establishing native vegetation under these conditions is exceedingly difficult and energy-intensive (Stringham et al. 2003).

Changes in soil physical and chemical properties wrought by cheatgrass invasion have the potential to affect soil biological communities, including bacteria, fungi, nematodes, and biological soil crusts (Belnap and Phillips 2001). Soil

organisms play vital roles in carrying out ecosystem processes, and community composition may be particularly important in semi-arid ecosystems where resource availability fluctuates dramatically throughout the year (Wall and Virginia 1999). Soil biotic communities respond to variability in plant litter quality and quantity, rooting structures, and soil resources that are directly and indirectly related to individual plant species. It is well established that biomass and composition of microbial communities differ in soils beneath different species of plants (Grayston et al. 1998, Bardgett et al. 1999, Kuske et al. 2002) and that invasive plant species have the potential to alter soil biotic communities in a variety of ecosystems (Kourtev et al. 2002, Hawkes et al. 2006, Callaway et al. 2008, Jordan et al. 2008). Because of the growing spatial extent of cheatgrass dominance across the northern Great Basin, we felt it was important to determine if and how soil communities are responding to the grass's invasion.

Land managers use a variety of strategies to combat the growth and spread of cheatgrass and re-establish native plants in invaded areas. One of the more promising restoration techniques exploits the propensity of cheatgrass to acquire inorganic soil N in the fall or winter while native plants are dormant. Properly timed applications of a readily available carbon substrate, such as beet sugar, sawdust, or newspaper clippings, to cheatgrass grasslands can stimulate microbial N-immobilization, decrease plant-available N, reduce cheatgrass biomass and seed production, and potentially give the native perennial bunchgrasses a chance to re-establish (Blumenthal et al. 2003, Witwicki 2005). Details of the mechanisms behind this process are not fully understood, but it seems likely that soil microbial and microinvertebrate populations play important roles, as they are responsible for the majority of nutrient transformations in the soil. We wanted to assess the impact of this practice on both native and cheatgrass-invaded soil communities.

Our objectives in this study were to characterize microbial and nematode abundance, composition, and functional diversity in big sagebrush/perennial bunchgrass and cheatgrass plots paired on similar soils in the northern Great Basin.

Carbon and N levels were experimentally manipulated to test the hypothesis that cheatgrass production is suppressed at low soil N levels. Soil samples were collected directly under four cover types: sagebrush, bunchgrass, interspace (which included biological soil crusts), and cheatgrass. We were interested in what hierarchical factors were most important for structuring soil microbial and microinvertebrate communities (i.e., sampling date, site, nutrient treatment, or cover type) in the sagebrush steppe. We also wanted to examine the effects of cheatgrass on the functional and taxonomic diversity of soil communities.

Materials and Methods

Site descriptions and sample collection

Six field sites were located in the Snake River Plain, three each near Boise, Idaho, and Ontario, Oregon, USA (Table 3.1). Climate in this region is characterized by cold, wet winters and warm, dry summers. Average annual precipitation ranges from 200 to 300 mm, with 60 percent received from October through March (Bates et al. 2006). Sites were chosen to be representative of Wyoming big sagebrush (*Artemisia tridentata* Nutt. ssp. *wyomingensis* Beetle & Young)/bluebunch wheatgrass (*Pseudoroegneria spicata* [Pursh] A. Löve) ecological sites as defined by the Natural Resources Conservation Service (USDA NRCS). This designation takes into consideration climate, soil, and hydrologic conditions in addition to potential plant community composition and productivity. We also compared elevation, aspect, landscape position, and soil morphological characteristics to ensure that sites could be used as replicates. Soils are well-drained shallow loams or silt loams derived from loess over igneous material. At all but one site, a restrictive duripan layer begins between 25-55 cm (Table 3.1). All sites were historically dominated by Wyoming big sagebrush and perennial bunchgrasses, but one or more burn events over the past 50 years converted large areas to annual grasslands (Table 3.1). At each of the six sites, we established a "native" plot and an "annual" plot located no more than 3 km apart. Dominant plant

species in native plots included Wyoming big sagebrush, bluebunch wheatgrass, Sandberg bluegrass (*Poa secunda* J. Presl), and bottlebrush squirreltail (*Elymus elymoides* [Raf.] Swezey). Interspaces (i.e., areas that lack vascular plant cover) in native plots were generally covered by semi-continuous biological soil crust consisting of cyanobacteria, mosses, and lichens (Table 3.1). Vegetation in annual plots was dominated by cheatgrass but also included Sandberg bluegrass and the exotic annual medusahead (*Taeniatherum caput-medusae* [L.] Nevski) at some sites. There was no remaining shrub component and less than 5% native bunchgrass cover (excluding Sandberg bluegrass). Biological soil crust cover was very low in annual plots (Table 3.1) and consisted of only mosses and cyanobacteria.

Within each native and annual plot, we established three 5-m^2 subplots that were amended with N, C, or water (control) in October 2003, before annual grass germination. Nitrogen was applied as NH_4NO_3 at a rate of 100 kg N ha^{-1}, and C was applied in the form of cane sugar at a rate of 2000 kg C ha^{-1}. Treatments were hand dispersed and followed by an application of deionized water to simulate a 0.5 mm rainfall event. Soil sampling for community level physiological profiling (CLPP) took place five days after treatment application. Samples for phospholipid fatty acid (PLFA), terminal restriction fragment polymorphism (TRFLP) and nematode analyses were collected one month later. Soil samples were collected again at the height of the growing season in May 2004. In native plots, soils were taken under three different cover types: sagebrush, bunchgrass or interspace. In annual plots, soil samples were collected under cheatgrass, the dominant vegetation, bringing the total number of samples per sampling date to 72 (6 sites x 3 treatments x 4 cover types). For each sample, 10 soil cores were collected using a 2 cm diameter soil corer to a depth of 10-12 cm (approx. 400 g of soil). Soils were stored in coolers with icepacks, returned to the lab, and placed in a 4°C refrigerator. The following day, the soils were homogenized, passed through a 2 mm sieve, and processed for pH_{water}, gravimetric soil moisture (oven-dried at 105°C for 24 h), and water potential (filter paper method,

(Kaya and Stock 1997). Total soil C and N from pre-treated plots were determined by combustion on a Costech Elemental Analyzer, Model 4010 (Costech Analytical Technologies, Inc., Valencia, CA) (Table 3.2).

Community level physiological profiles (CLPP)

Soil microbial functional diversity was assessed using Biolog EcoPlates™ (Biolog Inc., Hayward, CA, USA). Each 96-well plate contains 31 different carbon substrates and one water blank replicated three times. Each well also contains tetrazolium violet dye that is reduced as a result of microbial respiratory activity. 1 g of soil was placed in 99 mL phosphate buffer with $MgCl_2$ (Fisher Scientific, Pittsburgh, PA, pH = 7.2) and placed in a refrigerator overnight. The following day, samples were shaken for 20 min at 160 rpm on a clinical rotator, resulting in a well-mixed soil suspension. Under a laminar flow hood, 100 μL aliquots of suspension were pipetted into the wells of an EcoPlate™ (one plate per sample). Plates were incubated in the dark at 20°C. Color development for each well was determined using a Bio-tek $PowerWave_X340$ spectrophotometer at a wavelength of 596 nm (OD_{596}). The absorbance values, which represent the microbial community's ability to use a particular substrate, were recorded at 24-h intervals for 5 consecutive days (Sinsabaugh et al. 1999). The average well color development (AWCD) method was used to standardize for differences in initial inoculum density (Garland 1996). We subtracted the average optical density value of the three water wells from the 93 remaining wells and changed all resulting negative values to zero. We then calculated the AWCD for each plate, divided the data by the AWCD, and averaged the three substrate replicates (Kohler et al. 2005). We used data from the day when the AWCD $\geq$ 0.2 (Garland 1996, Grayston et al. 1998), thereby creating a matrix of data obtained from 48, 72, 96, and 120 h readings. The 31 carbon substrates were categorized into biochemical guilds (amino acids, amines/amides, carbohydrates, carboxylic acids, and polymers) for community ordinations and statistical analyses (Kohler et al. 2005). Because CLPPs provide only

a qualitative assessment of functional diversity and, with few exceptions, every substrate was used to some degree in each sample, we did not use the absorbance values to calculate ecological diversity indices.

Lipid analysis

Fatty acids are found in membranes of all living cells and can serve as biomarkers to identify groups of microorganisms (Sinsabaugh et al. 1999). These signature lipids are used to generate a taxonomic fingerprint of the microbial community as well as estimates of microbial biomass, relative taxa abundances, and fungal to bacterial ratios (Bailey et al. 2002a, Bailey et al. 2002b). We used a hybrid phospholipid fatty acid (PLFA) and fatty-acid methyl ester (FAME) technique (Smithwick et al. 2005) based on a modified Bligh and Dyer (1959) method. Within one week of collection, soils were sieved as above and 12 g subsamples were weighed, freeze-dried, and stored in vacuum-sealed bags in a freezer until further processing. Samples were homogenized using a mortar and pestle, and 4 g subsamples were weighed into hexane-rinsed Teflon™ centrifuge tubes. Whole fatty acids were extracted from soils using a chloroform-methanol extractant in phosphate buffer (4 mL $CHCl_3$, 8 mL MeOH, 3.6 mL phosphate buffer). Samples were covered, shaken for 1 h, and centrifuged for 10 min at 2500 rpm. The supernatant liquid was decanted into hexane-rinsed glass test tubes, and 3.6 mL 0.1 M phosphate buffer (pH = 7.0) and 4 mL $CHCl_3$ were added to each sample. Test tubes were shaken by hand for 1 min and stored at room temperature in the dark overnight to allow for phase separation. After aspirating off the top layer, the chloroform phase was dried using N_2 gas. Saponification and methylation followed the Microbial ID Inc. (Hayward, CA) protocol. For saponification, we added 1 mL of a NaOH-MeOH solution to test tubes and placed the tubes in a 95°C water bath for 30 min. For methylation, we added 2 mL of a 1:1.2 MeOH-6 N HCl solution to tubes and heated them in an 80°C water bath for 10 min. Extraction was performed by adding 1.25 mL of a 1:1 MTBE-hexane solution.

The nomenclature used for fatty acids is as follows: the number before the colon is the total number of carbon atoms, the number following the colon is the number of double bonds, and 'ω' indicates the location of the double bond from the methyl end of the molecule. The prefixes '*i*' and '*a*' refer to iso and anteiso-branched fatty acids, while '*cy*', 'Me', and 'OH' indicate cyclopropane, methyl, and hydroxy groups, respectively. Total bacterial biomass was estimated by summing the abundances of gram-positive bacterial biomarkers *i*14:0, *i*15:0, *a*15:0, *i*16:0, *i*17:0 and gram-negative bacterial biomarkers *cy*17:0, 16:1ω9c, 17:1ω9c, 18:1ω7c, *cy*19:0. Actinobacteria were identified as 10Me16:0, 10Me17:0, and 10Me18:0. Saprotrophic and arbuscular mycorrhizal (AM) fungal biomass were estimated using fatty acids18:2ω6,9c and 16:1ω5c, respectively (Balser et al. 2004). Protozoan biomarkers were 20:4ω6c and 20:2ω6,9c. Total microbial biomass was estimated using the aforementioned markers in addition to the following non-specific fatty acids: 11Me18:1ω7c, 14:0, 15:0, 15:0 3OH, *a*15:1, i15:1, 16:0, 16:1 2OH, 16:1 3OH, 16:1ω7c, 17:0, 17:1ω8c, 18:0, 18:1ω5c, 18:1ω9c, 18:3ω6,9,12c, 19:0, 19:0 2OH, 19:1ω8t, 20:0, 21:0, and 22:0. For community analysis, the relative proportions (mol %) of all 41 biomarkers were calculated and used to create data matrices for both the fall and spring sampling dates.

Terminal Restriction Fragment Length Polymorphism (TRFLP)

We extracted DNA from soil samples using the UltraClean DNA Extraction kit (MoBio Laboratories, Carlsbad, CA, USA) following the alternative DNA extraction protocol for maximum yields as supplied by the manufacturer. Extracted DNA was stored at -20ºC. Soil bacterial rDNA was examined by amplifying the 16S region using 0.62 μL of each universal primer 8F (5'-AGAGTTTGATCCTGGCTCAG-3', FAM-labeled) (Edwards et al. 1989) and 907R (5'-CCGTCAATTCCTTTRAGTTT-3', HEX-labeled) (Muyzer et al. 1993). Each 50 μL reaction also contained 33 μL dH_2O, 5 μL ThermoPol PCR Buffer (New England Biolabs, Beverly, MA, USA), 4

μL dNTPs, 4 μL $MgCl_2$, 2 μL BSA (New England Biolabs, Beverly, MA, USA), 0.5 μL Taq DNA Polymerase (New England Biolabs, Beverly, MA, USA), and 0.6 μL of soil DNA template. The thermocycling protocol consisted of an initial denaturation for 3 min at 95ºC followed by 30 cycles of 95ºC for 30 sec, 55ºC for 1 min, and 72ºC for 45 sec, concluding with 7 min at 72ºC. Each extract was amplified twice and the product was examined using gel electrophoresis. Samples that showed faint product bands were re-amplified with minor modifications to the protocol (including changes in the volume of $MgCl_2$, BSA, or template) until two strong products were confirmed. Duplicate amplicons were pooled and purified using the UltraClean DNA Purification Kit (MoBio Laboratories, Carlsbad, CA, USA) following the manufacturer's protocol. Products were again verified with gel electrophoresis prior to separate restriction digestions with *MspI* and *AluI* (New England Biolabs, Beverly, MA, USA). For the digestion, 7 μL of the restriction enzyme cocktail (5.25 μL dH_20, 1.5 μL NEB 2 Buffer [New England Biolabs, Beverly, MA, USA] and 0.25 μL restriction enzyme) was combined with 8 μL PCR product. Products were vortexed and incubated for 4 hours at 37°C. Digested products were stored at -20ºC prior to submission to the Center for Genome Research and Biocomputing (CGRB, Oregon State University, Corvallis, OR, USA) for concentration and re-purification and subsequent analysis on an ABI 3100 capillary DNA sequencer. For the spring sampling date, a presence/absence data matrix consisting of 952 terminal restriction fragments (TRFs) was created using GeneMapper 4.0 software (Applied Biosystems, Foster City, CA, USA) (Bastias et al. 2007). Samples from the fall sampling date were not processed due to high processing costs.

To compare fungal communities, we amplified the ITS region using universal primers ITS1F (5'-CTTGTTCATTTAGAGGAAG-TAA-3', HEX-labeled) (Gardes and Bruns 1993) and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al. 1990). Because the fungal signal was weaker than the bacterial, these reactions were carried out in 40 μL to concentrate the DNA. The reactions consisted of 30 μL dH_2O,

4 μL 10x TempaseII PCR buffer (GeneChoice, Inc., Frederick, MD, USA), 4 μL 10x dNTP, 0.45 μL $MgCl_2$ (25mM), 0.35 μL BSA (New England Biolabs, Beverly, MA, USA), 0.29 μL of each primer, 0.2 μL TempaseII DNA polymerase (GeneChoice, Inc., Frederick, MD, USA) and 0.6 μL soil DNA template. Thermocycling was initiated with denaturation at 95ºC for 7 min and was followed by 35 cycles of 95ºC for 40 sec, 49ºC for 30 sec, and 72ºC for 48 sec, concluding with 10 min at 72ºC. Two strong amplicons were obtained prior to purification, digestion, and analysis following the bacterial protocol listed above, with the substitution of fungal restriction enzymes *Hin*fI and *Hae*III (New England Biolabs, Beverly, MA, USA). Digested products were stored at -20ºC but were not concentrated and re-purified as with the bacteria. Instead, subsamples were removed and submitted to the CGRB for genotyping. We used GeneMapper 4.0 software to create presence/absence data matrices consisting of 231 and 222 TRFs for the fall and spring sampling dates, respectively.

Soil nematodes

Soil nematodes were extracted using the sugar centrifugation technique (Kaya and Stock 1997). A subsample of 100 g of soil was mixed in 800 mL of water and stirred in a figure-eight pattern for 30 sec to 1 min. The mixture settled for 1 min, after which time it was poured over two stacked sieves of mesh sizes 250 μm and 38 μm. The silt and nematodes remaining on the bottom sieve were poured into a 50 mL centrifuge tube and centrifuged at 3000 rpm for 3 min. After pouring off half of the water, the tube was filled with a 1 M sucrose solution and centrifuged again at 3000 rpm for 3 min. Nematodes remaining in the supernatant were poured onto a small sieve, rinsed thoroughly with tap water, and transferred to a glass vial. Samples were refrigerated at 4°C until further processing could be completed. Nematode counts and genera identifications were performed on a Leica inverted interference contrast compound microscope. Nematodes were assigned to feeding groups according to Yeates et al.

(1993). We used six different trophic groups in our data matrices: bacteriovores, fungivores, omnivores, plant parasites, root associates, and predators.

Statistical analysis

Multivariate statistical analyses were performed using PC-Ord version 5.11 beta (McCune and Mefford 1999). Nonmetric multidimensional scaling (NMS) with the Sørensen distance measure was used to assess CLPP, PLFA, TRFLP, and nematode community patterns at the date, site, treatment, and cover type levels. Prior to performing NMS analyses, CLPP and nematode data were square root transformed and PLFA mol% data were arcsine square root transformed. Bacterial and fungal terminal restriction fragments that occurred in fewer than four samples were deleted from their respective TRFLP datasets. For NMS ordinations, the medium setting of 'autopilot' was used with random starting configurations and 50 runs with real data. Monte Carlo randomization tests were performed against real data to evaluate the significance of the *k*-dimensional solution. Outliers were identified as sample units with an average distance greater than 3.0 standard deviations from the mean that exhibited undue influence on the ordination results; these were deleted from the datasets. Statistical significance and strength of *a priori* within-in group membership were tested using one-way multi-response permutation procedures (MRPP), which provides a *p*-value and effect size, *A*, that is independent of the sample size. An *A*-statistic of ≥ 0.1 accompanied by a *p*-value of < 0.05 is considered to be significant for ecological data (McCune and Grace 2002). To test for interaction effects between treatment and cover type, a two-way factorial permutational multivariate analysis of variance (PerMANOVA) was performed (Anderson 2001). PerMANOVA produces output analogous to a traditional ANOVA table, with a pseudo *F*-statistic and *p*-value based on a user-supplied number of permutations (999 runs were used for all tests in this study). The Sørensen distance measure was used for outlier analysis, MRPP, and PerMANOVA for all datasets (we did not use blocked MRPP because this test does

not allow for the use of the Sørensen distance measure, and we wished to remain consistent with the distance measure used throughout the analyses). ANOVAs for CLPP guilds and PLFA taxa were performed in SAS 9.1 using PROC MIXED (SAS Institute, Cary, NC, USA). The six sites were treated as blocks in the model, with treatment and cover type as fixed effects.

Results

Values for CLPP substrate utilization, PLFA biomass and relative proportions of taxa, TRFLP species richness, and nematode generic richness for all treatment-cover type combinations are presented in Appendix A. Sampling date, site variability, treatment, and cover type contributed in varying degrees to differences in soil community substrate utilization patterns, structure, and composition. Sampling date was a significant effect only for PLFA communities (Table 3.3). In general, community structure was more variable in the fall, and the abundances of actinobacteria, gram-negative bacteria, and protozoa biomarkers were higher. Spring samples were more tightly clustered in ordination space and had greater total biomass and fungal abundance (Fig. 3.1). Because patterns were similar for both sampling dates for all other community analyses, only ordination results from the spring are presented.

Cover type effects

With the exception of nematode composition, the strongest differences in soil community function, structure, or composition were due to cover type effects (Table 3.3). Communities from sagebrush, bunchgrass, interspace, or cheatgrass soils had strongly different CLPP, PLFA (Fig. 3.2), and bacterial and fungal TRFLP patterns (Fig. 3.3). Sagebrush soil communities generally had low variability in CLPP and PLFA structure and greater variability in TRFLP peak profiles. In all analyses except fungal TRFLP, bunchgrass and cheatgrass soil communities were more similar to each

other than to other cover types, while interspace soil communities fell at the far end of the community gradient (Figs. 3.2 and 3.3).

Cover type was a significant grouping factor for CLPP when treatment was controlled for (Table 3.4). Sagebrush soils had low variability in CLPP patterns relative to cheatgrass and interspace soils. Interspace soils were located relatively far away from the plant soils in ordination space (Fig. 3.2). None of the abiotic parameters (pH, gravimetric soil moisture, water potential, total soil C, N, and C:N) were correlated with the first or second axes. Amino acid utilization was higher in sagebrush soils than cheatgrass or interspace soils, but was not significantly different than bunchgrass soils. Carboxylic acid utilization was higher in bunchgrass soils than cheatgrass or interspace soils, but did not differ from sagebrush soils (Fig. 3.4). Interspace and cheatgrass soils utilized significantly higher amounts of carbohydrates than sagebrush and bunchgrasses (Fig. 3.4).

Cover type was the most important factor structuring PLFA soil communities during the spring (Table 3.3). Sagebrush soils fell far from the three other cover types in PLFA mol % space (Fig. 3.2) Variability was low in sagebrush and bunchgrass soils and increased under cheatgrass soils. Total PLFA biomass was positively correlated with axis 2 and highest in sagebrush soils, while the percentage of fungi was negatively correlated with axis 2 and greatest in interspace soils (Fig. 3.2). Total, gram-positive, and gram-negative bacteria and actinobacteria were all highest in sagebrush soils; there were no differences between bunchgrass, cheatgrass, or interspace soils for the same taxa. Fungi decreased significantly in cheatgrass soils compared to the other three cover types. Arbuscular mycorrhizal fungal biomass (16:1ω5c) was lowest in interspace soils (Fig. 3.5).

