

University of Montana

ScholarWorks at University of Montana

Graduate Student Theses, Dissertations, &
Professional Papers

Graduate School

2002

Arbuscular mycorrhizae in mine revegetation as affected by fungal source plant species and abiotic stress

October Seastone Moynahan
The University of Montana

Follow this and additional works at: <https://scholarworks.umt.edu/etd>

Let us know how access to this document benefits you.

Recommended Citation

Moynahan, October Seastone, "Arbuscular mycorrhizae in mine revegetation as affected by fungal source plant species and abiotic stress" (2002). *Graduate Student Theses, Dissertations, & Professional Papers*. 9430.

<https://scholarworks.umt.edu/etd/9430>

This Dissertation is brought to you for free and open access by the Graduate School at ScholarWorks at University of Montana. It has been accepted for inclusion in Graduate Student Theses, Dissertations, & Professional Papers by an authorized administrator of ScholarWorks at University of Montana. For more information, please contact scholarworks@mso.umt.edu.

INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

**ProQuest Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600**

UMI[®]



**Maureen and Mike
MANSFIELD LIBRARY**

The University of

Montana

Permission is granted by the author to reproduce this material in its entirety, provided that this material is used for scholarly purposes and is properly cited in published works and reports.

****Please check "Yes" or "No" and provide signature****

Yes, I grant permission

 X

No, I do not grant permission

Author's Signature:

 October S. Mayrath

Date:

 5-30-02

Any copying for commercial purposes or financial gain may be undertaken only with the author's explicit consent.

**Arbuscular Mycorrhizae in Mine Revegetation
as Affected by Fungal Source, Plant Species, and Abiotic Stress**

by

October Seastone Moynahan

B.S., Bates College, Lewiston, Maine, 1994

M.S., The University of Montana, Missoula, Montana, 1996

Presented as partial fulfillment of the requirements for the degree of

Doctor of Philosophy

The University of Montana

February 2002


Approved by



Dr. Catherine Zabinski, Research Director



Dr. Aonia Sala, Program Director



Dr. David Strobel, Dean, Graduate School

6-3-02
Date

UMI Number: 3048329

UMI[®]

UMI Microform 3048329

Copyright 2002 by ProQuest Information and Learning Company.
All rights reserved. This microform edition is protected against
unauthorized copying under Title 17, United States Code.

ProQuest Information and Learning Company
300 North Zeeb Road
P.O. Box 1346
Ann Arbor, MI 48106-1346

Arbuscular Mycorrhizae In Mine Revegetation As Affected By Fungal Source, Plant Species, And Abiotic Stress

Directors: Dr. Catherine Zabinski CZ
Dr. Anna Sala AS

Arbuscular mycorrhizae (AM) are plant-fungal symbioses that contribute to plant growth through a variety of functions, including enhanced phosphorus uptake. My research examines the role of AM in promoting plant growth in mine waste-contaminated soils. Through a series of greenhouse and field experiments, I examined effects of AM on plant growth while varying the AM fungal source, nonmycorrhizal microbial community, soil abiotic characteristics, and plant species. The AM fungal sources were 1) sterilized inoculum (nonmycorrhizal), 2) inoculum from a metals-contaminated site (possibly adapted to abiotic stress), and 3) inoculum from an uncontaminated site (potentially more diverse and beneficial to plant communities). In most cases, AM from metals-contaminated soils significantly increased plant growth, although effects varied from no difference to a 9-fold increase in biomass. Enhanced biomass in one of the experiments corresponded with phosphorus uptake by plants, but not with tissue metal (Cd, Cu, Fe, Pb, Zn) levels, indicating AM benefits were general mycorrhizal benefits, rather than direct alterations to plant metal-resistance. The microbial amendment (without mycorrhizae) from a metals-contaminated soil eliminated the positive biomass effect seen with the addition of metals-contaminated soil AM fungi. Soil abiotic characteristics were varied in two experiments: a greenhouse experiment including a series of limed tailings treatments, and a field experiment with compost-amended mine wastes. In the greenhouse, AM effects were most pronounced with a low level of lime addition, and in the field, plants growing in compost-amended wastes showed the greatest response to AM fungi. Positive AM effects were much more pronounced for metals-sensitive plant species than metal-resistant species, suggesting that AM inoculation may potentially increase the number of plant species used for revegetation. These experiments show that AM can increase plant success in mine revegetation, but AM effects vary with fungal inoculum source, plant species, and soil abiotic conditions.

In addition to my scientific research, I completed an E.P.A.-sponsored internship focused on incorporating academic research into restoration management strategies. My final chapter summarizes my internship experience, including a discussion of impediments to and strategies for increasing information transfer between academic scientists and restoration managers.