As with CLPP and PLFA, both bacterial and fungal TRFLP profiles were strongly patterned by cover type (Table 3.3). Bacterial communities under sagebrush were clearly separated from cheatgrass and interspace communities in ordination space, with bunchgrass communities falling in an intermediate position (Fig. 3.3).

Similarly, fungal community composition under sagebrush differed from the other cover types, but bunchgrass and interspace samples were tightly clustered and separated from cheatgrass samples (Fig. 3.3). Three fungal TRFs were indicator species in sagebrush soils (1FHae85, 1FHae106, 1FHae126), and interspace and cheatgrass soils each had one indicator species (1FHin220 and 1FHae498, respectively) (Table 3.5). Two TRFs, 1FHae427 and 1FHin188, were only found in native soils (under sagebrush, bunchgrass, and interspace) and never in annual soils (Table 3.5).

Treatment effects

Nitrogen and C treatment effects were variable across sampling dates and cover types for the different community analyses. Treatments had no effect on CLPP or nematode composition in either the fall or spring (Table 3.3), even when cover type was controlled for (Table 3.4). There were significant treatment effects on PLFA community structure and bacterial and fungal TRF composition when cover type was accounted for, as well as an interaction effect in the latter (Table 3.4). The carbon treatment significantly increased PLFA fungal to bacterial ratios in sagebrush, bunchgrass, and cheatgrass soils in the fall, but the effect only remained significant for bunchgrass soils in the spring (Fig. 3.6).

The mean number of fungal TRFs, or potential species, was highest in untreated sagebrush soils and decreased significantly in N and C treatment plots (Fig. 3.7). The number of fungal TRFs increased significantly under bunchgrasses in N treated plots and interspace and cheatgrass in C treated plots (Fig. 3.7). The same two TRFs that were found only in native soils were also only recovered from control plots and never from N or C treated plots (Table 3.5).

Site effects

Site effects were not statistically significant in CLPP, PLFA, or TRFLP analyses (Table 3.3). However, nematode community composition and structure responded most strongly to site variability during both the fall and spring samplings (Table 3.3). Nematode communities consistently showed strong separation by site and secondarily by cover type for both sampling dates (Fig. 3.8). This was confirmed by PerMANOVA tests: there were significant differences between cover types when blocked for sites (F = 2.07, p = .00 in fall; F = 2.90, p = .00 in spring). Bacterial feeding nematodes were negatively correlated with axis 2 in the direction of the Oregon sites, and fungal feeders were correlated with axis 3 in the direction of Kuna Butte, ID. Plant parasites were more abundant at the Mayfield and Bowns Creek, ID sites. pH tended to increase in the direction of samples collected under cheatgrass cover and decrease in samples collected from interspaces (Fig. 3.8).

Discussion

Sagebrush grasslands are inherently patchy in resource distribution (Bolton et al. 1993, Smith et al. 1994), and we expected microbial community characteristics to respond to resource gradients created by differing plant life forms and their spatial relationships. We were interested in the direction and magnitude of these differences and how they compared to areas dominated by cheatgrass, which represents a life form, annual grass, that rarely features prominently in this ecosystem. Cheatgrass soil communities tended to be intermediate between sagebrush and interspace communities in measures of community function, structure, and composition.

After controlling for treatment differences, CLPPs were clearly delineated by cover type. Variability in CLPP patterns was relatively low in sagebrush soil samples at both sampling dates. However, CLPPs from bunchgrass, cheatgrass, and interspace soils tended to be more variable, especially during the fall. For bunchgrass samples, three different species were sampled and thus we may have detected increased

microbial functional variability as a result. Furthermore, cheatgrass genetic diversity across the Great Basin can be high (Novak and Mack 2001), which may have added to CLPP variability in annual soils. Carbohydrate use was significantly greater in interspace and cheatgrass soils, especially in sugar-amended plots. Presumably, substrate quality in the interspace is low (i.e. high C:N) because there are few organic inputs, with the exception being areas where biological soil crusts are prevalent. Higher carbohydrate use under cheatgrass might be indicative of high inputs of readily available plant carbohydrates or low quality root exudates (Evans et al. 2001).

Amino acid utilization was higher in sagebrush soils compared to cheatgrass and interspace soils. Higher amounts of soil organic matter and total N have also been found under sagebrush canopies when compared to cheatgrass and interspace soils (Bolton et al. 1990, Norton et al. 2004, Witwicki 2005). Because N in sagebrush ecosystems tends to be conserved (i.e. N mineralization and nitrification rates are relatively low), native microbial communities may be adept at using dissolved organic nitrogen (DON) when inorganic N sources are scarce. Amino acids and other forms of DON could play an important role in meeting microbial and native plant requirements in this N-limited ecosystem (Schimel and Bennett 2004). It is likely that DON is not the preferred N source for a fast-growing annual like cheatgrass (McLendon and Redente 1991, James 2008, Vasquez et al. 2008), thus restoring the balance of DON to DIN may be another tool in reclaiming cheatgrass-invaded landscapes.

Other studies have found or postulated increases in microbial biomass in cheatgrass-invaded soils (Bolton et al. 1993). Our results did not confirm this as we found lower total, gram-positive, gram-negative, actinobacterial, and protozoan PLFA biomass in cheatgrass soils when compared to sagebrush soils and no differences between cheatgrass, bunchgrass, and interspace soils for any of the same taxa. We also saw a significant decrease in fungal biomass under cheatgrass when compared to sagebrush, bunchgrass, and interspace soils. This finding lends weight to our hypothesis that cheatgrass promotes simplification of the soil ecosystem and a

potential decline in soil quality, as fungal growth can contribute to soil aggregate stability (Frey et al. 1999) and be a food source for higher level trophic groups such as fungal-feeding nematodes and microarthropods (Hunt et al. 1987).

With the loss in fungal biomass, an important consideration in the conversion from sagebrush to cheatgrass grasslands is the potential for decreasing fungi:bacteria ratios and a shift to a bacterial-dominated soil system. Fungi and bacteria, in very broad terms, can play substantially different roles in the soil ecosystem: their food web roles, growth forms, ability to decompose plant materials, carbon storage capacities, and nutrient requirements and acquisition strategies are different (Wardle 2002). Conversion from a fungal- to bacterial-dominated community would signal a major shift in belowground resources and create a positive feedback to maintain conditions that created that shift. Our data do not support this scenario, as bacterial biomass remained relatively low in both fall and spring. But our fungi:bacteria ratios are trending in the direction of shifting dominance, especially when compared to bunchgrass and interspace soils.

Absent a change in fungi:bacteria ratios, we did find significant differences in the composition of the fungal community between sagebrush and cheatgrass soils. Sagebrush soils harbor a diverse fungal community, as demonstrated by TRFLP profiles, and many of the sagebrush TRFs were not found in cheatgrass plots. Bennett et al. (2009) also found that fungal diversity was lost in fragmented soil landscapes. Conserving fungal diversity could be important in restoration efforts, as sagebrush-associated fungi may aid in germination, establishment, and growth throughout the life of the shrub (Goodwin 1992, Frost et al. 2001).

Because Witwicki (2005) found clear differences in plant biomass and tissue C and N in the same treated plots as examined in this study, we expected to find equally clear differences in soil communities due to both direct and indirect treatment effects. Indeed, research being done at different sites around the Great Basin has shown that sugar applications can create a high degree of similarity in microbial substrate use

patterns, even between sites that are hundreds of miles apart (Chapter 2, this thesis). Although we did see some evidence for treatment effects, most notably in PLFA community structure and fungal TRF richness, it played a secondary role to cover type in influencing soil communities. It is important to consider the effects of restoration treatments on soil communities, specifically, the potential to inadvertently hinder restoration efforts by adversely affecting soil communities. At least one TRF of native-plant associated fungi was completely eliminated where N and C treatments were applied, and we saw a significant decrease in fungal TRF richness in sagebrush soils from treated plots.

Site differences accounted for a portion of the variability in community patterns. At a landscape scale, sites spatially near one another generally grouped together in ordinations. The Kuna Butte, ID, site, however, tended to have nematode community patterns more similar to the spatially distant Oregon sites, which were dominated by bacterial and fungal feeding nematodes. From a qualitative perspective, the native plots at Kuna Butte were much more degraded than those at the other two Idaho sites, Mayfield and Bowns Creek. At Kuna Butte, sagebrush recruitment was low, Sandberg bluegrass was the dominate bunchgrass, and biological crust cover was low and consisted of early-successional species. If nematode trophic groups can be thought of as following successional patterns (Bongers 1990), we would expect to find bacterial feeders colonizing a degraded area first, followed by fungal feeders, then root associates and plant parasites as the vegetative component was re-established, and finally culminating in a small but robust population of omnivores and predators. The high numbers of plant and root associates at Mayfield and Bowns Creek might indicate that these sites are, in fact, healthy, intact sagebrush sites as compared to some of the other sites sampled in Oregon and Idaho (Bongers and Ferris 1999).

Although we did not explicitly address food web dynamics in this study, we did examine several levels of the soil trophic system at different times of the year. We found that microbial community function and structure were more variable in the fall

than in the spring. During the growing season, plants produce root exudates that may serve as a common substrate on which rhizosphere microorganisms can proliferate. Communities under each plant cover type became more tightly clustered in ordination space during the spring, signifying that they are influenced by the active root growth and taking on a characteristic plant-specific fingerprint (Ladygina and Hedlund 2010). For nematodes, the community shifted to a more bacterial-dominated one between fall and spring. At first this seems counterintuitive given the proliferation of plant root biomass belowground over the course of the growing season. However, this too may be driven by the association of bacterial feeding nematodes with cheatgrass cover; as above- and belowground cheatgrass biomass increases, so too does the population of bacteriovores. The loss of plant- and fungal-associated and increase in bacterial-feeding nematodes in cheatgrass soils seems to corroborate our hypothesis that cheatgrass-invaded areas promote bacterial-dominated soil communities.

Cheatgrass-invaded areas have been likened to agricultural systems where one dominant plant species establishes a monoculture, triggering the loss of spatial heterogeneity in resource distribution, depletion of soil organic matter, and simplification of soil food webs. We saw clear differences in soil community function, structure, and composition between native (particularly sagebrush) and cheatgrass-invaded soils across sampling dates, sites, and nutrient treatments. Other researchers have documented changes in nutrient cycling (Evans et al. 2001) and soil organic matter storage (Norton et al. 2004) as big sagebrush/perennial bunchgrass ecosystems are converted to cheatgrass grasslands. Our results demonstrate that the soil biological component is also altered with this conversion, raising the possibility that these community shifts could be an explanatory factor for changes described in the above studies. Understanding and facilitating the re-establishment of native soil communities may aid in advancing the overall recovery of the sagebrush steppe biome.

Acknowledgements

We generously thank L. Goldstein, T. Lair, T. Wojtowicz, R. Yarwood, J. Smith, D. McKay, T. Jennings, and L. Kluber for field, laboratory, and data analysis assistance. P. Doescher was instrumental in designing the experiment. This study was funded by the U.S. Geological Survey Forest and Rangeland Ecosystem Science Center's Coordinated Intermountain Restoration Project and the U.S. Department of Agriculture Initiative for Future Agriculture and Food Systems program (CREES Agreement No. 2001-52103-11322). Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

References

Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology **26**:32-46.

Bailey, V. L., A. D. Peacock, J. L. Smith, and H. Bolton, Jr. 2002a. Relationships between soil microbial biomass determined by chloroform fumigation-extraction, substrate-induced respiration, and phospholipid fatty acid analysis. Soil Biology & Biochemistry **34**:1385-1389.

Bailey, V. L., J. L. Smith, and H. Bolton. 2002b. Fungal-to-bacterial ratios in soils investigated for enhanced C sequestration. Soil Biology & Biochemistry **34**:997-1007.

Balser, T. C., K. K. Treseder, and M. Ekenler. 2004. Using lipid analysis and hyphal length to quantify AM and saprotrophic fungal abundance along a soil chronosequence. Soil Biology & Biochemistry **37**:601-604.

Bardgett, R. D., J. L. Mawdsley, S. Edwards, P. J. Hobbs, J. S. Rodwell, and W. J. Davies. 1999. Plant species and nitrogen effects on soil biological properties of temperate upland grasslands. Functional Ecology **13**:650-660.

Bastias, B. A., I. C. Anderson, Z. Xu, and J. W. G. Cairney. 2007. RNA- and DNA-based profiling of soil fungal communities in a native Australian eucalypt forest and adjacent *Pinus elliotti* plantation. Soil Biology & Biochemistry **39**:3108-3114.

Bates, J. D., T. Svejcar, R. F. Miller, and R. A. Angell. 2006. The effects of precipitation timing on sagebrush steppe vegetation. Journal of Arid Environments **64**:670-697.

Belnap, J. and S. L. Phillips. 2001. Soil biota in an ungrazed grassland: response to annual grass (*Bromus tectorum*) invasion. Ecological Applications **11**:1261-1275.

Bennett, L. T., S. Kasel, and J. Tibbits. 2009. Woodland trees modulate soil resources and conserve fungal diversity in fragmented landscapes. Soil Biology & Biochemistry **41**:2162-2169.

Bligh, E. G. and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemical Physiology **37**:911-917.

Blumenthal, D. M., N. R. Jordan, and M. P. Russelle. 2003. Soil carbon addition controls weeds and facilitates prairie restoration. Ecological Applications **13**:605-615.

Bolton, H., Jr., J. L. Smith, and S. O. Link. 1993. Soil microbial biomass and activity of a disturbed and undisturbed shrub-steppe ecosystem. Soil Biology & Biochemistry **25**:545-552.

Bolton, H., Jr., J. L. Smith, and R. E. Wildung. 1990. Nitrogen mineralization potentials of shrub-steppe soils with different disturbance histories. Soil Science Society of America Journal **54**:887-891.

Bongers, T. 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia **83**:14-19.

Bongers, T. and H. Ferris. 1999. Nematode community structure as a bioindicator in environmental monitoring. Trends in Ecology and Evolution **14**:224-228.

Briske, D. D., S. D. Fuhlendorf, and F. E. Smeins. 2005. State-and-transition models, thresholds, and rangeland health: a synthesis of ecological concepts and perspectives. Rangeland Ecology and Management **58**:1-10.

Callaway, R. M., D. Cipollini, K. Barto, G. C. Thelen, S. G. Hallett, D. Prati, K. Stinson, and J. Klironomos. 2008. Novel weapons: invasive plant suppresses fungal mutualists in America but not in its native Europe. Ecology **89**:1043-1055.

Chen, J. and J. M. Stark. 2000. Plant species effects and carbon and nitrogen cycling in a sagebrush-crested wheatgrass soil. Soil Biology & Biochemistry **32**:47-57.

Crooks, J. A. 2002. Characterizing ecosystem-level consequences of biological invasions: the role of ecosystem engineers. Oikos **97**:153-166.

D'Antonio, C. M. and P. M. Vitousek. 1992. Biological invasions by exotic grasses, the grass/fire cycle, and global change. Annual Review of Ecology and Systematics **23**:63-87.

Davies, K. W., J. D. Bates, and R. F. Miller. 2007. The influence of *Artemisia tridentata* spp. *wyomingensis* on microsite and herbaceous vegetation heterogeneity. Journal of Arid Environments **69**:441-457.

Edwards, U., T. Rogall, H. Blocker, M. Emde, and E. C. Bottger. 1989. Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. Nucleic Acids Research **17**:7843-7853.

Evans, R. D., R. Rimer, L. Sperry, and J. Belnap. 2001. Exotic plant invasion alters nitrogen dynamics in an arid grassland. Ecological Applications **11**:1301-1310.

Frey, S. D., E. T. Elliott, and K. Paustian. 1999. Bacterial and fungal abundance and biomass in conventional and no-tillage agroecosystems along two climatic gradients. Soil Biology & Biochemistry **31**:573-585.

Frost, S. M., P. D. Stahl, and S. E. Williams. 2001. Long-term reestablishment of arbuscular mycorrhizal fungi in a drastically disturbed semiarid surface mine soil. Arid Land Research and Management **15**:3-12.

Gardes, M. and T. D. Bruns. 1993. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. Molecular Ecology **2**:113-118.

Garland, J. L. 1996. Analytical approaches to the characterization of samples of microbial communities using patterns of potential C source utilization. Soil Biology & Biochemistry **28**:213-221.

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.

Grayston, S. J., S. Q. Wang, C. D. Campbell, and A. C. Edwards. 1998. Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biology & Biochemistry **30**:369-378.

Halvorson, J. J., H. Bolton, and J. L. Smith. 1997. The pattern of soil variables related to *Artemisia tridentata* in a burned shrub-steppe site. Soil Science Society of America Journal **61**:287-294.

Hawkes, C. V., J. Belnap, C. D'Antonio, and M. K. Firestone. 2006. Arbuscular mycorrhizal assemblages in native plant roots change in the presence of invasive exotic grasses. Plant & Soil **281**:369-380.

Hilty, J., L. Bond, M. Wicklow-Howard, and R. Rychert. 2001. Functional diversity of soil microbial communities: effects of exotic plant invasion and implications for restoration of semi-arid rangelands. Submitted to Bureau of Land Management, Idaho State Office and Lower Snake River District, Biology Department, Boise State University.

Hunt, H. W., D. C. Coleman, E. R. Ingham, R. E. Ingham, E. T. Elliott, J. C. Moore, S. L. Rose, C. P. P. Reid, and C. R. Morley. 1987. The detrital food web in a shortgrass prairie. Biology and Fertility of Soils 3:57-68.

James, J. J. 2008. Effect of soil nitrogen stress on the relative growth rate of annual and perennial grasses in the Intermountain West. Plant and Soil **310**:201-210.

Jordan, N. R., D. L. Larson, and S. C. Huerd. 2008. Soil modification by invasive plants: effects on native and invasive species of mixed-grass prairies. Biological Invasions **10**:177-190.

Kaya, H. K. and S. P. Stock. 1997. Techniques in insect nematology. Pages 281-324 *in* L. Lacey, editor. Manual of techniques in insect pathology. Academic Press, San Diego, CA.

Knapp, P. A. 1996. Cheatgrass (*Bromus tectorum L*) dominance in the Great Basin desert. Global Environmental Change **6**:37-52.

Kohler, F., J. Hamelin, F. Gillet, J. Gobat, -M., and A. Buttler. 2005. Soil microbial community changes in wooded mountain pastures due to simulated effects of cattle grazing. Plant and Soil **278**:327-340.

Kourtev, P. S., J. G. Ehrenfeld, and M. Haggblom. 2002. Exotic plant species alter the microbial community structure and function in the soil. Ecology **83**:3152-3166.

Kuske, C. R., L. O. Ticknor, M. E. Miller, J. M. Dunbar, J. A. Davis, S. M. Barns, and J. Belnap. 2002. Comparison of soil bacterial communities in rhizospheres of three plant species and the interspaces in an arid grassland. Applied and Environmental Microbiology **68**:1854-1863.

Ladygina, N. and K. Hedlund. 2010. Plant species influence microbial diversity and carbon allocation in the rhizosphere. Soil Biology & Biochemistry **42**:162-168.

Laycock, W. A. 1991. Stable states and thresholds of range condition on North American rangelands: a viewpoint. Journal of Range Management **44**:427-433.

McCune, B. and J. B. Grace. 2002. Analysis of ecological communities. MjM Software Design, Gleneden Beach, OR, USA.

McCune, B. and M. J. Mefford. 1999. PC-ORD. Multivariate analysis of ecological data. MjM Software, Gleneden Beach, OR, USA.

McLendon, T. and E. F. Redente. 1991. Nitrogen and phosphorus effects on secondary succession dynamics on a semi-arid sagebrush site. Ecology **72**:2016-2024.

Muyzer, G., E. C. d. Waal, and A. G. Uitierlinden. 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Applied and Environmental Microbiology **59**:695-700.

Norton, J. B., T. A. Monaco, J. M. Norton, D. A. Johnson, and T. A. Jones. 2004. Soil morphology and organic matter dynamics under cheatgrass and sagebrush-steppe plant communities. Journal of Arid Environments **57**:445-466.

Novak, S. J. and R. N. Mack. 2001. Tracing plant introduction and spread: genetic evidence from *Bromus tectorum* (cheatgrass). BioScience **51**:114-122.

Schimel, J. P. and J. Bennett. 2004. Nitrogen mineralization: challenges of a changing paradigm. Ecology **85**:591-602.

Sinsabaugh, R. L., M. J. Klug, H. P. Collins, P. E. Yeager, and S. O. Petersen. 1999. Characterizing soil microbial communities. Pages 318-348 *in* G. P. Robertson, D. C. Coleman, C. S. Bledsoe, and P. Sollins, editors. Standard soil methods for long-term ecological research. Oxford University Press, New York, NY.

Smith, J. L., J. J. Halvorson, and H. Bolton, Jr. 1994. Spatial relationships of soil microbial biomass and C and N mineralization in a semi-arid shrub-steppe ecosystem. Soil Biology & Biochemistry **26**:1151-1159.

Smithwick, E. A. H., M. G. Turner, K. L. Metzger, and T. C. Balser. 2005. Variation in NH_4^+ mineralization and microbial communities with stand age in lodgepole pine (*Pinus contorta*) forests, Yellowstone National Park (USA). Soil Biology & Biochemistry **37**:1546-1559.

Stringham, T. K., W. C. Krueger, and P. L. Shaver. 2003. State and transition modeling: an ecological process approach. Journal of Range Management **56**:106-113.

Vasquez, E., R. Sheley, and T. Svejcar. 2008. Nitrogen enhances the competitive ability of cheatgrass (*Bromus tectorum*) relative to native grasses. Invasive Plant Science and Management **1**:287-295.

Wall, D. H. and R. A. Virginia. 1999. Controls on soil biodiversity: insights from extreme environments. Applied Soil Ecology **13**:137-150.

Wardle, D. A. 2002. Communities and ecosystems: linking the aboveground and belowground components. Princeton University Press, Princeton, NJ, USA.