ACKNOWLEDGEMENTS

I am especially grateful to Cathy Zabinski, my research advisor, for teaching me to think creatively as well as critically, and for encouraging me to balance personal and professional priorities as a scientist. Many thanks to Anna Sala, my program advisor, for guidance and encouragement, and for always making me feel valued and welcome as part of her lab group. I also thank my committee members, Ray Callaway, Bill Holben, Johnnie Moore, Del Kilgore, and Scott Brown, for technical advice and careful review of my dissertation work. Jon Graham, although not officially a committee member, helped me enormously with experimental design and statistical analysis.

This work would not have been possible without endorsement and financial support from the U.S. Environmental Protection Agency, Montana Department of Environmental Quality (Abandoned Mine Lands and Permitting and Enforcement), The Reclamation Research Unit at Montana State University, the Montana Department of Natural Resources and Conservation, and the Bertha Morton Fellowship. I am especially thankful to Penny Kukuk and the National Science Foundation-funded Training Within Environmental Biology program at the University of Montana (NSF-GRT #9553611 to P. Kukuk, C. Brewer, and F. Allendorf), for financial and programmatic support throughout my PhD program. The Training WEB support allowed me to create a research program with applications for management, and to develop and pursue avenues for their dissemination.

I am very fortunate to have enjoyed the friendship and technical assistance of many fellow graduate and undergraduate students, especially Dawn White, Beth Newingham, Brad Cook, Sara Barth, and Kendra Hinxman. I also benefited from laboratory and field assistance from Davin Ringen, Kevin Simonin, Maritt Hartjes, Lauren Quinn, Zach Franz, Marilyn Pratter, Tom Seastone, Heather Tone, Patricia Sioux, Shannon Phelps, Lorna McIntire, Molly White, Maureen O'Mara, Chris Richey, Adam Collins, and Scott Bogan. Thanks to the Division of Biological Sciences Staff and the Murdock Environmental Geochemistry Lab for administrative and technical support. All of the graduate students in Anna Sala's and Ray Callaway's lab groups provided technical help, advice, constructive criticism, and wonderful friendship throughout this process. Additional thanks to Stuart Jennings and Dennis Neuman (Reclamation Research Unit, MSU), Scott Brown (US EPA), Bob Wintergerst (Deerlodge National Forest), Pete Strazdas (Montana DEQ), for appreciating my ideas and helping me bring my research into restoration practice.

The support and encouragement of my friends and family has been essential throughout my academic development. I am especially grateful to my husband and best friend, Brendan Moynahan, for amazing love and support, not to mention countless hours of editing, consoling, and carrying heavy buckets of mine tailings to and from the greenhouse.

TABLE OF CONTENTS

Abstract.....	ii
Acknowledgements.....	iii
List of Figures	vi
List of Tables	vii
 CHAPTER 1: Introduction: Arbuscular Mycorrhizae in Hardrock Mine Waste Revegetation	
Waste Revegetation	1
Arbuscular Mycorrhizae and Plant Communities	2
Positive Interactions in Stressful Conditions	3
Revegetation of Hardrock Mine Wastes.....	4
Scope of Dissertation.....	6
References	10
 CHAPTER 2: Plant Growth Effects Due to Arbuscular Mycorrhizal and Microbial Inoculum From Metals-Contaminated and Uncontaminated Sources.....	
Abstract.....	14
Introduction	15
Methods	16
Results.....	20
Discussion	25
Discussion	27
Acknowledgements.....	31
References	32
 CHAPTER 3: Mycorrhizal Dependency of <i>Deschampsia cespitosa</i> Across a pH- and Metals-induced Stress Gradient.....	
Abstract.....	41
Abstract.....	42
Introduction	43
Methods	47
Results.....	52
Discussion	55
Discussion	55
Acknowledgements.....	61
References	62
 CHAPTER 4: Mycorrhizal Source Effects on Six Native Plants with Potential for Mine Tailings Revegetation	
Abstract.....	71
Abstract.....	72
Introduction	73

Methods	77
Results	81
Discussion	83
Acknowledgements	87
References	88
CHAPTER 5: Effects of Compost and Arbuscular Mycorrhizal Inocula on Simple Native Plant Communities Transplanted for Mine Waste Revegetation.....	97
Abstract.....	98
Introduction	99
Methods	104
Results	111
Discussion	116
Acknowledgements.....	120
References	121
CHAPTER 6: Incorporating Ecological Concepts into Mine Restoration Practices.....	132
Introduction	133
Ecological Restoration of Mine Wastes.....	134
Information Exchange: Basic Science and Management.....	135
The Internship.....	137
Workshop Assessment	141
Conclusions and Recommendations.....	145
References	148
APPENDIX A: Quality Assurance / Quality Control for ICP-AES of Soil Water Extracts and Soil and Plant Digest	149
APPENDIX B: Workshop Evaluation Form.....	153