Westoby, M., B. Walker, and I. Noy-Meir. 1989. Opportunistic management for rangelands not at equilibrium. Journal of Range Management **42**:266-274.

White, T. J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315-322 *in* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, editors. PCR protocols: a guide to methods and applications. Academic Press, San Diego, CA, USA.

Witwicki, D. 2005. Sugar application and nitrogen pools in Wyoming big sagebrush communities and exotic annual grasslands. Master of Science thesis. Oregon State University, Corvallis.

Yeates, G. W., T. Bongers, R. G. M. De Goede, D. W. Freckman, and S. S. Georgieva. 1993. Feeding habits in soil nematode families and genera--an outline for soil ecologists. Journal of Nematology **25**:315-331.

Table 3.1 Summary of site characteristics for three sites each in eastern Oregon and southwestern Idaho sampled in fall 2003 and spring 2004.

Site	Plant community	Last known fire(s)[1]	Dominant vegetation	Biological soil crust cover (%)[2]	Bare ground (%)	Litter (%)	Soil depth (cm)[3]
Bowns Creek, ID	Native	Unknown (>50 years)	*Artemisia tridentata ssp. wyomingensis, Pseudoroegneria spicata, Poa secunda,*	77.8	1.4	20.3	42
	Annual	1982	*Bromus tectorum, Taeniatherum caput-medusae, P. secunda*	16.7	8.1	71.7	39
Mayfield, ID	Native	Unknown (>50 years)	*A. tridentata, Elymus elymoides, P. secunda*	61.1	13.3	23.9	25
	Annual	1985	*B. tectorum, T. caput-medusae, P. secunda*	10.6	1.9	87.5	29
Kuna Butte, ID	Native	Unknown (>50 years)	*A. tridentata, P. secunda*	51.9	2.8	44.4	30
	Annual	1987	*B. tectorum, P. secunda*	9.7	3.6	86.7	55
Lincoln Bench, OR	Native	Unknown (>50 years)	*A. tridentata, P. spicata, Achnatherum thurberianum, E. elymoides, P. secunda*	68.3	1.7	27.8	36
	Annual	1999	*B. tectorum, T. caput-medusae, P. secunda*	0.0	4.4	92.5	40
Shell Rock Butte, OR	Native	Unknown (>50 years)	*A. tridentata, P. spicata, P. secunda*	66.9	10.0	23.1	45
	Annual	1996	*B. tectorum, P. secunda*	17.2	20.6	62.2	46
Two Forks, OR	Native	Unknown (>50 years)	*A. tridentata, P. spicata, A. thurberianum, P. secunda*	71.1	4.4	24.2	>94
	Annual	1996, 2002	*B. tectorum, P. secunda*	4.2	95.3	0.6	>94

[1]Prior to sampling. Native and annual plots at Lincoln Bench and Two Forks burned in 2006.
[2]Percent cover of cyanobacteria, lichen, and moss.
[3]Depth to clay, hardpan, or bedrock.

Table 3.2 Estimates of soil chemical properties by date and cover type. Values are mean (standard error) from control plots at all sites (n = 6).

Date	Cover type	Soil pH	Total C ($g\ kg^{-1}$ soil)	Total N ($g\ kg^{-1}$ soil)	Soil C:N
Fall 2003	Sagebrush	6.86 (.10)	14.1 (1.1)	1.14 (.08)	12.3 (.3)
	Bunchgrass	6.86 (.08)	10.6 (0.9)	0.95 (.09)	11.2 (.2)
	Interspace	6.86 (.06)	7.6 (0.7)	0.74 (.07)	10.3 (.3)
	Cheatgrass	7.15 (.09)	11.2 (1.1)	1.01 (.09)	11.1 (.2)
Spring 2004	Sagebrush	6.94 (.15)	10.2 (0.7)	0.91 (.06)	11.2 (.2)
	Bunchgrass	7.07 (.12)	9.1 (0.6)	0.85 (.05)	10.7 (.3)
	Interspace	6.98 (.06)	6.2 (0.6)	0.64 (.05)	9.5 (.3)
	Cheatgrass	7.26 (.12)	9.3 (1.0)	0.87 (.08)	10.6 (.2)

Table 3.3 Multi-response permutation procedures (MRPP) *A*-statistics[1] (*P*-values) for soil community analyses for each hierarchical level in the study. Values were generated using Sorensen distance measure with square root- (CLPP) or arcsine square root- (PLFA) transformed data. Shading indicates statistical significance[2].

	Date	CLPP	PLFA	TRFLP bacteria	TRFLP fungi	Nematodes
Sampling date		.03 (.00)	.10 (.00)	ND	.09 (.00)	.05 (.00)
Site	Fall	.02 (.02)	.02 (.16)	ND	.04 (.00)	.12 (.00)
	Spring	.03 (.00)	.06 (.00)	.04 (.00)	.05 (.00)	.16 (.00)
Treatment	Fall	.00 (.30)	.06 (.00)	ND	.01 (.03)	-.01 (.84)
	Spring	.01 (.10)	.02 (.01)	.02 (.00)	.03 (.00)	-.01 (.95)
Cover type	Fall	.07 (.00)	.05 (.00)	ND	.03 (.00)	.03 (.00)
	Spring	.06 (.00)	.15 (.00)	.06 (.00)	.07 (.00)	.04 (.00)

[1] $A = 0$ when heterogeneity within groups equals expectation by chance; $A_{max} = 1$ when all items are identical within groups.
[2] *A*-statistic ≥ 0.10 accompanied by *P*-value < 0.05 is considered significant for ecological data (McCune and Grace 2002).
ND = not determined

Table 3.4 Two-way factorial PerMANOVA results from soil community analyses, spring 2004.

		CLPP		**PLFA**		**Bacterial TRFLP**		**Fungal TRFLP**		**Nematodes**	
Source	**d.f**	**F**	***P*-value**	**F**	***P*-value**	**F**	**P-value**	**F**	***P*-value**	**F**	***P*-value**
Treat	2	1.52	.08	3.05	.00	2.13	.00	3.67	.00	0.65	.89
Cover	3	3.87	.00	11.98	.00	3.48	.00	4.94	.00	2.86	.00
Treat x Cover	6	1.17	.18	0.67	.88	1.15	.09	1.53	.01	0.55	.99

Table 3.5 Indicator species analysis results for soil fungi as determined by terminal restriction fragment length polymorphism (TRFLP), spring 2004. Cover type data are from control plots only.

Cover type	Allele	Indicator value	*P*-value
Sagebrush	1FHae106	66.7	0.0060
	1FHae126	50.0	0.0430
	1FHae85	50.0	0.0342
Interspace	1FHin220	50.0	0.0396
Cheatgrass	1FHae498	50.0	0.0402
Native[1]	1FHin188	83.3	0.0006
Native	1FHae427	61.1	0.0158
Treatment			
Carbon	1FHin261	43.0	0.0002
	1FHae168	25.0	0.0022
	1FHin264	21.4	0.0118
Control	1FHin188	56.1	0.0002
	1FHae427	46.2	0.0002

[1]Native cover type indicates alleles that were found in sagebrush, bunchgrass, and interspace soils, but did not occur in cheatgrass soils.

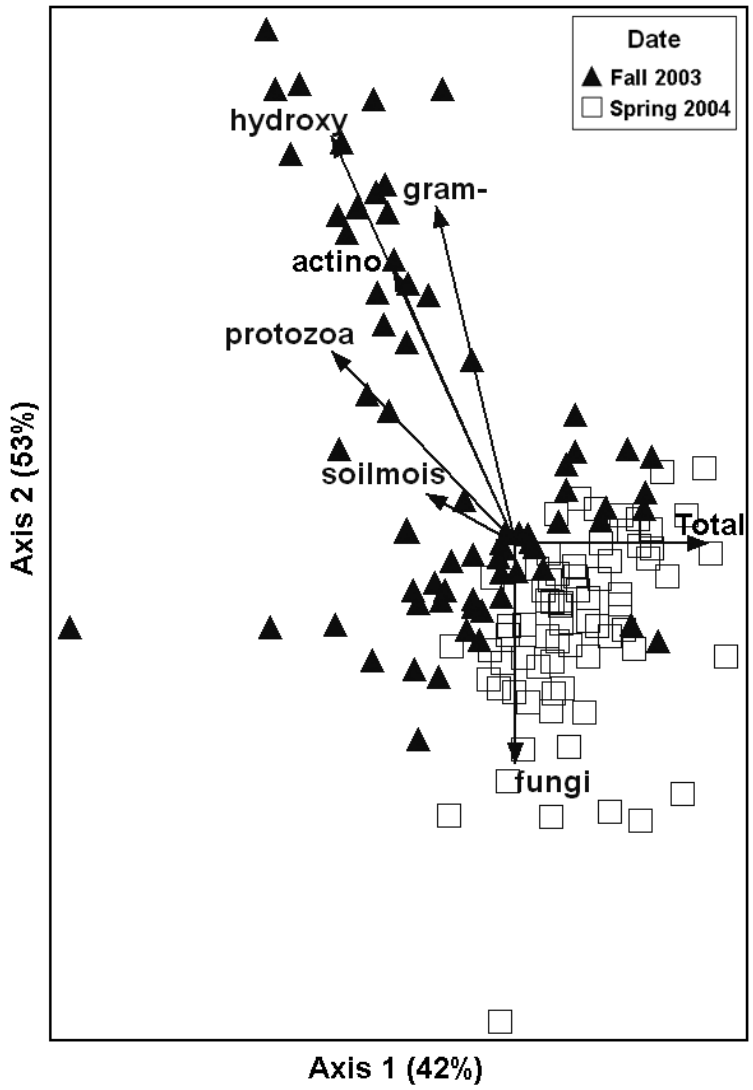

Figure 3.1 Nonmetric multidimensional scaling (NMS) ordination of soil samples collected in fall 2003 and spring 2004 for PLFA analysis. Samples are from all six study sites and treatment/cover type combinations. Points represent samples in mol % biomarker space. The ordination presented is a 2-dimensional solution; final stress = 12.99; final instability = 0.00001; cumulative R^2 = .94. Vectors represent the direction and magnitude of correlation between sample units and PLFA taxa or environmental variables: actinobacteria (r = -.49, .74), gram- bacteria (r = -.40, .82), fungi (r = .01, -.67), hydroxy groups (r = -.61, .91), protozoa (r = -.61, .62), soil moisture (r = -.42, .32), total lipids (r = .62, .03).

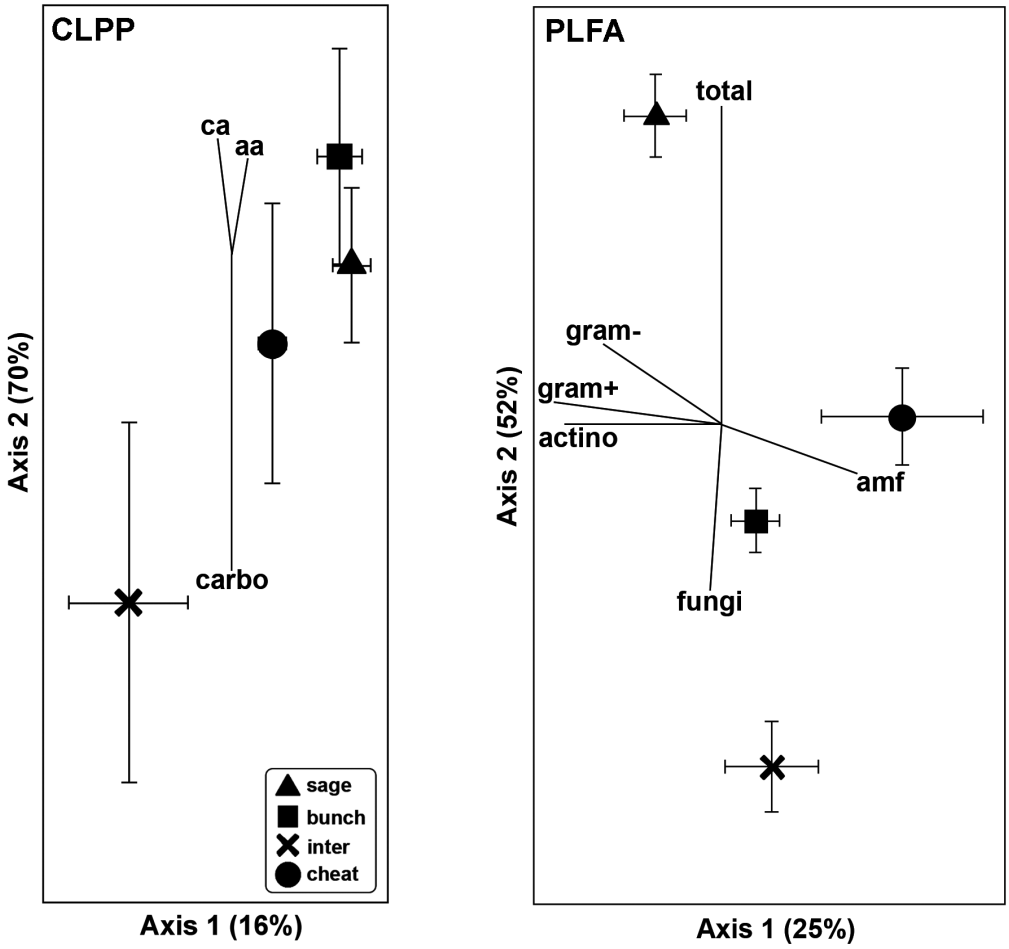

Figure 3.2 Nonmetric multidimensional scaling (NMS) ordinations of soil samples collected in spring 2004 for community level physiological profiling (CLPP) and phospholipid fatty acid (PLFA) analysis. Each point represents the mean axes scores of 18 replicate samples in carbon substrate and mol % biomarker space, respectively; bars are standard error of the mean. The CLPP ordination presented is a 2-dimensional solution; final stress = 18.83; final instability = 0.00001; cumulative R^2 = .86. The PLFA ordination presented is a 3-dimensional solution (axes 1 and 2 shown); final stress = 10.25; final instability = 0.00001; cumulative R^2 = .94. Vectors represent the direction and magnitude of correlation between sample units and CLPP guilds or PLFA taxa for the axes shown. Aa = amino acids (r = .25, .48), carbo = carbohydrates (r = -.06, -.85), ca = carboxylic acids (r = -.24, .53), actino = actinobacteria (r = -.74, -.01), amf = arbuscular mycorrhizal fungi (r = .70, -.34), fungi (r = -.21, -.63), gram- bacteria (r = -.65, .44), gram+ bacteria (r = -.77, .23), and total lipids (r = .02, .87). Legend is the same for both graphs.

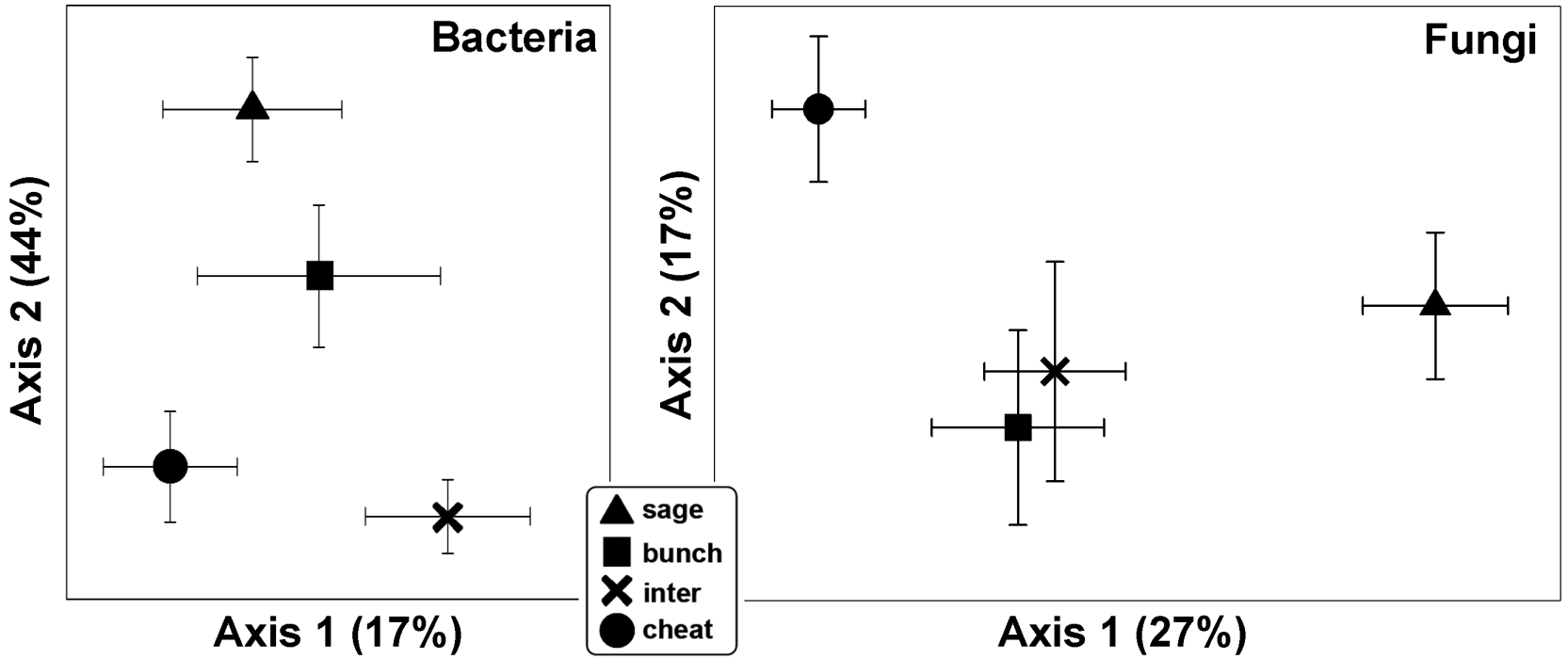

Figure 3.3 Nonmetric multidimensional scaling (NMS) ordinations of soil samples collected in spring 2004 for bacterial (left panel) and fungal (right panel) terminal restriction fragment length polymorphism (TRFLP). Each point represents the mean axes scores of 18 replicate samples in binary allele space; bars are standard error of the mean. The bacterial TRFLP ordination presented is a 3-dimensional solution (axes 1 and 2 shown); final stress = 16.19; final instability = .0003; cumulative R^2 = .81. The fungal TRFLP ordination presented is a 3-dimensional solution (axes 1 and 2 shown); final stress = 20.91; final instability = .0004; cumulative R^2 = .64. Legend is the same for both graphs.

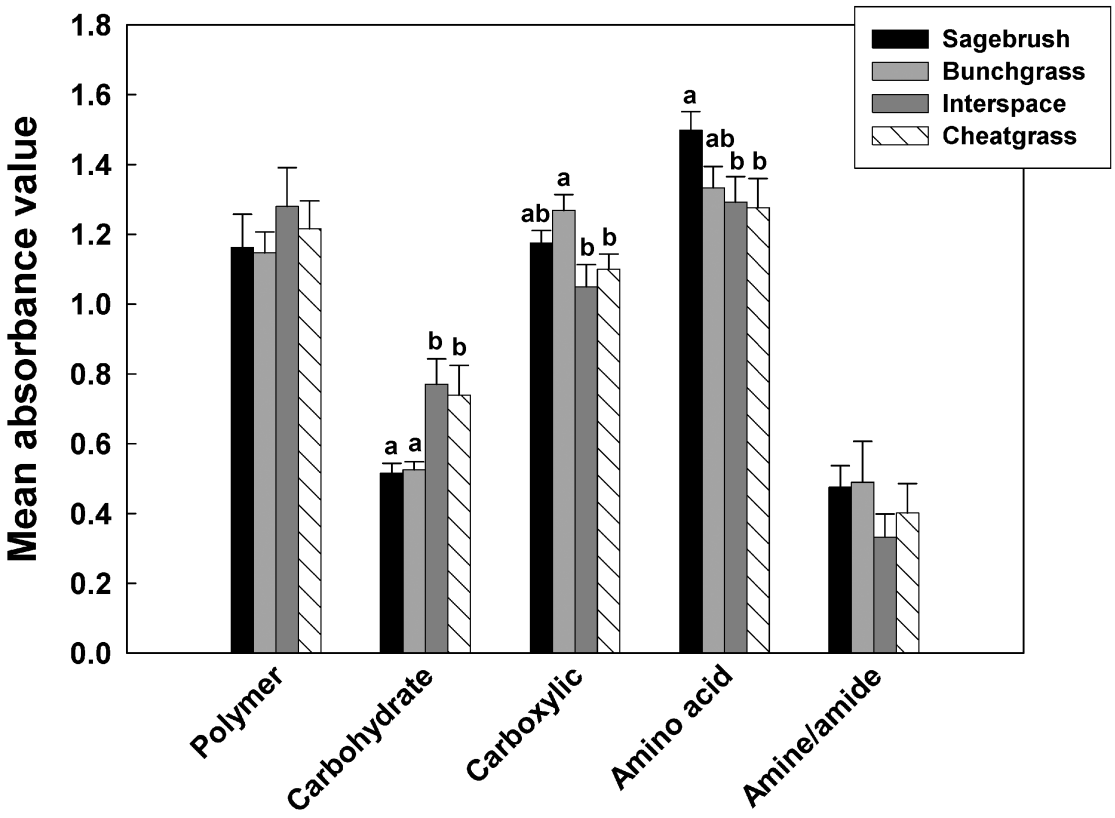

Figure 3.4 Comparison of CLPP guilds from soil samples collected in spring 2004 from all six study sites. Bars represent means with standard errors for each of the four cover types. Bars within guilds with different letters are significantly different (least square means, $P < 0.05$).

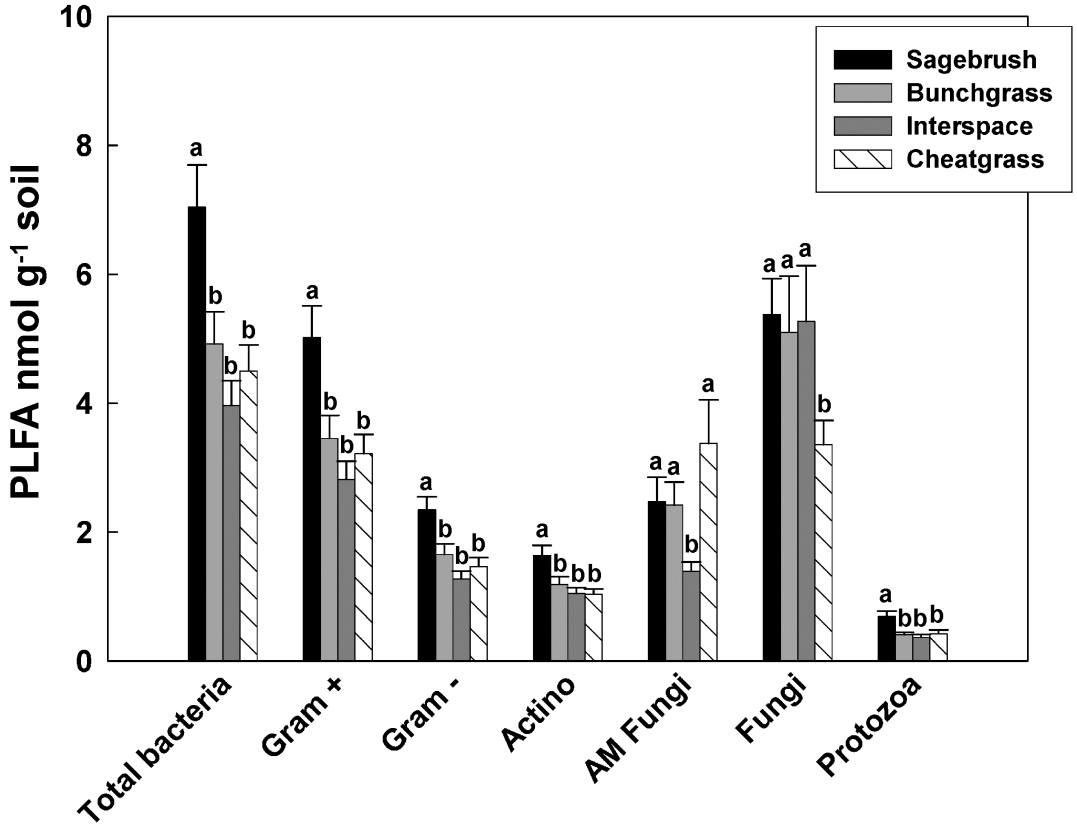

Figure 3.5 Comparison of PLFA biomarker taxa from soil samples collected in spring 2004 from all six study sites. Bars represent means with standard errors for each of the four cover types. Bars within taxonomic groups with different letters are significantly different (least square means, $P < 0.05$).

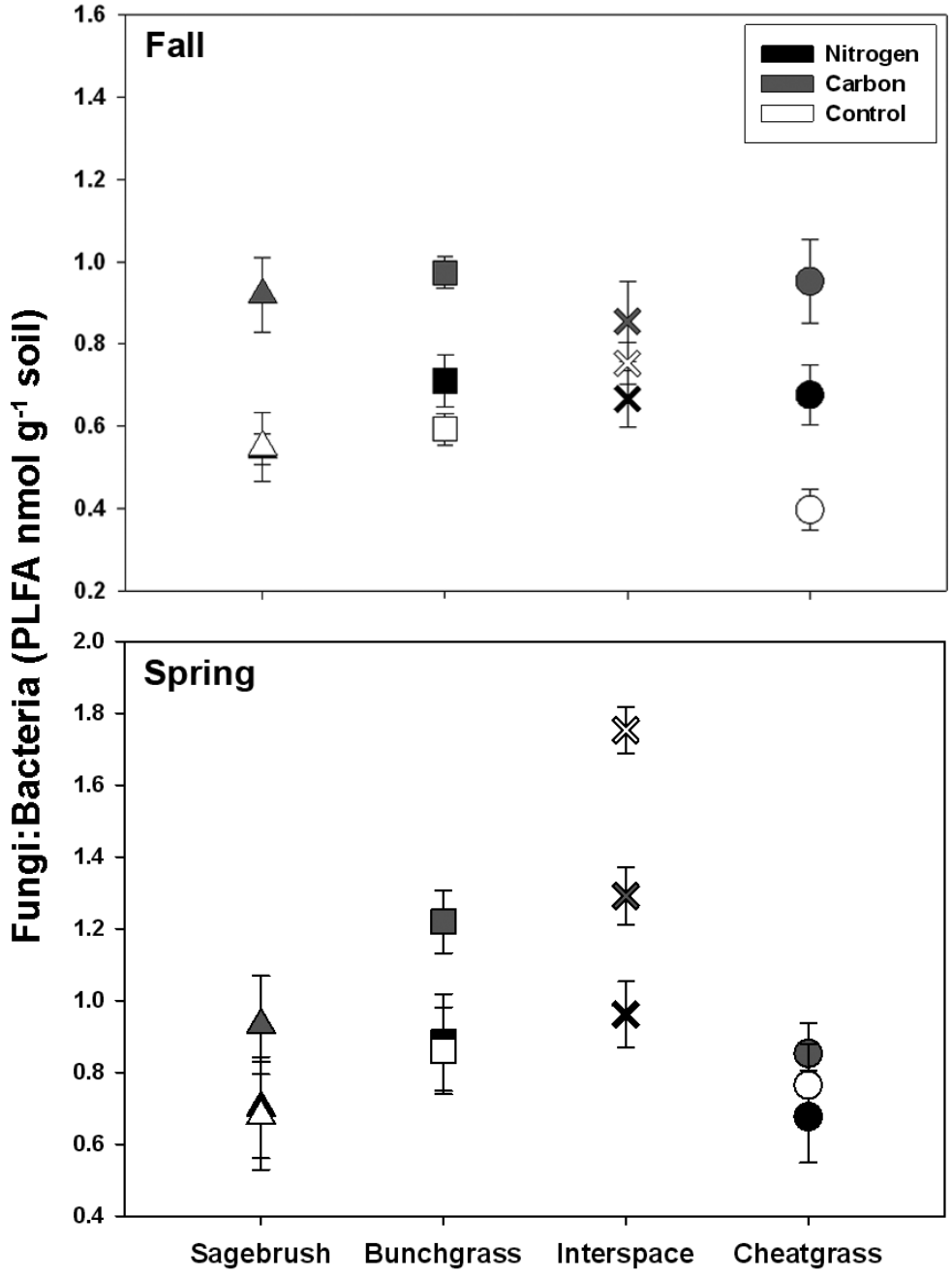

Figure 3.6 Comparison of fatty acid fungal to bacterial ratios from sagebrush, bunchgrass, interspace, and cheatgrass soils from treated and control plots sampled in fall 2003 (top) and spring 2004 (bottom). Legend is the same for both graphs.

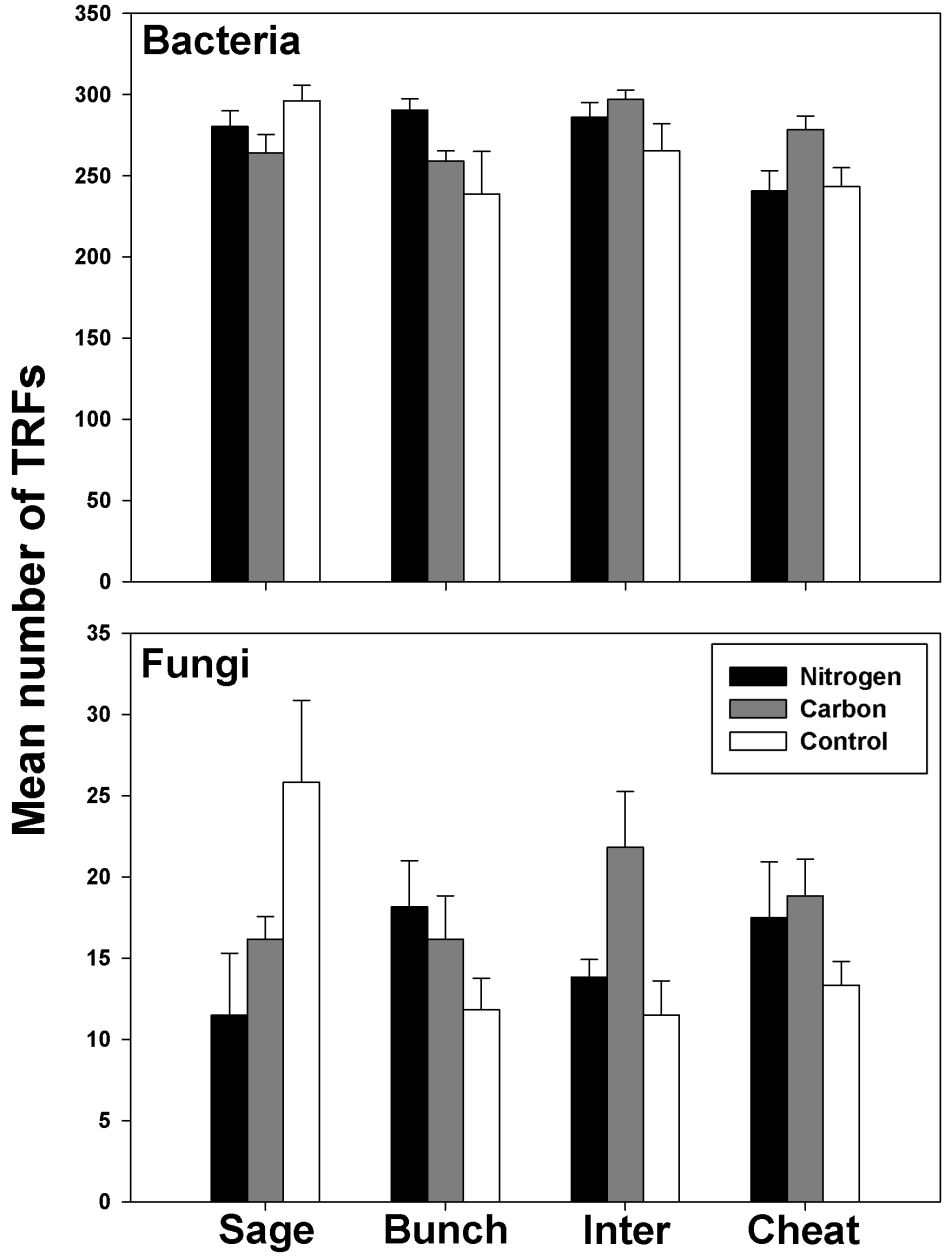

Figure 3.7 Comparison of bacterial (top) and fungal (bottom) terminal restriction fragments (TRFs) recovered from sagebrush, bunchgrass, interspace, and cheatgrass soils from treated and control plots sampled in spring 2004. Bars are means with standard error bars.

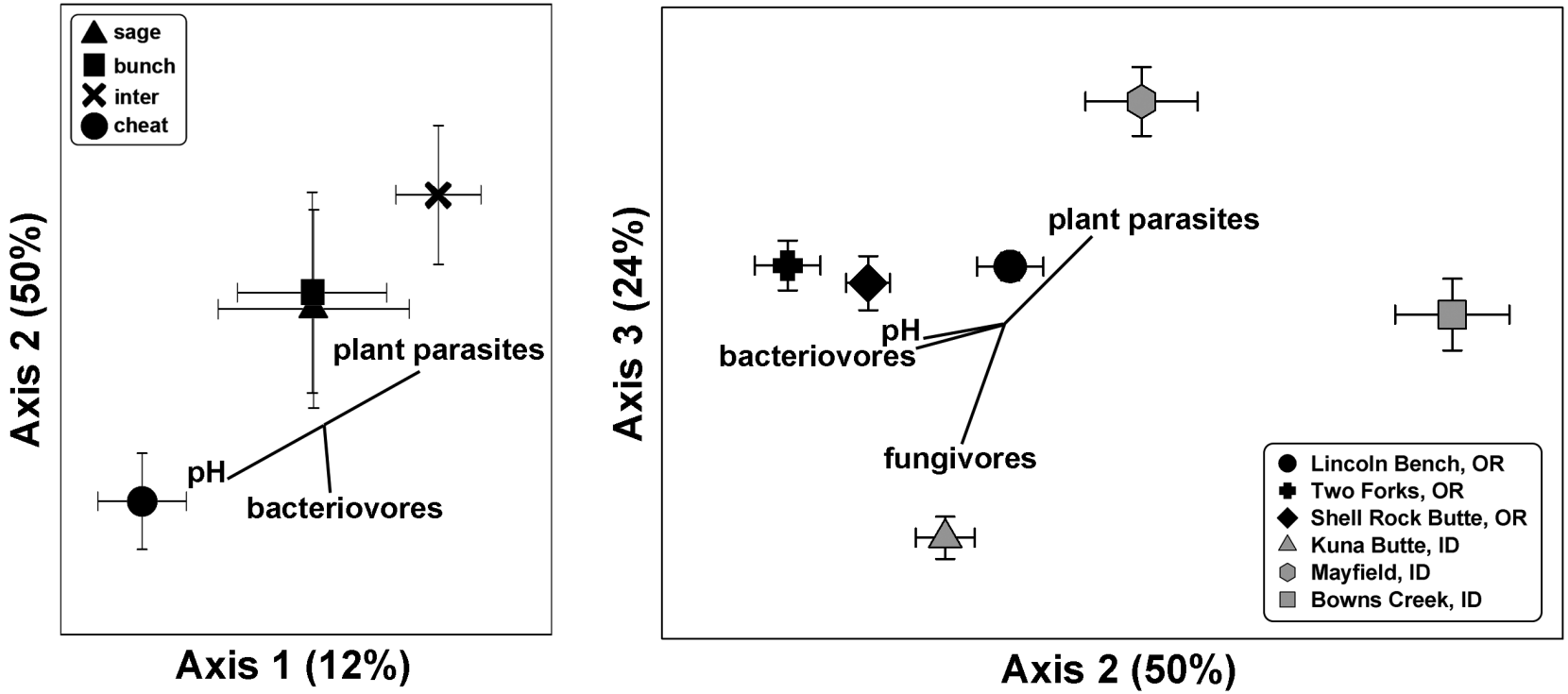

Figure 3.8 NMS ordination of soil samples collected in spring 2004 for nematode community analysis. Points represent sample units in nematode genus space coded by cover type (left panel) and site (right panel). The ordination presented is a 3-dimension solution (axes 1 and 2 shown in left panel, axes 2 and 3 shown in right panel); final stress = 14.61; final instability = .00001; cumulative R^2 = .85. Vectors represent the direction and magnitude of correlation between sample units and nematode trophic groups and pH for the axes shown: bacteriovores (r = .20, -.47, -.25), fungivores (r = .37, -.32, -.56), plant parasites (r = .51, .46, .49), and pH (r = -.51, -.46, -.01).

Chapter 4

The contribution of soil fungi to inorganic nitrogen cycling in sagebrush and cheatgrass-influenced soils

Nicole M. DeCrappeo, Elizabeth J. DeLorenze, Elizabeth A. Brewer, Stephanie A. Yarwood, David A. Pyke, David D. Myrold, and Peter J. Bottomley

Abstract

Exotic plant species can become ecosystem engineers in their new habitats and dramatically alter above- and belowground properties and processes. Cheatgrass (*Bromus tectorum* L.) is one such exotic annual grass that can potentially convert diverse, nitrogen (N)-limited sagebrush steppe ecosystems to homogenous, N-mineralizing environments. Although many studies have characterized pools and fluxes of inorganic N (i.e., ammonium and nitrate) in sagebrush and cheatgrass rhizosphere soils, little research has focused on the relative contributions of specific microbial groups to N cycling in these soils. Previous work had determined that soil community structure and composition from cheatgrass-invaded and uninvaded sagebrush/perennial bunchgrass plots differed significantly, most notably in fungal abundance and diversity. To assess the role of fungi in N cycling in Wyoming big sagebrush (*Artemisia tridentata* ssp. *wyomingensis* Nutt.) and cheatgrass rhizosphere soils, we added the fungal protein synthesis inhibitor cycloheximide (CHX) to a $^{15}NH_4^+$ pool dilution experiment. We determined gross NH_4^+ production and consumption, net mineralization and nitrification, and microbial biomass C and N at 0-24 and 24-48 h. There were no differences between control sagebrush and cheatgrass soils in any of the measured gross or net N cycling rates. Gross NH_4^+ mineralization and consumption significantly decreased in both soil types in the presence of CHX. Cycloheximide-treated sagebrush soils exhibited increases in net N mineralization and nitrification relative to cheatgrass soils during the 24-48 h incubation period. Our results suggest that N cycling has not been measurably affected by cheatgrass invasion into sagebrush steppe ecosystems of the Snake River Plain and that soil fungi play a large role in N cycling in both soil types. However, because of the accumulation of NH_4^+ in sagebrush soils after 24 h, we posit that bacterial communities in these soils are dependent on fungi for organic matter decomposition and can become carbon limited in their absence.

Keywords: *Bromus tectorum*, cheatgrass, invasive plant species, *Artemisia tridentata*, Wyoming big sagebrush, Snake River Plain, soil communities, soil fungi, nitrogen cycling, isotope dilution, cycloheximide.

Introduction

Cheatgrass (*Bromus tectorum* L.) invasion in the northern Great Basin has transformed landscapes from biologically diverse shrub/perennial bunchgrass ecosystems to homogenous annual grasslands, greatly reducing fire return intervals, reducing native biodiversity, and altering nutrient inputs and cycling rates (D'Antonio and Vitousek 1992). Soil nitrogen (N) is particularly important in the story of cheatgrass invasion because, after water, it is the limiting resource in sagebrush steppe (Smart et al. 1999). Intact sagebrush ecosystems have been described as having tight nutrient cycles, where available N is quickly taken up by a diversity of plants and microorganisms (Norton et al. 2007). In areas grazed by livestock, disturbed by fire, or colonized by exotic plant species, concentrations of inorganic N (NH_4^+ and NO_3^-) can increase and be lost from the system by leaching, volatilization, or denitrification (Schimel 1986, Smart et al. 1999, Norton et al. 2004). Indeed, many researchers have concluded that cheatgrass promotes the conversion of a predominantly N immobilizing system to a "leaky" N mineralizing environment (Smith et al. 1994, Norton et al. 2004, Norton et al. 2007). Two factors contribute to this potential conversion. First, the many fine, shallow roots of cheatgrass modify soil structure such that formerly protected organic matter begins to breakdown, increasing the availability of both carbon and mineral N (Norton et al. 2004). Second, frequent fires, fed by highly flammable cheatgrass litter, volatilize organic N to inorganic forms (D'Antonio and Vitousek 1992). The conversion to an N mineralizing environment is meaningful because invasive annuals such as cheatgrass tend to prefer readily available inorganic N and can outcompete native plants for this resource (Huenneke et al. 1990).

Nitrogen transformations are carried out or stimulated by soil biota, including bacteria, saprophytic and mycorrhizal fungi, and micro- and macroinvertebrates.

Understanding their function in N cycling is crucial to interpreting results of plant interaction studies. For example, the outcome of a plant competition experiment could be explained by intrinsic factors, such as differences in plant growth rates or N use efficiencies, or by extrinsic factors, such as soil pH, associations with arbuscular mycorrhizal fungi, or the density of bacterial-feeding nematodes in the rhizosphere. Plant species have a direct effect on the composition of the microbial community primarily through the quality and quantity of litter and root exudates (Grayston et al. 1998, Kuske et al. 2002). This creates a feedback loop in which microbes that are most efficient at recycling species-specific plant compounds thrive and release plant-available nutrients as a by-product of their metabolic activities (Bever et al. 1997, Klironomos 2002). In the context of N cycling, microbial communities associated with sagebrush or cheatgrass rhizospheres may be well adapted to using specific forms of N if these prove to be available in appreciable amounts.

Soil bacteria and fungi have diverse nutrient requirements and resource-use efficiencies, and while they share roles as decomposers and nutrient recyclers, the specifics of their interactions with plants, contributions to ecosystem processes, and relative contributions to inorganic N pools may be very different (Boyle et al. 2008). It is clear that cheatgrass invasion fundamentally alters fungal and bacterial communities of sagebrush ecosystems (Chapter 3, this thesis). We do not yet understand if and how these compositional changes in microbial communities affect plant competition or ecosystem processes, nor do we know if they represent another barrier to successful restoration of native perennials and shrubs in cheatgrass soils.

The goal of this study was to determine the contribution of soil fungi to N mineralization and inorganic N consumption in Wyoming big sagebrush (*Artemisia tridentata* Nutt. ssp. *wyomingensis* Beetle & Young) and cheatgrass rhizospheres. Our hypothesis was that fungi would be more important to N cycling in sagebrush soils than in cheatgrass soils. Our specific objectives were to 1) quantify differences in gross and net N cycling rates in sagebrush and cheatgrass soils, and 2) measure effects of a fungal protein-synthesis inhibitor on these rates.

Materials and Methods

Site descriptions and soil collection

Soils were collected from sites in the Snake River Plain, USA. The region has a Mediterranean climate with cold, wet winters and warm, dry summers. Average annual precipitation ranges from 20 to 30 cm, with 60 percent received from October through March (Bates et al. 2006). Six study sites had been established in 2003, three each near Vale, OR, and Boise, ID (Table 4.1). The westernmost site was located approximately 160 km away from the easternmost site. Sites were chosen to be representative of Wyoming big sagebrush/bluebunch wheatgrass (*Pseudoroegneria spicata* [Pursh] A. Löve) ecological sites as defined by the Natural Resources Conservation Service (USDA NRCS). This designation takes into consideration climate, soil, and hydrologic conditions in addition to potential plant community composition and productivity. We also compared elevation, aspect, landscape position, and soil morphological characteristics to ensure that sites could be used as replicates.

All sites were historically dominated by Wyoming big sagebrush and perennial bunchgrasses, but one or more burn events over the past 50 years contributed to a loss of shrubs and conversion to annual grasslands. At each of the six sites, we established a "native" sagebrush/perennial bunchgrass plot and an "annual" cheatgrass-dominated plot located on similar soils no more than 3 km apart. Dominant plant species in native plots included Wyoming big sagebrush, bluebunch wheatgrass, Sandberg bluegrass (*Poa secunda* J. Presl), and bottlebrush squirreltail (*Elymus elymoides* [Raf.] Swezey). Vegetation in annual plots was dominated by cheatgrass but also included Sandberg bluegrass and the exotic annual medusahead (*Taeniatherum caput-medusae* L. Nevski). All plots had been fenced since at least 2003 to exclude cattle. In April 2008, we collected approximately 1.2 kg of soil from underneath mature sagebrush shrubs and cheatgrass plants at their respective plots at each site. We sampled soils in Idaho two weeks after sampling in Oregon to account for a lag in plant phenology at the Idaho sites. Soils were collected from 0-10 cm using a 2-cm diameter corer, transported to the laboratory in coolers with ice packs, and sieved to < 2 mm within 48

h of field collection. Subsamples were removed to determine gravimetric water content, pH, and electrical conductivity; the remaining soil was air-dried overnight.

^{15}N isotope dilution experiment

Approximately 150 g of soil from each of the 12 site-by-soil type combinations (6 sites x 2 soil types) was placed into a sealable plastic bag and re-wetted to field moisture with an aqueous solution of $(^{15}NH_4)_2SO_4$ and the fungal protein synthesis inhibitor cycloheximide (CHX) (Velvis 1997). $^{15}NH_4^+$ (98 atom%) was added at a concentration of 2 mg N kg^{-1} dry soil to label the NH_4^+ pool to approximately 2 atom% ^{15}N. Cycloheximide was added at a concentration of 2 g kg^{-1} dry soil (Boyle et al. 2008). After adding the solutions to each bag, the soil was thoroughly homogenized by hand, placed in sealed canning jars, and incubated at 25ºC. One-third of the jars were destructively sampled at each of three times: 3 h (T_0), 24 h, and 48 h. At each sample time, 20 g of wet soil was removed for extraction with 50 mL 0.05M K_2SO_4 to determine inorganic N concentrations. Another 20 g portion was subjected to chloroform fumigation extraction (CFE) for 24 h and extracted with 50 mL 0.05M K_2SO_4 for microbial biomass measurements. Gravimetric soil moisture was determined by drying 10 g of soil at 105°C for 24 h in order to express N-cycling rates by dry soil weight. Soil extracts were frozen at -20°C until further analysis.

Soluble C and N pools

Soluble total organic C (TOC, measured as non-purgeable organic C due to varying amounts of inorganic C in these soils) and total N (TN) of fumigated and unfumigated extracts were measured with a Shimadzu TOC analyzer (TOC-V CSH with TNM-1 unit, Shimadzu, Kyoto, Japan). Microbial biomass C (MBC) and N (MBN) were calculated as the difference between fumigated and unfumigated TOC and TN values, respectively. Inorganic N (NH_4^+-N and NO_3^--N) pools were measured colorimetrically on an Astoria Pacific series 300 autoanalyzer (Astoria Pacific, Inc., Clackamas, OR). Cycloheximide degrades under the alkaline conditions used in the autoanalyzer

procedure, therefore it was necessary to correct for the amount of NH_4^+-N contributed by CHX remaining in the soil extracts. The percentage of CHX remaining in solution was first determined by calculating the difference between CHX-treated and control TN values and dividing by the amount of CHX–N added to soils (CHX remaining in solution ranged between 39-85% of the amount added at the beginning of the experiment). We ran standards that encompassed a range of concentrations for CHX (0, 33, 67, 100, 133% of CHX remaining in solution) and NH_4^+-N (0.5, 1.0, 1.5, 2.0 ppm) on the autoanalyzer and created regression equations specific to the percent of CHX remaining in each soil extract. We then used the raw autoanalyzer NH_4^+-N value in the appropriate regression equation to calculate a corrected value. The mean difference between raw and corrected CHX NH_4^+-N values was ±0.41 mg L^{-1}.

Isotopic determination of NH_4^+-N

Isotopic determination of NH_4^+-N in soil extracts was performed following the diffusion procedure of Stark and Hart (1996). We added a 100 µL $(NH_4)_2SO_4$ natural abundance spike to each sample to ensure that total N was high enough for accurate atom% determination. After adding MgO to each sample to create an alkaline environment, extracts were shaken on an orbital shaker at 25ºC for 10 d in order to diffuse NH_4^+ onto acidified discs. Discs were dried and wrapped in tin cups; $\%^{15}N$ was measured on a continuous flow isotope ratio mass spectrometer (CF-IRMS, PDZ Europa, England). As with the autoanalyzer procedure, it was necessary to account for NH_4^+ contributed by CHX degradation during the alkali diffusion process. Using a range of CHX diffusion standards (0, 33, 67, 100, 133% of CHX remaining in solution), we created a regression equation to determine the amount of NH_4^+-N added for the percent of CHX remaining in each soil extract.

Rate calculations

We determined gross and net N-cycling rates for three time periods: 0-24 h, 24-48 h, and 0-48 h. Gross NH_4^+ production and consumption rates were calculated using the

Kirkham and Bartholomew (1954) equations. Because there were significant changes in NH_4^+ and NO_3^- pool sizes over the course of the experiment, we used the equation for unequal rates of production and consumption. Net N mineralization and net nitrification were calculated as the change in NH_4^++NO_3^- and NO_3^- concentrations, respectively, over the course of the experiment. Corrected CHX NH_4^+-N autoanalyzer and diffusion values were used in all relevant calculations and statistical analyses. Ammonium values from cheatgrass soils sampled from Two Forks, OR, were determined to be outliers and not used in any of the rate calculations or subsequent analyses.

Statistical analyses

Data were analyzed using SAS 9.2 software (SAS Institute Inc., Cary, NC). Differences in soil chemical and biological properties between control sagebrush and cheatgrass soils (n = 6) were analyzed using two-sample t-tests. Data from the 48 h incubation experiment were analyzed in PROC MIXED as a randomized complete block design with split-plot treatments. Site was a random blocking factor, soil type (sagebrush or cheatgrass) was a fixed whole-plot factor, and treatment (control or CHX) was a fixed split-plot factor. Multiple comparisons of means were performed using Tukey's Honestly Significant Difference. To assess the magnitude of site variability on gross and net rate estimates, an F-statistic and p-value for the random effect of site was generated using PROC GLM. Changes in gross and net N cycling rates and microbial biomass C, N, and C:N over time were analyzed using repeated measures ANOVA with unstructured covariance. Negative gross rates and microbial biomass values were not used in any of the analyses. For all tests, $p \leq 0.05$ was the criterion for statistical significance, and $0.05 < p \leq 0.10$ was considered marginally significant.

Results

Gross and net N cycling rates

At the beginning of the experiment, soluble TOC, NO_3^-, MBC, and MBN were all higher in sagebrush soils than cheatgrass soils (Table 4.2). There were no significant differences between control sagebrush and cheatgrass soils for any of the measured gross or net rates over the course of the 48 h experiment (Fig. 4.1). Gross NH_4^+ production decreased by about 50% in both soil types in the presence of CHX (Fig. 4.1A). There was nearly an order of magnitude decline in NH_4^+ consumption by the CHX treatment for both soil types, but, similar to gross NH_4^+ production, no difference between sagebrush and cheatgrass soils within a treatment (Fig. 4.1B). Net N mineralization was controlled by an interaction between soil type and CHX treatment (Fig. 4.1C). Rates for control sagebrush and cheatgrass soils were similar and over an order of magnitude lower than CHX-treated soils. Net N mineralization in CHX-treated sagebrush soils, however, was nearly twice as high as treated cheatgrass soils. Net nitrification was similar between soil types and treatments but tended to be about two-fold higher in CHX-treated sagebrush soils (Fig. 4.1D). Site did not significantly contribute to variability in any of the measured rates (Table 4.3).

Changes in rates over time

There were no significant differences in gross NH_4^+ production rates between 0-24 h and 24-48 h, but CHX rates did start to approach those of control samples during the second 24 h period (Fig. 4.2A). For NH_4^+ consumption, there was a treatment-by-time interval interaction with control rates decreasing and CHX rates slightly increasing over time (Fig. 4.2B). Net N mineralization and nitrification were both highest in CHX-treated sagebrush soils (Figs. 4.2C and D), and there was a marginally significant three-way interaction between soil type, treatment, and time interval for net nitrification. Net rates for control soils and CHX-treated cheatgrass soils did not change over time.

Microbial biomass

Microbial biomass C and N levels were consistently higher in control sagebrush soils than cheatgrass soils at 0, 24, and 48 h, while CHX-treated soils exhibited much more variability over the course of the experiment (Figs. 4.3A and B). Microbial biomass C significantly decreased over time in both control and CHX-treated soils (Fig. 4.3A). However, there was a marginally significant soil type-by-treatment interaction due to higher MBC in CHX-treated cheatgrass soils as compared to control soils, as well as a significant treatment-by-time interaction due to a sharp decrease in MBC in CHX-treated sagebrush soils at 24 h (Fig. 4.3A).

There were no changes in MBN over time (Fig. 4.3B). As with MBC, there was a decrease and subsequent increase in CHX-treated sagebrush soils after 24 and 48 h, respectively, which contributed to the significant soil type-by-treatment interaction. Microbial biomass C:N ratios were similar between control and CHX samples at each sampling time, but did decrease significantly over the course of the experiment (Fig. 4.3C).

Discussion

Despite the large spatial extent of our experimental sites—six sites located over a 160 km range—site did not significantly contribute to variability within the dataset, and we were able to discern patterns related to soil type and treatment. This gives us some confidence that our scope of inference can be applied to Wyoming big sagebrush ecosystems of the Snake River Plain with loam or silt loam surface soils. Although N dynamics in sagebrush-perennial bunchgrass communities have been examined extensively, most studies have been conducted at plots or along transects at a single site (Bolton et al. 1990, Chen and Stark 2000, Evans et al. 2001, Smith et al. 2002, Booth et al. 2003, Saetre and Stark 2005, Hooker et al. 2008, James et al. 2008), somewhat limiting researchers' ability to generalize to the larger ecosystem. This may help explain why the present study's results do not agree with those that found differences in gross and net N cycling rates between sagebrush and cheatgrass soils at

specific sites. Booth et al. (2003), for example, reported that gross NH_4^+ production and consumption were twice as high in cheatgrass soils as they were in sagebrush soils in northern Utah. At another northern Utah site, Saetre and Stark (2005) found that cheatgrass soils had faster gross N mineralization rates and approximately three times as much 2 M KCl-extractable NO_3^- as sagebrush soils throughout the course of a 10-d wetting experiment. It is worth noting that soils at both Utah sites were silt loams of lacustrine origin high in calcium carbonate, whereas our soils are derived primarily from weathered volcanic materials. In addition to seeing no difference in N cycling rates between soil types, the gross NH_4^+ production and consumption rates in our study were between two to ten times higher than those found by others (Chen and Stark 2000, Booth et al. 2003, James et al. 2008). That we found no differences between sagebrush and cheatgrass net N mineralization and nitrification rates was especially surprising given other researchers' characterization of cheatgrass soils as more "leaky" in regards to N cycling (Booth et al. 2003, Norton et al. 2007). Interestingly, it was sagebrush soils that had higher NO_3^- concentrations at the start of our experiment. This may be partially explained by the timing of soil collection. In the Snake River Plain, both native and exotic plants are actively growing in April, but cheatgrass has been acquiring soil resources throughout the winter and early spring. At the time of sampling for this experiment, it is possible that cheatgrass plants had already taken up readily available soil NO_3^-. Timing issues aside, our net nitrification data certainly suggest that sagebrush soils convert as much NH_4^+ to NO_3^- under optimal conditions and should not be considered "conservative" in this respect.

Nitrogen process rates were variously affected by the CHX treatment. Cycloheximide inhibits the anabolic process of protein synthesis in fungi. It does not necessarily prevent fungi from performing catabolic reactions, such as mineralizing organic matter to meet respiratory needs, if intracellular and extracellular enzymes have been synthesized before addition of the inhibitor (Boyle et al. 2008, Rousk et al. 2008). Gross NH_4^+ production in CHX-treated soils may have continued in our experiment because previously produced fungal enzymes or newly produced bacterial

enzymes were contributing to organic matter mineralization. Contrary to the results of Boyle et al. (2008), we did not see stimulation of gross NH_4^+ production in the presence of CHX but instead saw an approximately 50% decrease in this rate. This slowing of NH_4^+ production suggests that fungi play an important role in producing enzymes that mineralize organic matter in both sagebrush and cheatgrass soils.

Ammonium consumption, which includes microbial NH_4^+ assimilation and oxidation of NH_4^+ to NO_3^-, was greater than gross NH_4^+ production in control soils over the course of the experiment. Both soil types started with high NH_4^+ concentrations, which may have accounted for the additional NH_4^+ consumed and NO_3^- produced. Cycloheximide dramatically decreased the consumption of NH_4^+. Although we expected CHX to strongly inhibit fungal N uptake in sagebrush soils, we were surprised that it was equally effective in cheatgrass soils given the lower fungal biomass and species richness found in these soils (Chapter 3, this thesis). The implication is that fungi are important consumers of N in sagebrush ecosystems. Further, we can infer that bacteria did not compensate for or take advantage of the lack of fungal competition, and that they potentially play a more minor role in N cycling processes in this system. Additional evidence for this comes from phospholipid fatty acid (PLFA) data from the same study sites showing that bacteria account for only 16% of the PLFA biomass (Chapter 3, this thesis), a percentage well below that of grassland (Bardgett and McAlister 1999, Grayston et al. 2004) and forest soils (Brant et al. 2006).

Cycloheximide had differential effects depending on soil type for net N mineralization (from 0-48 h) and nitrification (from 24-48 h). Cycloheximide-treated cheatgrass soils had higher net N mineralization rates than control soils, but net nitrification did not differ. The net N mineralization increase can therefore be attributed to accumulated NH_4^+ or NO_3^- that could not be immobilized by fungi due to protein synthesis inhibition. Net nitrification, on the other hand, remained constant presumably because autotrophic nitrifying bacteria were already performing that transformation in cheatgrass soils. Inhibiting fungi did not affect this process rate

because fungi were not contributing to nitrification in cheatgrass soils to begin with. Production and consumption remained coupled in CHX-treated cheatgrass soils; in other words, net rates were close to what were expected given the amount of NH_4^+ produced and consumed.

In CHX-treated sagebrush soils, however, there were increases in both net rates after 24 h. There are two competing explanations for these results. The first is that fungi in sagebrush soils are primarily responsible for breaking down organic matter into smaller, more easily degradable compounds. These compounds can then be used by bacteria as an energy source (i.e., C). In our experiment, after fungal enzyme production ceased and usable decomposition products were less abundant, bacteria may have become C limited and been unable to assimilate NH_4^+ into biomass. Net nitrification increased as a result of high substrate availability (i.e., accumulation of NH_4^+) for nitrifying bacteria.

A second explanation for increased net rates is that fungi in sagebrush soils are good competitors for soil resources, and when fungi are effectively removed from the system bacteria take full advantage of substrate availability and increase mineralization and nitrification. However, because the final concentrations of NH_4^+ and NO_3^- in CHX-treated sagebrush soils were higher than expected given the amount of NH_4^+ produced and consumed over the experiment, alternative organic N pools must have been exploited under this scenario. One possible N source was the microbial biomass pool, which had sufficient N to drive the high net rates observed. However, if fungi were accessing this pool, we would expect this to hasten ^{15}N pool dilution and lead to higher gross NH_4^+ production, which we did not see. An alternative explanation is that fungi were performing heterotrophic nitrification, directly oxidizing organic N to NO_3^- and thereby bypassing the NH_4^+ pathway. Evidence for heterotrophic nitrification has been found in some forest (Schimel et al. 1984, Killham 1987) and grassland soils (Laughlin et al. 2008). Future analysis of $^{15}NO_3$-labeled soils and bacterial-inhibited soils that were part of the present pool

dilution experiment will help clarify the role of heterotrophic nitrification in the sagebrush steppe.

Traditional isotope dilution experiments take place over 48 h, long enough for microorganisms to mineralize organic matter and cycle nutrients, but not long enough to allow for significant changes in community composition and structure. Because we were purposely manipulating the microbial community in this experiment, we thought it was important to quantify inorganic and microbial N pools at 24 h. We were also concerned about the possibility of CHX breakdown during the 48 h incubation: if CHX degraded, it would have the undesired effects of permitting fungal protein synthesis and contributing to faster pool dilution via the addition of CHX-derived $^{14}NH_4^+$. We confirmed that there was no CHX breakdown during the experiment by examining total soluble C and N at 0, 24, and 48 h (data not shown). But we also discovered valuable information about these soils from the change in rates over 0-24 and 24-48 h, such as the apparent adaptive ability of sagebrush soil communities to oxidize more NH_4^+ from 24-48 h (Fig. 4.2D). We recommend that researchers conducting similar experiments analyze soil extracts at both 24 and 48 h.

Microbial community analyses done previously on soils from the same study area showed that PLFA fungal:bacterial ratios were not different between sagebrush and cheatgrass soils (Chapter 3, this thesis). Fungal:bacterial ratios have been shown to decrease with increasing N availability (de Vries et al. 2006) and pH (Högberg et al. 2007) and increase with larger quantities of stored soil C (Bailey et al. 2002). In the current study, we did not find differences in N availability or pH between sagebrush or cheatgrass soils, and this may help to explain why fungi:bacteria did not change. However, it is important to note that total microbial biomass (determined in this study by CFE) and PLFA fungal and bacterial biomass were higher under sagebrush, and fungal and bacterial community composition were dramatically different under the two plants, with a higher proportion of rare and unique species being found in sagebrush soils (Chapter 3, this thesis). Perhaps in sagebrush steppe ecosystems of the Snake River Plain, the significant change brought about by cheatgrass invasion is not

the conversion from an N-limited system to an N-replete one, but the loss of microbial biomass and species diversity. These losses seem to have had only minor affects on N cycling processes thus far, but there may be many other functionally significant roles that microorganisms play to maintain sagebrush/perennial shrublands or cheatgrass grasslands.

Acknowledgements

We would like to thank Upekala Wijayratne and Kevin Knutson for laboratory help and Manuela Huso of the Quantitative Services Group of the College of Forestry at Oregon State University for statistical advice. This study was funded by the U.S. Geological Survey Forest and Rangeland Ecosystem Science Center Coordinated Intermountain Restoration Project. Any use of trade names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

References

Bailey, V. L., J. L. Smith, and H. Bolton. 2002. Fungal-to-bacterial ratios in soils investigated for enhanced C sequestration. Soil Biology & Biochemistry **34**:997-1007.

Bardgett, R.D., and McAllister, E., 1999. The measurement of soil fungal:bacterial biomass ratios as an indicator of ecosystem self-regulation in temperate meadow grasslands. Biology and Fertility of Soils **29**:282–290.

Bates, J. D., T. Svejcar, R. F. Miller, and R. A. Angell. 2006. The effects of precipitation timing on sagebrush steppe vegetation. Journal of Arid Environments **64**:670-697.

Bever, J. D., K. M. Westover, and J. Antonovics. 1997. Incorporating the soil community into plant population dynamics: the utility of the feedback approach. Journal of Ecology **85**:561-573.

Bolton, H., Jr., J. L. Smith, and R. E. Wildung. 1990. Nitrogen mineralization potentials of shrub-steppe soils with different disturbance histories. Soil Science Society of America Journal **54**:887-891.

Booth, M. S., J. M. Stark, and M. M. Caldwell. 2003. Inorganic N turnover and availability in annual- and perennial-dominated soils in a northern Utah shrub-steppe ecosystem. Biogeochemistry **66**:311-330.

Boyle, S. A., R. R. Yarwood, P. J. Bottomley, and D. D. Myrold. 2008. Bacterial and fungal contributions to soil nitrogen cycling under Douglas fir and red alder at two sites in Oregon. Soil Biology & Biochemistry **40**:443-451.

Brant, J. B., D. D. Myrold, and E. W. Sulzman. 2006. Root controls on soil microbial community structure in forest soils. Oecologia **148**:650-659.

Chen, J., and J. M. Stark. 2000. Plant species effects and carbon and nitrogen cycling in a sagebrush-crested wheatgrass soil. Soil Biology & Biochemistry **32**:47-57.

D'Antonio, C. M., and P. M. Vitousek. 1992. Biological invasions by exotic grasses, the grass/fire cycle, and global change. Annual Review of Ecology and Systematics **23**:63-87.

de Vries, F. T., E. Hoffland, N. van Eekeren, L. Brussaard, and J. Bloem. 2006. Fungal/bacterial ratios in grasslands with contrasting nitrogen management. Soil Biology & Biochemistry **38**:2092-2103.

Evans, R. D., R. Rimer, L. Sperry, and J. Belnap. 2001. Exotic plant invasion alters nitrogen dynamics in an arid grassland. Ecological Applications **11**:1301-1310.

Grayston, S.J., S.Q. Wang, C.D. Campbell, and A.C. Edwards. 1998. Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biology & Biochemistry 30:369-378.

Grayston, S. J., C. D. Campbell, R. D. Bardgett, J. L. Mawdsley, C. D. Clegg, K. Ritz, B. S. Griffiths, J. S. Rodwell, S. J. Edwards, W. J. Davies, D. J. Elston, and P. Millar. 2004. Assessing shifts in microbial community structure across a range of grasslands of differing management intensity using CLPP, PLFA and community DNA techniques. Applied Soil Ecology **25**:63-84.

Högberg, M. N., P. Högberg, and D. D. Myrold. 2007. Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three? Oecologia **150**:590-601.

Hooker, T. D., J. M. Stark, U. Norton, A. J. Leffler, M. Peek, and R. Ryel. 2008. Distribution of ecosystem C and N within contrasting vegetation types in a semiarid rangeland in the Great Basin, USA. Biogeochemistry **90**:291-308.

Huenneke, L., S. P. Hamburg, R. Koide, H. A. Mooney, and P. M. Vitousek. 1990. Effects of soil resources on plant invasion and community structure in Californian serpentine grassland. Ecology **71**:478-491.

James, J. J., K. W. Davies, R. L. Sheley, and Z. T. Ananderud. 2008. Linking nitrogen partitioning and species abundance to invasion resistance in the Great Basin. Oecologia **156**:637-648.

Killham, K. 1987. A new perfusion system for the measurement and characterization of potential rates of soil nitrification. Plant and Soil **97**:267-272.

Kirkham, D. and W. V. Bartholomew. 1954. Equations for following nutrient transformations in soil, utilizing tracer data. Soil Science Society of America Proceedings **18**:33-34.

Klironomos, J. N. 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature **417**:67-70.

Kuske, C.R., L.O. Ticknor, M.E. Miller, J.M. Dunbar, J.A. Davis, S.M. Barns, and J. Belnap. 2002. Comparison of soil bacterial communities in rhizospheres of three plant species and the interspaces in an arid grassland. Applied and Environmental Microbiology 68:1854-1863.

Laughlin, R. J., R. J. Stevens, C. Müller, and C. J. Watson. 2008. Evidence that fungi can oxidize NH_4^+ to NO_3^- in a grassland soil. European Journal of Soil Science **59**:285-291.

Norton, J. B., T. A. Monaco, J. M. Norton, D. A. Johnson, and T. A. Jones. 2004. Soil morphology and organic matter dynamics under cheatgrass and sagebrush-steppe plant communities. Journal of Arid Environments **57**:445-466.

Norton, J. B., T. A. Monaco, and U. Norton. 2007. Mediterranean annual grasses in western North America: kids in a candy store. Plant and Soil **298**:1-5.

Rousk, J., L. A. Demoling, and E. Baath. 2008. Contrasting short-term antibiotic effects on respiration and bacterial growth compromises the validity of the selective respiratory inhibition technique to distinguish fungi and bacteria. Microbial Ecology.

Saetre, P., and J. M. Stark. 2005. Microbial dynamics and carbon and nitrogen cycling following re-wetting of soils beneath two semi-arid plant species. Oecologia **142**:247-260.

Schimel, D. S. 1986. Carbon and nitrogen turnover in adjacent grassland and cropland ecosystems. Biogeochemistry **2**:345-357.

Schimel, J.P., M.K. Firestone, and K.S. Killham. 1984. Identification of heterotrophic nitrification in a Sierran forest soil. Applied and Environmental Microbiology **48**:802-806.

Smart, D. R., J. M. Stark, and V. Diego. 1999. Resource limitations to nitric oxide emissions from a sagebrush-steppe ecosystem. Biogeochemistry **47**:63-86.

Smith, J. L., J. J. Halvorson, and H. Bolton, Jr. 1994. Spatial relationships of soil microbial biomass and C and N mineralization in a semi-arid shrub-steppe ecosystem. Soil Biology & Biochemistry **26**:1151-1159.

Smith, J. L., J. J. Halvorson, and H. J. Bolton. 2002. Soil properties and microbial activity across a 500 m elevation gradient in a semi-arid environment. Soil Biology & Biochemistry **34**:1749-1757.

Stark, J. M., and S. C. Hart. 1996. Diffusion technique for preparing salt solutions, Kjeldahl digests, and persulfate digests for nitrogen-15 analysis. Soil Science Society of America Journal **60**:1846-185

Velvis, H., 1997. Evaluation of the selective respiratory inhibition method for measuring the ratio of fungal:bacterial activity in acid agricultural soils. Biology and Fertility of Soils **25**:354–360.

Table 4.1 Physical, chemical, and biological properties of sagebrush and cheatgrass rhizosphere soils collected from paired native and annual plots at six Snake River Plain study sites.

Site/Plot type	Latitude Longitude	Surface soil texture	Bulk density ($g\ cm^{-2}$)[1]	pH	EC ($\mu S\ cm^{-1}$)	% Soil moisture	Total soil C[1] ($g\ kg^{-1}$ soil)	Total soil N[1] ($g\ kg^{-1}$ soil)	Soil C:N[1]	Fungi:Bacteria PLFA[1]
Lincoln Bench, OR										
Sagebrush	43º53'20"N 117º08'13"W	Silt loam	1.22	6.95	529	17.7	8.5	0.80	10.7	0.50
Cheatgrass	43º53'33"N 117º08'15"W	Silt loam	1.06	7.52	597	15.5	12.4	1.11	11.1	0.53
Two Forks, OR										
Sagebrush	43º48'47"N 117º18'01"W	Loam	1.12	7.81	625	14.5	10.9	1.04	10.5	0.71
Cheatgrass	43º48'32"N 117º18'15"W	Loam	1.16	6.43	804	12.0	7.1	0.70	10.3	0.45
Shell Rock Butte, OR										
Sagebrush	43º45'45"N 117º15'55"W	Silt loam	1.28	7.06	537	16.0	11.0	0.95	11.6	0.63
Cheatgrass	43º45'46"N 117º15'50"W	Silt loam	1.14	7.15	491	15.2	10.1	0.94	10.7	0.93
Kuna Butte, ID										
Sagebrush	43º24'00"N 116º29'00"W	Silt loam	1.37	7.51	583	10.5	12.8	1.09	11.8	0.73
Cheatgrass	43º24'00"N 116º29'00"W	Silt loam	1.26	7.78	764	8.4	9.7	0.92	10.5	0.82
Mayfield, ID										
Sagebrush	43º22'00"N 115º51'00"W	Loam	1.45	7.68	555	14.1	8.9	0.80	11.2	0.89
Cheatgrass	43º22'00"N 115º50'00"W	Loam	1.08	7.42	461	16.6	10.6	0.98	10.8	0.70
Bowns Creek, ID										
Sagebrush	43º20'00"N 115º56'00"W	Silt loam	1.43	7.15	446	18.2	8.8	0.77	11.4	0.62
Cheatgrass	43º20'00"N 115º55'00"W	Silt loam	1.38	7.11	481	14.7	5.7	0.57	9.9	1.17

[1]Analysis performed on soils collected from the same plots in May 2004.

Table 4.2 Soil C and N concentrations and microbial biomass estimates from sagebrush and cheatgrass rhizosphere soils collected at paired native and annual plots at six Snake River Plain study sites. Values are mean (SE), n = 6. Different letters indicate significant differences in soil type ($p \leq 0.05$).

	Sagebrush	Cheatgrass
Total soluble organic C (mg kg^{-1} soil)	42.0 (8.5) a	15.5 (2.3) b
Total soluble N (mg kg^{-1} soil)	8.5 (1.3) a	6.0 (1.9) a
NH_4^+-N (mg kg^{-1} soil)	2.6 (0.4) a	2.7 (0.9) a
NO_3^--N (mg kg^{-1} soil)	4.6 (0.9) a	1.4 (0.2) b
Microbial biomass C (mg kg^{-1} soil)	157.9 (4.3) a	98.5 (9.1) b
Microbial biomass N (mg kg^{-1} soil)	23.6 (2.0) a	13.8 (1.1) b
Microbial biomass C:N	6.9 (0.4) a	7.2 (0.6) a

Table 4.3 Tests for the significance of site, the random effect in the mixed model ANOVA, for gross and net N cycling rates in soils from six sites in the northern Great Basin.

N rate measurement	d.f.	F-value	p-value
Gross NH_4^+ production	5	1.01	0.51
NH_4^+ consumption	5	2.61	0.19
Net mineralization	5	0.08	0.99
Net nitrification	5	0.79	0.60

Figure 4.1 Gross and net N cycling rates from control and cycloheximide-treated sagebrush and cheatgrass rhizosphere soils collected at six Snake River Plain sites. Bars represent mean (SE) rates over the 48 h incubation period; $n = 6$ for sagebrush soils, $n = 5$ for cheatgrass soils. Significant main effects variables (soil type and treatment) and interactions are designated as ***$p < 0.001$, *$p < 0.05$, †$p < 0.10$. Different letters indicate significant differences among soil types within a treatment ($p < 0.05$). **A) Gross NH_4^+ production** ($n = 6$ for sagebrush soils, $n = 5$ for cheatgrass soils). **B) NH_4^+ consumption** ($n = 6$ for sagebrush soils, $n = 5$ for cheatgrass soils). **C) Net N mineralization** ($n = 6$ for 0-24 h control sagebrush soils and all cyloheximide sagebrush soils), $n = 5$ for cheatgrass soils). **D) Net nitrification** ($n = 6$ for 0-24 h control sagebrush soils and all cyloheximide sagebrush soils), $n = 5$ for cheatgrass soils).

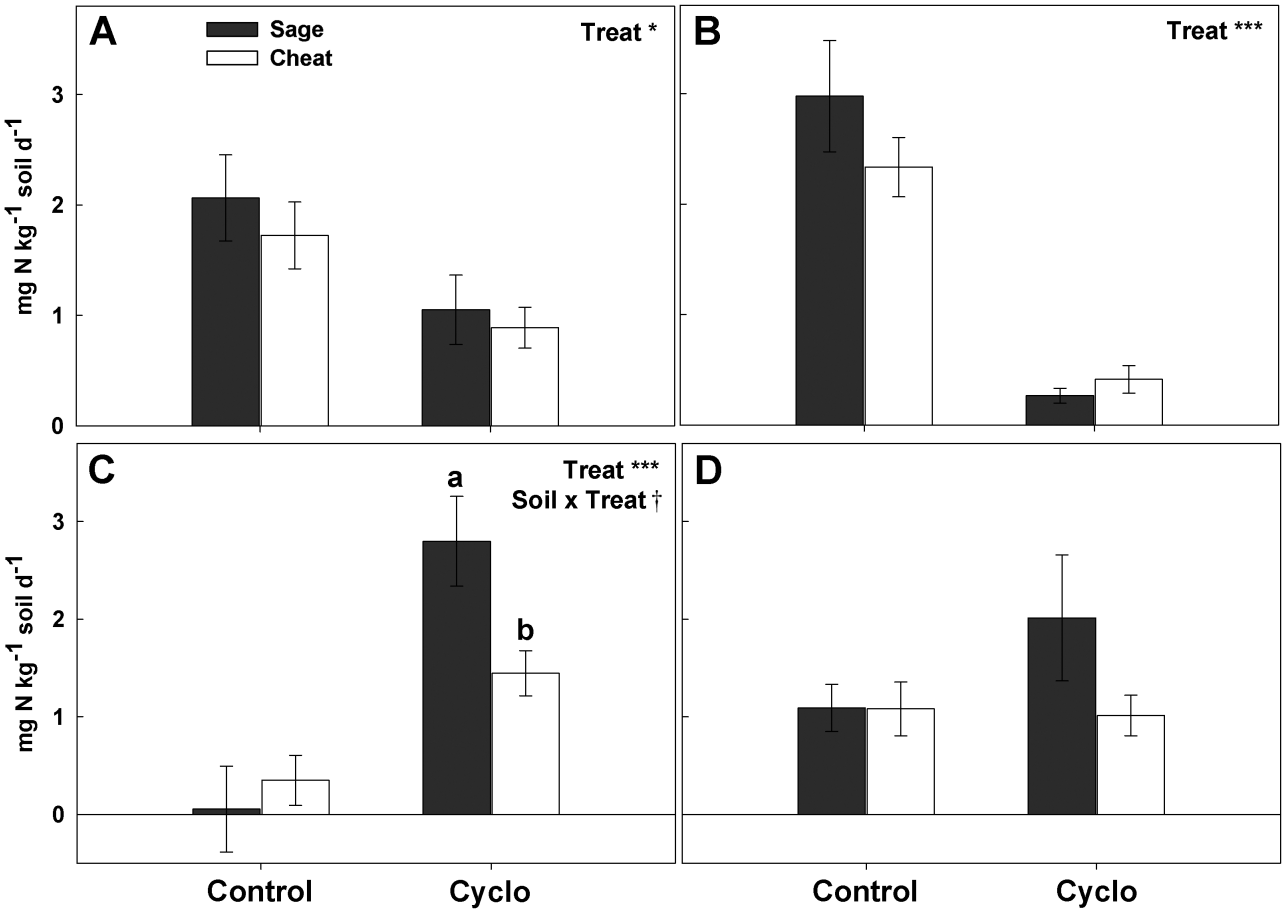

Figure 4.1 Gross and net N cycling rates from control and cycloheximide-treated sagebrush and cheatgrass rhizosphere soils collected at six Snake River Plain sites.

Figure 4.2 Changes in gross and net N cycling rates from control and cycloheximide-treated sagebrush and cheatgrass rhizosphere soils collected at six Snake River Plain sites. Points represent means (SE) of rates over the first ("0-24") and second 24 h ("24-48") of a 48 h incubation. Significant main effects variables and interactions from repeated measures ANOVA are designated as ***$p < 0.001$, *$p < 0.05$, †$p < 0.10$. **A) Gross NH_4^+ production** (n = 6 for 24-48 h sagebrush soils, n = 5 for all cheatgrass and 0-24 h control sagebrush soils). **B) NH_4^+ consumption** (n = 6 for control sagebrush and 24-48 cycloheximide sagebrush soils, n = 5 for all cheatgrass soils, n = 4 for 0-24 h cycloheximide sagebrush soils). **C) Net N mineralization** (n = 6 for 0-24 h control sagebrush and all cycloheximide sagebrush soils, n = 5 for all cheatgrass soils and 24-48 h control sagebrush soils). **D) Net nitrification** (n = 5 for 24-48 h control sagebrush, n = 6 for all other soils).

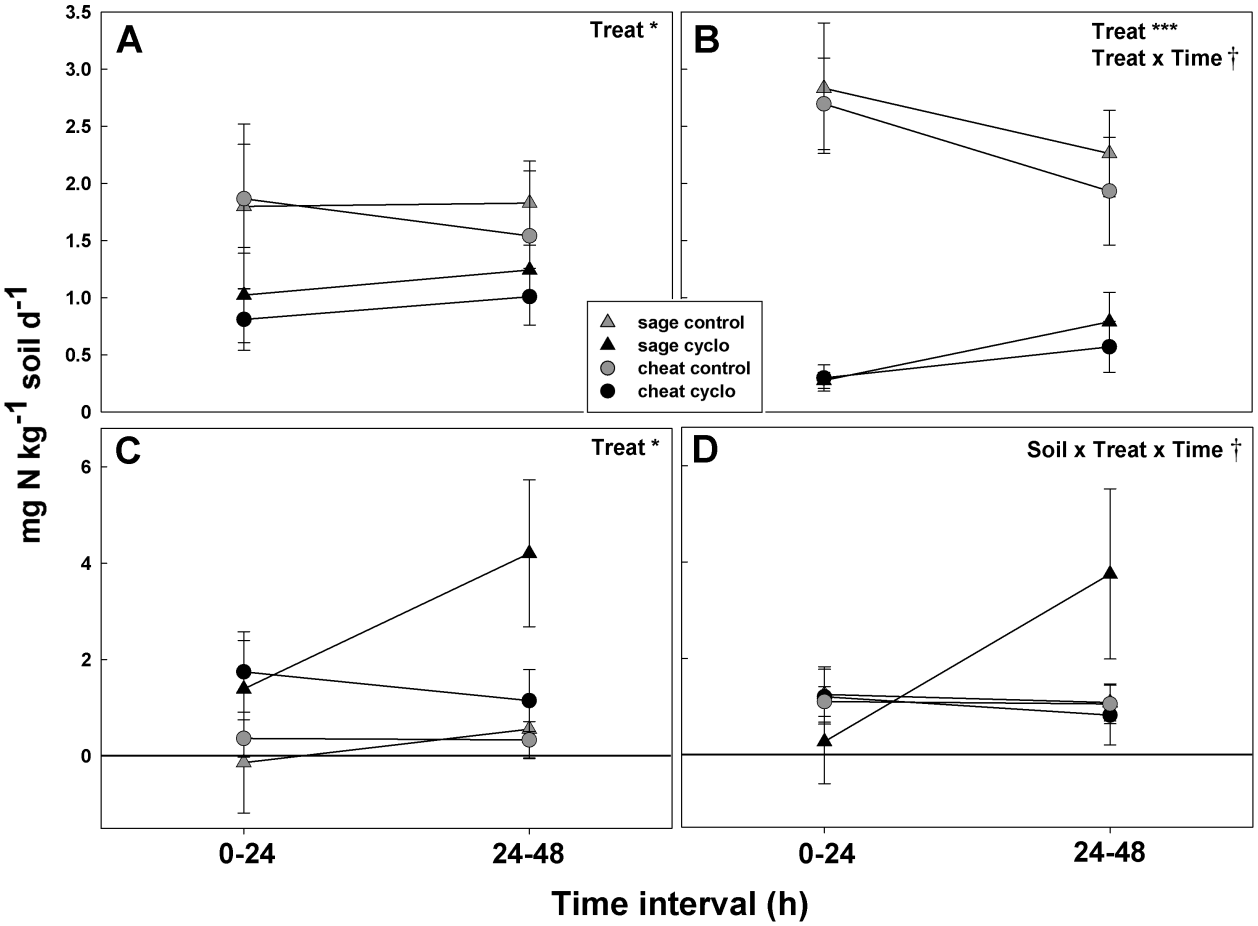

Figure 4.2 Changes in gross and net N cycling rates from control and cycloheximide-treated sagebrush and cheatgrass rhizosphere soils collected at six Snake River Plain sites.

Figure 4.3 Soil microbial biomass (MB) C, N, and C:N estimates from a 48 h isotope dilution experiment using control and cycloheximide-treated sagebrush and cheatgrass soils. Top panel shows MBC, middle panel shows MBN, and bottom panel shows MB C:N. Points represent means (SE); $n = 6$ for all control samples and cycloheximide-treated MBN samples. For cycloheximide-treated MBC and MB C:N, $n = 5$ at 0 h, $n = 4$ for sagebrush at 24 h, $n = 3$ for cheatgrass at 24 h and sagebrush at 48 h, and $n = 2$ for cheatgrass at 48 h. Significant main effects variables and interactions from repeated measures ANOVA are designated as ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, †$p < 0.10$.

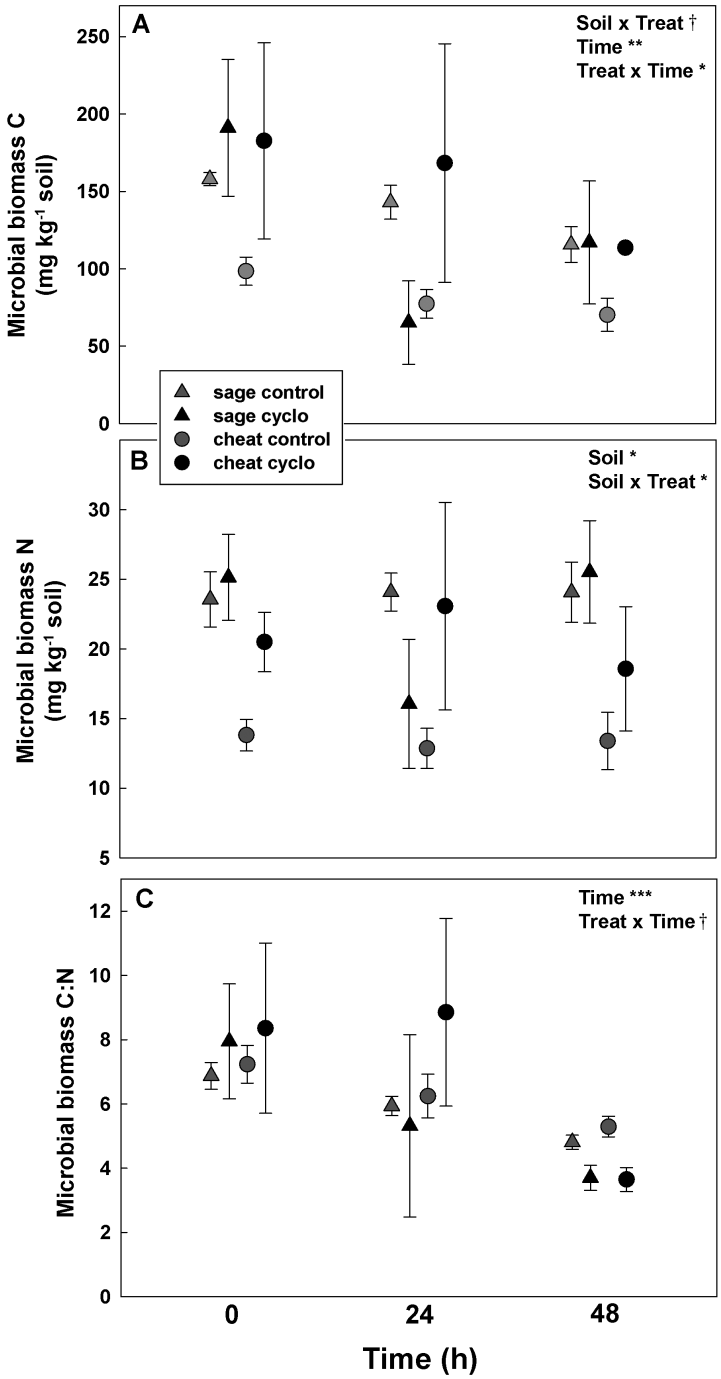

Figure 4.3 Soil microbial biomass (MB) C, N, and C:N estimates from a 48 h isotope dilution experiment using control and cycloheximide-treated sagebrush and cheatgrass soils.

Chapter 5

General Conclusions

Sagebrush steppe ecosystems of the Intermountain West, USA, are being dramatically altered by the invasion of cheatgrass (*Bromus tectorum* L.). The objectives of the studies described here were to: 1) characterize patterns in soil community functional diversity, structure, and composition at intact sagebrush and cheatgrass-invaded areas of the northern Great Basin; 2) assess impacts of restoration and management treatments on soil communities; and 3) determine if changes in soil fungal communities had altered nitrogen (N) cycling in sagebrush and cheatgrass soils.

At cheatgrass-invaded sites across the northern Great Basin, we found that soil communities, as measured by biological soil crust cover, community level physiological profiles (CLPP), phospholipid fatty acids (PLFA), and nematode trophic groups, were similar to one another at sites that shared similar environmental conditions. Restoration treatments (herbicide or sugar application) and seeded plant species had very little effect on community similarity when all sites were analyzed together. Soil communities that were most alike came from sites with similar edaphic factors, specifically high soil pH and percent bare ground. At the individual site level, we found that restoration treatments had varying effects on different components of the soil community. Cyanobacterial soil crust cover decreased at low precipitation sites after plant seeding using a low-impact rangeland drill; carbohydrate utilization and bacterial and fungal biomass increased in plots treated with sugar.

In paired intact and cheatgrass-invaded sagebrush sites in the Snake River Plain, our analyses indicated that vegetation type was most important in structuring microbial community composition and structure, whereas nitrogen (N) availability and site differences played minor roles. Sagebrush, bunchgrass, cheatgrass, and interspace soils differed at varying degrees in their function, structure, and composition as measured by community level physiological profiles (CLPP), phospholipid fatty acid (PLFA) analysis, and terminal restriction fragment length polymorphism (TRFLP) profiles. Sagebrush soil communities had higher bacterial and fungal biomass and higher fungal diversity than soil communities under cheatgrass. Whereas site was the

most important factor structuring soil communities in our first study, it did not explain community variability in this case. In the first study, sites were selected based on common ecological site descriptions, the amount of annual grass cover, and precipitation zones, but soil factors such as parent material and pH were not considered. This led to high degree of variability in soil communities at the site level. In the second study, we were very careful about choosing sites that had common soil textures, horizons, parent material, and depth to restrictive layers in addition to potential plant communities. This allowed us to use site as a random blocking factor in our analyses and discern patterns related to vegetation and nutrient treatments.

In examining N cycling at the same Snake River Plain sites, we found that there were no differences between control sagebrush and cheatgrass soils in any of the measured gross or net N cycling rates. Gross NH_4^+ mineralization and consumption significantly decreased in both soil types in the presence of the fungal protein synthesis inhibitor cycloheximide (CHX). Sagebrush soils treated with CHX exhibited increases in net N mineralization and nitrification relative to cheatgrass soils during the latter half of a 48 h isotope dilution experiment. Our results suggest that N cycling has not been measurably affected by cheatgrass invasion into sagebrush steppe ecosystems of the Snake River Plain. We found that soil fungi play a large role in N cycling in both sagebrush and cheatgrass soils and that bacterial communities in sagebrush soils may depend on fungi to provide them with carbon substrates.

There are several broad conclusions and recommendations we can make from these studies. 1) When choosing cheatgrass-invaded sites to be restored to sagebrush/perennial bunchgrass communities, managers should take into consideration soil physical, chemical, and biological properties in addition to potential plant community information. High productivity sites with high amounts of grass litter cover may not be the best choice for restoration, whereas low productivity sites with relatively high bare ground and arbuscular mycorrhizal fungi may be better suited to re-seeding efforts.

2) Decisions on the types of restoration treatments to be used should also account for soil factors. Sites with low aggregate stability and high cyanobacterial crust cover can be easily disturbed when even "low-impact" tools are used. However, managers must also consider that longer-term benefits of restoring plant communities may outweigh shorter-term losses in soil crust development. Sugar treatments can increase carbon (C)-to-nitrogen (N) ratios (Witwicki et al. submitted) and increase fungal biomass, but we did not determine if fungi are primarily responsible for immobilizing N or what direct effects increased fungal biomass has on cheatgrass or seeded plants. Although we did find that fungi play an important role in N cycling in sagebrush and cheatgrass soils, additional work is needed to understand how sugar treatments and fungi may work together in promoting native plant growth.

3) Cheatgrass-invaded areas of sagebrush systems in the Snake River Plain do not necessarily behave similarly to analogous sites in other areas of the Great Basin or in the Colorado Plateau. For example, results from our isotope dilution experiment showed that production and consumption of NH_4^+ and net N mineralization were not different between sagebrush and cheatgrass rhizospheres, and this contrasts with findings from other sites in the Intermountain West. Whenever possible, management and restoration decisions should be based on information from areas that have plant, soil, and environmental factors in common with the site to be restored.

Bibliography

Allcock, K., R. Nowak, B. Blank, T. Jones, T. Monaco, J. Chambers, R. Tausch, P. Doescher, V. Satyal, T. J., D. Ogle, L. St. John, M. Pellant, D. Pyke, E. Schupp, and C. Call. 2006. Integrating weed management and restoration on western rangelands. Ecological Restoration **24**:199-300.

Anderson, D. C., K. T. Harper, and S. R. Rushforth. 1982. Recovery of cryptogamic soil crusts from grazing in Utah winter ranges. Journal of Range Management **35**:355-359.

Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology **26**:32-46.

Bailey, V. L., A. D. Peacock, J. L. Smith, and H. Bolton, Jr. 2002a. Relationships between soil microbial biomass determined by chloroform fumigation-extraction, substrate-induced respiration, and phospholipid fatty acid analysis. Soil Biology & Biochemistry **34**:1385-1389.

Bailey, V. L., J. L. Smith, and H. Bolton. 2002b. Fungal-to-bacterial ratios in soils investigated for enhanced C sequestration. Soil Biology & Biochemistry **34**:997-1007.

Balser, T. C., K. K. Treseder, and M. Ekenler. 2004. Using lipid analysis and hyphal length to quantify AM and saprotrophic fungal abundance along a soil chronosequence. Soil Biology & Biochemistry **37**:601-604.

Bardgett, R. D., J. L. Mawdsley, S. Edwards, P. J. Hobbs, J. S. Rodwell, and W. J. Davies. 1999. Plant species and nitrogen effects on soil biological properties of temperate upland grasslands. Functional Ecology **13**:650-660.

Bardgett, R.D., and McAllister, E., 1999. The measurement of soil fungal:bacterial biomass ratios as an indicator of ecosystem self-regulation in temperate meadow grasslands. Biology and Fertility of Soils **29**:282–290.

Bastias, B. A., I. C. Anderson, Z. Xu, and J. W. G. Cairney. 2007. RNA- and DNA-based profiling of soil fungal communities in a native Australian eucalypt forest and adjacent *Pinus elliotti* plantation. Soil Biology & Biochemistry **39**:3108-3114.

Bates, J. D., T. Svejcar, R. F. Miller, and R. A. Angell. 2006. The effects of precipitation timing on sagebrush steppe vegetation. Journal of Arid Environments **64**:670-697.

Batten, K. M., K. M. Scow, K. F. Davies, and S. P. Harrison. 2006. Two invasive plants alter soil microbial community composition in serpentine grasslands. Biological Invasions **8**:217-230.

Belnap, J. 2002. Nitrogen fixation in biological soil crusts from southeast Utah, USA. Biology and Fertility of Soils **35**:128-135.

Belnap, J. and D. A. Gillette. 1998. Vulnerability of desert soil surfaces to wind erosion: impacts of soil texture and distrubance. Journal of Arid Environments **39**:133-142.

Belnap, J. and S. L. Phillips. 2001. Soil biota in an ungrazed grassland: response to annual grass (*Bromus tectorum*) invasion. Ecological Applications **11**:1261-1275.

Belnap, J., S. L. Phillips, S. K. Sherrod, and A. Moldenke. 2005. Soil biota can change after exotic plant invasion: does this affect ecosystem processes? Ecology **86**:3007-3017.

Belnap, J., R. Rosentreter, S. Leonard, J. H. Kaltenecker, J. Williams, and D. Eldridge. 2001. Biological soil crusts: ecology and management. United States Department of the Interior, Bureau of Land Management, National Science and Technology Center Information and Communications Group, Denver, CO.

Bennett, L. T., S. Kasel, and J. Tibbits. 2009. Woodland trees modulate soil resources and conserve fungal diversity in fragmented landscapes. Soil Biology & Biochemistry **41**:2162-2169.

Bever, J. D., K. M. Westover, and J. Antonovics. 1997. Incorporating the soil community into plant population dynamics: the utility of the feedback approach. Journal of Ecology **85**:561-573.

Blecker, S. W., L. L. Stillings, M. C. Amacher, J. A. Ippolito, and N. M. DeCrappeo. 2010. Ecosystem health in mineralized terrane; data from podiform chromite (Chinese Camp mining district, California), quartz alunite (Castle Peak and Masonic mining districts, Nevada/California), and Mo/Cu porphyry (Battle Mountain mining district, Nevada) deposits. U.S. Geological Survey Open-File Report 2010-1040.

Bligh, E. G. and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemical Physiology **37**:911-917.

Blumenthal, D. M., N. R. Jordan, and M. P. Russelle. 2003. Soil carbon addition controls weeds and facilitates prairie restoration. Ecological Applications **13**:605-615.

Bolton, H., Jr., J. L. Smith, and S. O. Link. 1993. Soil microbial biomass and activity of a disturbed and undisturbed shrub-steppe ecosystem. Soil Biology & Biochemistry **25**:545-552.

Bolton, H., Jr., J. L. Smith, and R. E. Wildung. 1990. Nitrogen mineralization potentials of shrub-steppe soils with different disturbance histories. Soil Science Society of America Journal **54**:887-891.

Bongers, T. 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia **83**:14-19.

Bongers, T. and H. Ferris. 1999. Nematode community structure as a bioindicator in environmental monitoring. Trends in Ecology and Evolution **14**:224-228.

Booth, M. S., J. M. Stark, and M. M. Caldwell. 2003. Inorganic N turnover and availability in annual- and perennial-dominated soils in a northern Utah shrub-steppe ecosystem. Biogeochemistry **66**:311-330.

Bowker, M. A., M. E. Miller, J. Belnap, T. D. Sisk, and N. C. Johnson. 2008. Prioritizing conservation effort through the use of biological soil crusts as ecosystem function indicators in an arid region. Conservation Biology **22**:1533-1543.

Boyle, S. A., R. R. Yarwood, P. J. Bottomley, and D. D. Myrold. 2008. Bacterial and fungal contributions to soil nitrogen cycling under Douglas fir and red alder at two sites in Oregon. Soil Biology & Biochemistry **40**:443-451.

Bradley, B. A., R. A. Houghton, J. F. Mustard, and S. P. Hamburg. 2006. Invasive grass reduces aboveground carbon stocks in shrublands of the Western US. Global Change Biology **12**:1815-1822.

Bradley, B. A. and J. F. Mustard. 2005. Identifying land cover variability distinct from land cover change: cheatgrass in the Great Basin. Remote Sensing of Environment **94**:204-213.

Brady, N. C. and R. R. Weil. 2002. The nature and property of soils. Prentice Hall, Upper Saddle River, NJ, USA.

Brant, J. B., D. D. Myrold, and E. W. Sulzman. 2006. Root controls on soil microbial community structure in forest soils. Oecologia **148**:650-659.

Briske, D. D., B. T. Bestelmeyer, T. K. Stringham, and P. L. Shaver. 2008. Recommendations for development of resilience-based state-and-transition models. Rangeland Ecology & Management **61**:359-367.

Briske, D. D., S. D. Fuhlendorf, and F. E. Smeins. 2005. State-and-transition models, thresholds, and rangeland health: a synthesis of ecological concepts and perspectives. Rangeland Ecology and Management **58**:1-10.

Brunson, J. L., D. A. Pyke, and S. S. Perakis. 2010. Yield responses of ruderal plants to sucrose in invasive-dominated sagebrush steppe of the northern Great Basin. Restoration Ecology:doi: 10.1111/j.1526-1100X.2009.00644.x.

Callaway, R. M., D. Cipollini, K. Barto, G. C. Thelen, S. G. Hallett, D. Prati, K. Stinson, and J. Klironomos. 2008. Novel weapons: invasive plant suppresses fungal mutualists in America but not in its native Europe. Ecology **89**:1043-1055.

Chen, J. and J. M. Stark. 2000. Plant species effects and carbon and nitrogen cycling in a sagebrush-crested wheatgrass soil. Soil Biology & Biochemistry **32**:47-57.

Crooks, J. A. 2002. Characterizing ecosystem-level consequences of biological invasions: the role of ecosystem engineers. Oikos **97**:153-166.

D'Antonio, C. M. and P. M. Vitousek. 1992. Biological invasions by exotic grasses, the grass/fire cycle, and global change. Annual Review of Ecology and Systematics **23**:63-87.

Davies, K. W., J. D. Bates, and R. F. Miller. 2007. The influence of *Artemisia tridentata* spp. *wyomingensis* on microsite and herbaceous vegetation heterogeneity. Journal of Arid Environments **69**:441-457.

de Vries, F. T., E. Hoffland, N. van Eekeren, L. Brussaard, and J. Bloem. 2006. Fungal/bacterial ratios in grasslands with contrasting nitrogen management. Soil Biology & Biochemistry **38**:2092-2103.

Edwards, U., T. Rogall, H. Blocker, M. Emde, and E. C. Bottger. 1989. Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. Nucleic Acids Research **17**:7843-7853.

Evans, R. D., R. Rimer, L. Sperry, and J. Belnap. 2001. Exotic plant invasion alters nitrogen dynamics in an arid grassland. Ecological Applications **11**:1301-1310.

Fierer, N. and R. B. Jackson. 2006. The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Sciences of the United States of America **103**:626-631.

Fierer, N., M. S. Strickland, D. Liptzin, M. A. Bradford, and C. C. Cleveland. 2009. Global patterns in belowground communities. Ecology Letters **12**:1238-1249.

Frey, S. D., E. T. Elliott, and K. Paustian. 1999. Bacterial and fungal abundance and biomass in conventional and no-tillage agroecosystems along two climatic gradients. Soil Biology & Biochemistry **31**:573-585.

Frost, S. M., P. D. Stahl, and S. E. Williams. 2001. Long-term reestablishment of arbuscular mycorrhizal fungi in a drastically disturbed semiarid surface mine soil. Arid Land Research and Management **15**:3-12.

Gardes, M. and T. D. Bruns. 1993. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. Molecular Ecology **2**:113-118.

Garland, J. L. 1996. Analytical approaches to the characterization of samples of microbial communities using patterns of potential C source utilization. Soil Biology & Biochemistry **28**:213-221.

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.

Grayston, S. J., C. D. Campbell, R. D. Bardgett, J. L. Mawdsley, C. D. Clegg, K. Ritz, B. S. Griffiths, J. S. Rodwell, S. J. Edwards, W. J. Davies, D. J. Elston, and P. Millar. 2004. Assessing shifts in microbial community structure across a range of grasslands of differing management intensity using CLPP, PLFA and community DNA techniques. Applied Soil Ecology **25**:63-84.

Grayston, S. J., S. Q. Wang, C. D. Campbell, and A. C. Edwards. 1998. Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biology & Biochemistry **30**:369-378.

Green, J. and B. J. M. Bohannan. 2006. Spatial scaling of microbial biodiversity. Trends in Ecology & Evolution **21**:501-507.

Halvorson, J. J., H. Bolton, and J. L. Smith. 1997. The pattern of soil variables related to *Artemisia tridentata* in a burned shrub-steppe site. Soil Science Society of America Journal **61**:287-294.

Halvorson, J. J., H. Bolton, J. L. Smith, and R. E. Rossi. 1994. Geostatistical analysis of resource islands under *Artemisia tridentata* in the shrub-steppe. Great Basin Naturalist **54**:313-328.

Hawkes, C. V., J. Belnap, C. D'Antonio, and M. K. Firestone. 2006. Arbuscular mycorrhizal assemblages in native plant roots change in the presence of invasive exotic grasses. Plant and Soil **281**:369-380.

Hempy-Mayer, K. and D. A. Pyke. 2008. Defoliation effects on *Bromus tectorum* seed production: implications for grazing. Rangeland Ecology & Management **61**:116-123.

Herrick, J. E., W. G. Whitford, A. G. de Soyza, J. W. Van Zee, K. M. Havstad, C. A. Seybold, and M. Walton. 2001. Field soil aggregate stability kit for soil quality and rangeland health evaluations. Catena **44**:27-35.

Hilty, J., L. Bond, M. Wicklow-Howard, and R. Rychert. 2001. Functional diversity of soil microbial communities: effects of exotic plant invasion and implications for restoration of semi-arid rangelands. Submitted to Bureau of Land Management, Idaho State Office and Lower Snake River District, Biology Department, Boise State University.

Hilty, J., D. J. Eldridge, R. Rosentreter, M. Wicklow-Howard, and M. Pellant. 2004. Recovery of biological soil crusts following wildfire in Idaho. Journal of Range Management **57**:89-96.

Högberg, M. N., P. Högberg, and D. D. Myrold. 2007. Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three? Oecologia **150**:590-601.

Hooker, T. D., J. M. Stark, U. Norton, A. J. Leffler, M. Peek, and R. Ryel. 2008. Distribution of ecosystem C and N within contrasting vegetation types in a semiarid rangeland in the Great Basin, USA. Biogeochemistry **90**:291-308.

Huenneke, L. F., S. P. Hamburg, R. Koide, H. A. Mooney, and P. M. Vitousek. 1990. Effects of soil resources on plant invasion and community structure in Californian serpentine grassland. Ecology **71**:478-491.

Hunt, H. W., D. C. Coleman, E. R. Ingham, R. E. Ingham, E. T. Elliott, J. C. Moore, S. L. Rose, C. P. P. Reid, and C. R. Morley. 1987. The detrital food web in a shortgrass prairie. Biology and Fertility of Soils **3**:57-68.

James, J. J. 2008. Effect of soil nitrogen stress on the relative growth rate of annual and perennial grasses in the Intermountain West. Plant and Soil **310**:201-210.

Johansen, J. R. 2001. Impacts of fire on biological soil crusts. Pages 385-397 *in* J. Belnap and O. Lange, editors. Biological soil crusts: structure, function, and management. Springer-Verlag, Berlin.

Jonasson, S., A. Michelsen, I. K. Schmidt, E. V. Nielsen, and T. V. Callaghan. 1996. Microbial biomass C, N and P in two arctic soils and responses to addition of NPK fertilizer and sugar: implications for plant nutrient uptake. Oecologia **106**:507-515.

Jones, D. L. and D. V. Murphy. 2007. Microbial response time to sugar and amino acid additions to soil. Soil Biology & Biochemistry **39**:2178-2182.

Jordan, N. R., D. L. Larson, and S. C. Huerd. 2008. Soil modification by invasive plants: effects on native and invasive species of mixed-grass prairies. Biological Invasions **10**:177-190.

Kaya, H. K. and S. P. Stock. 1997. Techniques in insect nematology. Pages 281-324 *in* L. Lacey, editor. Manual of techniques in insect pathology. Academic Press, San Diego, CA.

Kemmitt, S. J., D. Wright, K. W. T. Goulding, and D. L. Jones. 2006. pH regulation of carbon and nitrogen dynamics in two agricultural soils. Soil Biology & Biochemistry **38**:898-911.

Killham, K. 1987. A new perfusion system for the measurement and characterization of potential rates of soil nitrification. Plant and Soil **97**:267-272.

Kirkham, D. and W. V. Bartholomew. 1954. Equations for following nutrient transformations in soil, utilizing tracer data. Soil Science Society of America Proceedings **18**:33-34.

Klironomos, J. N. 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature **417**:67-70.

Knapp, P. A. 1996. Cheatgrass (*Bromus tectorum L*) dominance in the Great Basin desert. Global Environmental Change **6**:37-52.

Kohler, F., J. Hamelin, F. Gillet, J. Gobat, -M., and A. Buttler. 2005. Soil microbial community changes in wooded mountain pastures due to simulated effects of cattle grazing. Plant and Soil **278**:327-340.

Kourtev, P. S., J. G. Ehrenfeld, and M. Haggblom. 2002. Exotic plant species alter the microbial community structure and function in the soil. Ecology **83**:3152-3166.

Kuske, C. R., L. O. Ticknor, M. E. Miller, J. M. Dunbar, J. A. Davis, S. M. Barns, and J. Belnap. 2002. Comparison of soil bacterial communities in rhizospheres of three plant species and the interspaces in an arid grassland. Applied and Environmental Microbiology **68**:1854-1863.

Ladygina, N. and K. Hedlund. 2010. Plant species influence microbial diversity and carbon allocation in the rhizosphere. Soil Biology & Biochemistry **42**:162-168.

Laughlin, R. J., R. J. Stevens, C. Müller, and C. J. Watson. 2008. Evidence that fungi can oxidize NH_4^+ to NO_3^- in a grassland soil. European Journal of Soil Science **59**:285-291.

Laycock, W. A. 1991. Stable states and thresholds of range condition on North American rangelands: a viewpoint. Journal of Range Management **44**:427-433.

Mack, R. N. 1981. Invasion of *Bromus tectorum* L. into Western North America: an ecological chronicle. Agro-Ecosystems **7**:145-165.

Mack, R. N. and D. A. Pyke. 1984. The demography of *Bromus tectorum*: the role of microclimate, grazing and disease. Journal of Ecology **72**:731-748.

Martiny, J. B. H., B. J. M. Bohannan, J. H. Brown, R. K. Colwell, J. A. Fuhrman, J. L. Green, M. C. Horner-Devine, M. Kane, J. A. Krumins, C. R. Kuske, P. J. Morin, S. Naeem, L. Ovreas, A.-L. Reysenbach, V. H. Smith, and J. T. Staley. 2006. Microbial biogeography: putting microorganisms on the map. Nature Reviews Microbiology **4**:102-112.

McArthur, E. D. and S. K. Goodrich. 2004. The Intermountain setting. Pages 7-14 *in* S. B. Monsen, R. Stevens, and N. L. Shaw, editors. Restoring western ranges and wildlands, vol. 1. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fort Collins, CO.

McCune, B. and J. B. Grace. 2002. Analysis of ecological communities. MjM Software Design, Gleneden Beach, OR, USA.

McCune, B. and M. J. Mefford. 1999. PC-ORD. Multivariate analysis of ecological data. MjM Software, Gleneden Beach, OR, USA.

McLendon, T. and E. F. Redente. 1991. Nitrogen and phosphorus effects on secondary succession dynamics on a semi-arid sagebrush site. Ecology **72**:2016-2024.

McLendon, T. and E. F. Redente. 1992. Effects of nitrogen limitation on species replacement dynamics during early secondary succession on a semiarid sagebrush site. Oecologia **91**:312-317.

Melgoza, G., R. S. Nowak, and R. J. Tausch. 1990. Soil water exploitation after fire: competition between *Bromus tectorum* (cheatgrass) and two native species. Oecologia **83**:7-13.

Miller, M., J. Belnap, S. Beatty, and B. Webb. 2001. Components of spatial and temporal soil variation at Canyonlands National Park: implications for P dynamics and cheatgrass (*Bromus tectorum)* performance. Pages 154-162 *in* Shrubland ecosystem genetics and biodiversity. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Provo, UT.

Monaco, T. A., D. A. Johnson, J. M. Norton, T. A. Jones, K. J. Connors, J. B. Norton, and M. B. Redinbaugh. 2003. Contrasting responses of Intermountain West grasses to soil nitrogen. Journal of Range Management **56**:282-290.

Monsen, S. B., R. Stevens, and N. L. Shaw. 2004. Restoring western ranges and wildlands, vol. 1. Gen. Tech. Rep. RMRS-GTR-136-vol-1, U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fort Collins, CO.

Morton, J. B. 1998. Fungi. Pages 72-93 *in* D. M. Sylvia, J. J. Fuhrmann, P. G. Hartel, and D. A. Zuberer, editors. Principals and applications of soil microbiology. Prentice Hall, Upper Saddle River, NJ, USA.

Muyzer, G., E. C. d. Waal, and A. G. Uitierlinden. 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Applied and Environmental Microbiology **59**:695-700.

Norton, J. B., T. A. Monaco, J. M. Norton, D. A. Johnson, and T. A. Jones. 2004. Soil morphology and organic matter dynamics under cheatgrass and sagebrush-steppe plant communities. Journal of Arid Environments **57**:445-466.

Norton, J. B., T. A. Monaco, and U. Norton. 2007. Mediterranean annual grasses in western North America: kids in a candy store. Plant and Soil **298**:1-5.

Novak, S. J. and R. N. Mack. 2001. Tracing plant introduction and spread: genetic evidence from *Bromus tectorum* (cheatgrass). BioScience **51**:114-122.

Pimentel, D., R. Zuniga, and D. Morrison. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecological Economics **52**:273-288.

Porazinska, D. L., R. D. Bardgett, M. B. Blaauw, H. W. Hunt, A. N. Parsons, T. R. Seastedt, and D. H. Wall. 2003. Relationships at the aboveground-belowground interface: plants, soil biota, and soil processes. Ecological Monographs **73**:377-395.

Porazinska, D. L., L. W. Duncan, R. McSorley, and J. H. Graham. 1999. Nematode communities as indicators of status and processes of a soil ecosystem influenced by agricultural management practices. Applied Soil Ecology **13**:69-86.

Rogers, R. W. and R. T. Lange. 1971. Lichen populations on arid soil crusts around sheep watering places in South Australia. Oikos **22**:93-100.

Rosentreter, R. and D. J. Eldridge. 2003. Monitoring rangeland health: using a biological soil crust stability index. Pages 74-76 *in* Seed and soil dynamics in shrubland ecosystems. U.S. Dept. of Agriculture, Forest Service, Rocky Mountain Research Station, Laramie, WY.

Rousk, J., L. A. Demoling, and E. Baath. 2008. Contrasting short-term antibiotic effects on respiration and bacterial growth compromises the validity of the selective respiratory inhibition technique to distinguish fungi and bacteria. Microbial Ecology.

Rowe, H. I. and C. S. Brown. 2008. Native plant growth and seedling establishment in soils influenced by *Bromus tectorum*. Rangeland Ecology and Management **61**:630-639.

Rowe, H. I., C. S. Brown, and V. P. Claassen. 2007. Comparisons of mycorrhizal responsiveness with field soil and commercial inoculum for six native montane species and *Bromus tectorum*. Restoration Ecology **15**:44-52.

Saetre, P. and J. M. Stark. 2005. Microbial dynamics and carbon and nitrogen cycling following re-wetting of soils beneath two semi-arid plant species. Oecologia **142**:247-260.

Schimel, D. S. 1986. Carbon and nitrogen turnover in adjacent grassland and cropland ecosystems. Biogeochemistry **2**:345-357.

Schimel, J. P. and J. Bennett. 2004. Nitrogen mineralization: challenges of a changing paradigm. Ecology **85**:591-602.

Schimel, J.P., M.K. Firestone, and K.S. Killham. 1984. Identification of heterotrophic nitrification in a Sierran forest soil. Applied and Environmental Microbiology **48**:802-806.Sinsabaugh, R. L., M. J. Klug, H. P. Collins, P. E. Yeager, and S. O. Petersen. 1999. Characterizing soil microbial communities. Pages 318-348 *in* G. P. Robertson, D. C. Coleman, C. S. Bledsoe, and P. Sollins, editors. Standard soil methods for long-term ecological research. Oxford University Press, New York, NY.

Smart, D. R., J. M. Stark, and V. Diego. 1999. Resource limitations to nitric oxide emissions from a sagebrush-steppe ecosystem. Biogeochemistry **47**:63-86.

Smith, J. L., J. J. Halvorson, and H. Bolton, Jr. 1994. Spatial relationships of soil microbial biomass and C and N mineralization in a semi-arid shrub-steppe ecosystem. Soil Biology & Biochemistry **26**:1151-1159.

Smithwick, E. A. H., M. G. Turner, K. L. Metzger, and T. C. Balser. 2005. Variation in NH_4^+ mineralization and microbial communities with stand age in lodgepole pine (*Pinus contorta*) forests, Yellowstone National Park (USA). Soil Biology & Biochemistry **37**:1546-1559.

Sperry, L. J., J. Belnap, and R. D. Evans. 2006. *Bromus tectorum* invasion alters nitrogen dynamics in an undisturbed arid grassland ecosystem. Ecology **87**:603-615.

Staddon, W. J., L. C. Duchesne, and J. T. Trevors. 1997. Microbial diversity and community structure of postdisturbance forest soils as determined by sole-carbon-source utilization patterns. Microbial Ecology **34**:125-130.

Stark, J. M. and S. C. Hart. 1996. Diffusion technique for preparing salt solutions, Kjeldahl digests, and persulfate digests for nitrogen-15 analysis. Soil Science Society of America Journal **60**:1846-1855.

Stewart, G. and A. C. Hull. 1949. Cheatgrass (*Bromus tectorum* L.)--an ecologic intruder in southern Idaho. Ecology **30**:58-74.

Stringham, T. K., W. C. Krueger, and P. L. Shaver. 2003. State and transition modeling: an ecological process approach. Journal of Range Management **56**:106-113.

Vasquez, E., R. Sheley, and T. Svejcar. 2008. Nitrogen enhances the competitive ability of cheatgrass (*Bromus tectorum*) relative to native grasses. Invasive Plant Science and Management **1**:287-295.

Velvis, H. 1997. Evaluation of the selective respiratory inhibition method for measuring the ratio of fungal:bacterial activity in acid agricultural soils. Biology and Fertility of Soils **25**:354-360.

Wall, D. H. 1999. Biodiversity and ecosystem functioning. BioScience **49**:107-108.

Wall, D. H. and J. C. Moore. 1999. Interactions underground: soil biodiversity, mutualism, and ecosystem processes. BioScience **49**:109-117.

Wall, D. H. and R. A. Virginia. 1999. Controls on soil biodiversity: insights from extreme environments. Applied Soil Ecology **13**:137-150.

Wardle, D. A. 2002. Communities and ecosystems: linking the aboveground and belowground components. Princeton University Press, Princeton, NJ, USA.

Wardle, D. A., R. D. Bardgett, J. N. Klironomos, H. Setälä, W. H. van der Putten, and D. H. Wall. 2004. Ecological linkages between aboveground and belowground biota. Science **304**:1629-1633.

Westoby, M., B. Walker, and I. Noy-Meir. 1989. Opportunistic management for rangelands not at equilibrium. Journal of Range Management **42**:266-274.

White, T. J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315-322 *in* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, editors. PCR protocols: a guide to methods and applications. Academic Press, San Diego, CA, USA.

Witwicki, D. 2005. Sugar application and nitrogen pools in Wyoming big sagebrush communities and exotic annual grasslands. Master of Science thesis. Oregon State University, Corvallis.

Witwicki, D. L., P. S. Doescher, D. A. Pyke, S. S. Perakis, and N. M. DeCrappeo. submitted. Growth and 15N retention of Wyoming big sagebrush communities and exotic annual grasslands following nitrogen and sugar additions. Ecological Applications.

Yeates, G. W. 1979. Soil nematodes in terrestrial ecosystems. Journal of Nematology **11**:213-229.

Yeates, G. W., T. Bongers, R. G. M. De Goede, D. W. Freckman, and S. S. Georgieva. 1993. Feeding habits in soil nematode families and genera--an outline for soil ecologists. Journal of Nematology **25**:315-331.

Appendix

Appendix A. Cover type, treatment, and seasonal differences in CLPP substrate use, PLFA microbial biomass and biomarkers, TRFLP species richness, and nematode generic richness from three field sites each in eastern Oregon and southwestern Idaho.

	CLPP[2]					PLFA[3]			
Cover type-Treatment[1]	Polymers	Carbohydrates	Carboxylic acids	Amino Acids	Amines	Microbial biomass (nmol PLFA g^{-1} soil)	Gram+ bacteria (mol %)	Gram- bacteria (mol %)	Actinobacteria (mol %)
FALL									
Sage-N	1.24 (0.09)	0.49 (0.01)	1.29 (0.04)	1.28 (0.06)	0.54 (0.05)	34.24 (5.94)	11.19 (0.36)	6.91 (0.71)	4.81 (0.50)
Sage-C	1.28 (0.06)	0.57 (0.03)	1.17 (0.05)	1.32 (0.07)	0.55 (0.10)	50.82 (9.19)	11.95 (0.62)	4.88 (0.31)	3.54 (0.17)
Sage-X	1.15 (0.07)	0.52 (0.02)	1.33 (0.04)	1.22 (0.09)	0.53 (0.11)	62.32 (14.23)	10.23 (0.74)	7.09 (0.80)	5.17 (0.57)
Bunch-N	1.24 (0.05)	0.72 (0.12)	1.21 (0.11)	1.11 (0.07)	0.44 (0.15)	43.01 (12.77)	9.54 (0.67)	7.48 (0.44)	5.87 (0.46)
Bunch-C	1.27 (0.08)	0.75 (0.13)	1.15 (0.07)	1.09 (0.12)	0.57 (0.18)	50.57 (8.94)[4]	13.01 (0.33)[4]	4.91 (0.12)[4]	3.77 (0.07)[4]
Bunch-X	1.07 (0.14)	0.54 (0.03)	1.36 (0.07)	1.22 (0.08)	0.48 (0.17)	58.63 (13.61)	9.90 (0.75)	7.09 (0.42)	5.73 (0.53)
Inter-N	1.19 (0.30)	0.79 (0.08)	1.09 (0.09)	1.23 (0.20)	0.42 (0.09)	45.37 (8.88)	9.92 (0.74)	7.87 (0.53)	5.29 (0.16)
Inter-C	1.22 (0.24)	1.05 (0.06)	0.98 (0.11)	1.02 (0.16)	0.34 (0.09)	79.08 (15.28)[4]	11.71 (0.81)[4]	6.46 (0.67)[4]	5.10 (0.61)[4]
Inter-X	1.66 (0.31)	0.94 (0.09)	0.78 (0.10)	1.26 (0.20)	0.24 (0.09)	37.64 (8.87)	9.54 (0.81)	7.43 (0.38)	5.86 (0.25)
Cheat-N	1.01 (0.13)	0.65 (0.08)	1.24 (0.07)	1.29 (0.16)	0.51 (0.18)	29.10 (5.79)	11.15 (0.76)	5.18 (0.50)	3.59 (0.23)
Cheat-C	1.33 (0.18)	0.84 (0.17)	1.09 (0.09)	1.02 (0.13)	0.54 (0.24)	68.71 (12.66)	12.16 (0.61)	5.81 (0.61)	4.24 (0.42)
Cheat-X	1.19 (0.13)	0.72 (0.12)	1.25 (0.13)	1.06 (0.14)	0.46 (0.19)	77.67 (13.63)[4]	9.44 (0.42)[4]	8.56 (0.33)[4]	6.12 (0.37)[4]
SPRING									
Sage-N	0.98 (0.17)	0.54 (0.04)	1.20 (0.09)	1.55 (0.13)	0.47 (0.15)	48.07 (7.00)	10.97 (0.32)	5.26 (0.21)	3.79 (0.11)
Sage-C	1.20 (0.16)	0.48 (0.01)	1.21 (0.05)	1.48 (0.08)	0.47 (0.08)	39.45 (8.70)	11.23 (0.38)	5.18 (0.22)	3.59 (0.07)
Sage-X	1.31 (0.16)	0.54 (0.08)	1.12 (0.04)	1.46 (0.06)	0.48 (0.10)	48.32 (7.66)	11.08 (0.18)	5.09 (0.20)	3.47 (0.17)
Bunch-N	1.33 (0.08)	0.52 (0.04)	1.14 (0.08)	1.48 (0.13)	0.36 (0.26)	19.33 (3.37)	11.62 (0.39)	5.43 (0.39)	4.10 (0.17)
Bunch-C	1.05 (0.08)	0.54 (0.03)	1.37 (0.06)	1.20 (0.09)	0.48 (0.16)	37.78 (8.29)	11.08 (0.36)	4.93 (0.28)	3.71 (0.18)
Bunch-X	1.06 (0.12)	0.51 (0.05)	1.30 (0.07)	1.32 (0.08)	0.63 (0.20)	34.19 (2.55)	11.47 (0.31)	5.97 (0.31)	4.02 (0.12)
Inter-N	1.30 (0.19)	0.71 (0.11)	1.13 (0.15)	1.30 (0.08)	0.11 (0.03)	20.79 (3.37)	11.68 (0.48)	5.25 (0.25)	4.52 (0.28)
Inter-C	1.17 (0.25)	0.60 (0.04)	1.07 (0.06)	1.50 (0.14)	0.59 (0.12)	26.37 (5.51)	12.49 (0.46)	5.05 (0.26)	4.33 (0.14)
Inter-X	1.37 (0.14)	1.00 (0.15)	0.94 (0.10)	1.07 (0.09)	0.30 (0.08)	31.12 (5.54)	8.78 (1.14)	4.53 (0.49)	3.41 (0.42)
Cheat-N	1.13 (0.04)	0.67 (0.05)	1.19 (0.08)	1.35 (0.13)	0.26 (0.10)	25.25 (2.45)	10.94 (0.61)	4.94 (0.61)	3.64 (0.23)
Cheat-C	1.22 (0.17)	0.64 (0.04)	1.09 (0.04)	1.36 (0.05)	0.61 (0.17)	36.72 (3.67)	11.64 (0.59)	4.75 (0.35)	3.47 (0.20)
Cheat-X	1.30 (0.17)	0.91 (0.25)	1.02 (0.09)	1.12 (0.21)	0.34 (0.14)	26.26 (6.13)	9.93 (0.83)	5.34 (0.48)	3.44 (0.24)

[1]Data are means (standard error). n=6 per cover type-treatment combination, except where noted.
[2]CLPP values are average substrate utilization measured as absorbance values.
[3]PLFA mol % values are weighted averages (weighted by the total microbial biomass per sample).
[4]n=5

Appendix A continued.

			PLFA (cont.)			TRFLP		Nematodes
Cover type-Treatment	AMF (mol%)	Fungi (mol %)	Protozoa (mol %)	Fungi: Bacteria	Stress ratio[5]	Fungal OTUs[6]	Bacterial OTUs[7]	Generic richness
FALL								
Sage-N	4.92 (0.99)	9.00 (0.40)	2.36 (0.61)	0.54 (0.04)	1.62 (0.40)	12.0 (5.0)	ND	13.7 (1.7)
Sage-C	4.55 (0.64)	14.87 (1.65)	2.09 (0.81)	0.92 (0.09)	1.46 (0.30)	10.7 (2.2)	ND	15.2 (1.0)
Sage-X	4.11 (1.17)	7.61 (0.96)	4.87 (1.03)	0.55 (0.08)	1.42 (0.47)	10.5 (3.1)	ND	14.3 (0.8)
Bunch-N	6.71 (0.40)	10.96 (0.90)	4.36 (1.01)	0.71 (0.06)	1.37 (0.19)	13.0 (2.0)	ND	14.8 (1.1)
Bunch-C	7.17 (1.25)[4]	16.90 (0.60)[4]	3.23 (0.88)[4]	0.97 (0.04)[4]	1.58 (0.27)[4]	11.7 (4.0)	ND	13.7 (1.1)
Bunch-X	6.12 (0.68)	9.35 (0.59)	4.19 (0.87)	0.59 (0.04)	1.15 (0.18)	11.2 (3.3)	ND	13.3 (0.4)
Inter-N	4.61 (0.77)	10.69 (1.23)	6.12 (1.25)	0.67 (0.07)	1.01 (0.17)	17.0 (4.5)	ND	11.2 (1.0)
Inter-C	2.94 (0.43)[4]	14.50 (1.70)[4]	5.87 (1.71)[4]	0.85 (0.10)[4]	0.90 (0.18)[4]	8.3 (2.5)	ND	10.8 (0.8)
Inter-X	3.95 (0.74)	11.77 (0.92)	4.66 (0.95)	0.75 (0.05)	0.85 (0.16)	21.2 (8.0)	ND	11.2 (0.8)
Cheat-N	8.99 (1.91)	9.82 (0.28)	3.30 (1.22)	0.68 (0.07)	0.98 (0.16)	13.0 (2.5)	ND	10.7 (1.0)
Cheat-C	5.19 (1.27)	15.08 (1.30)	2.61 (0.49)	0.95 (0.10)	0.94 (0.12)	12.8 (1.8)	ND	13.0 (1.0)
Cheat-X	6.28 (0.96)[4]	6.56 (0.53)[4]	4.79 (0.46)[4]	0.40 (0.05)[4]	0.82 (0.09)[4]	10.2 (3.3)	ND	12.3 (1.0)
SPRING								
Sage-N	4.22 (0.44)	10.58 (0.54)	1.48 (0.11)	0.70 (0.04)	4.42 (0.50)	11.5 (3.8)	280 (10)	14.7 (0.8)
Sage-C	6.96 (0.85)	14.99 (0.89)	1.44 (0.19)	0.93 (0.07)	4.11 (0.45)	16.2 (1.4)	264 (11)	13.5 (2.0)
Sage-X	5.46 (1.15)	10.58 (0.57)	1.63 (0.13)	0.68 (0.05)	3.27 (0.33)	25.8 (5.0)	296 (9)[4]	13.2 (1.5)
Bunch-N	7.11 (0.59)	15.40 (1.17)	1.50 (0.19)	0.88 (0.10)	N/A	18.2 (2.8)	290 (7)	13.3 (1.4)
Bunch-C	7.08 (0.63)	19.85 (1.40)	1.33 (0.15)	1.22 (0.13)	3.83 (0.71)	16.2 (2.7)	259 (6)	14.5 (1.8)
Bunch-X	9.35 (1.44)	14.09 (1.00)	1.26 (0.09)	0.86 (0.11)	4.60 (0.54)	11.8 (1.9)	239 (26)	13.8 (0.5)
Inter-N	6.42 (1.00)	15.85 (1.11)	1.69 (0.12)	0.96 (0.12)	N/A	13.8 (1.1)	286 (9)	13.2 (1.4)
Inter-C	4.84 (0.48)	20.18 (1.58)	1.16 (0.14)	1.29 (0.16)	N/A	21.8 (3.4)	297 (6)	12.0 (1.1)
Inter-X	5.00 (0.94)	23.07 (2.61)	1.42 (0.26)	1.75 (0.48)	N/A	11.5 (2.1)	265 (17)	12.3 (0.9)
Cheat-N	10.31 (2.15)	10.02 (0.36)	1.50 (0.10)	0.68 (0.08)	4.21 (0.23)	17.5 (3.4)	241 (12)	12.8 (0.7)
Cheat-C	11.08 (3.14)	13.24 (1.20)	1.31 (0.24)	0.85 (0.06)	3.83 (0.66)	18.8 (2.3)	279 (8)	13.0 (1.5)
Cheat-X	13.12 (2.81)	10.18 (0.78)	1.52 (0.23)	0.76 (0.11)	3.56 (0.46)	13.3 (1.5)	243 (12)	14.2 (1.2)

[5]Stress ratio = *cy*19:0/18:1ω7c. N/A indicates that ratio could not be calculated because 18:1ω7c = 0.

[6]OTU = operational taxonomic unit. ND = not determined.

[7]Bacterial TRFLP procedure was not performed on fall samples.