LIST OF FIGURES

CHAPTER 1:

- Figure 1 Model of soil ecosystem components contributing to recovery of mine-disturbed systems..... 6

CHAPTER 2:

- Figure 1 Total biomass of plants grown with all combinations of AM and MC inoculum treatments 39
- Figure 2 Estimated marginal means of total phosphorus content in plants grown with different AM inoculum sources 40

CHAPTER 3:

- Figure 1 Total biomass of nonmycorrhizal plants across lime treatments 68
- Figure 2 Total biomass of plants with all lime and AM treatments 69
- Figure 3 Plant-perceived stress and mycorrhizal dependency versus pH 70

CHAPTER 4:

- Figure 1 Total biomass of all plant species with AM treatments..... 95
- Figure 2 Root mass ratios of all plant species with AM treatments . 96

CHAPTER 5:

- Figure 1 Final transplant growth measurements 129
- Figure 2 Aboveground biomass, comparing AM effects within compost treatments for each plant species..... 130
- Figure 3 Number of flowering stalks, comparing AM effects within compost treatments for each plant species..... 131

LIST OF TABLES

CHAPTER 2:

Table 1	Geochemical properties of mine tailings growth substrate	37
Table 2	Metals concentrations of <i>D. cespitosa</i> root and shoot tissues	38

CHAPTER 3:

Table 1	Geochemical properties of AM inoculum source soils and mine tailings growth substrate from each lime treatment	66
Table 2	Mean AM colonization levels and plant growth characteristics of all treatment	67

CHAPTER 4:

Table 1	Geochemical characteristics of mine tailings growth substrate and AM inoculum source soils	93
Table 2	Mean AM colonization levels and plant growth characteristics of all treatments	94

CHAPTER 5:

Table 1	Soil geochemical characteristics of substrate and compost plots (whole plots)	126
Table 2	Soil biological characteristics of substrate and compost plots (whole plots)	127
Table 3	ANOVA results for aboveground biomass and number of flowering stalks	128

CHAPTER 1

Introduction:

Arbuscular Mycorrhizae in Hardrock Mine Waste Revegetation

“Ecologists have learned much about ecosystem structure by dissecting communities and examining their parts and processes. The true test of our understanding, however, is the ability to recreate them”.

John Ewel, 1990

Restoration ecologists recognize reconstruction of ecosystems on disturbed lands as a unique opportunity to test ecological principles that have been developed through the study of intact, functioning systems (Bradshaw 1987). My dissertation research examines two active areas in plant ecology research that may be especially relevant to restoration: 1) the role of soil microorganisms in determining plant community structure; and 2) the importance of positive interactions for structuring plant communities growing in stressful abiotic conditions. Specifically, I examine positive plant-microbe interactions as part of the piecewise reconstruction of functional plant communities in the context of mine waste revegetation.

Arbuscular Mycorrhizae and Plant Communities

Plant community dynamics often depend on associations with soil microorganisms (Bolton et al. 1992, Bever et al. 1997, Van der Heijden et al. 1998) such as mycorrhizal fungi. There are several types of mycorrhizae, and my research focuses on arbuscular mycorrhizae (AM, sometimes known as VAM), an often-mutualistic symbiosis between a plant root and a specialized fungus, in which the fungus forms structures inside plant cortical cells and extends into the soil. The fungus benefits from a direct source of carbon supplied by the plant, while the plant potentially receives increased nutrient uptake, improved water status, pathogen protection, improved soil structure and possibly enhanced metal resistance (Bolton et al. 1992, Newsham 1995, Martin et al. 2001).

Positive Interactions in Stressful Conditions

The abundance and diversity of plant species are determined by a balance of ecological processes and species interactions that may shift across environmental gradients and successional stages (Connell and Slatyer 1977, Walker and Chapin 1987, Bertness and Callaway 1994, Stachowicz 2001). The relative importance of facilitation and competition in structuring communities shifts under different conditions (Callaway 1997, Brooker and Callaghan 1998), with competition more important in low stress environments, and positive interactions more common with high physical stress and/or high consumer pressure (Bertness and Callaway 1994). Because disturbed lands present co-occurring stressors to plant communities, it may be especially important to include facilitative interactions as part of ecological restoration strategies (Pyke and Archer 1991, Young et al. 2001).

While most studies focus on positive interactions between plants, the undeniable dependence of plant communities on soil microbes suggests that positive interactions with microorganisms also may be especially important in abiotically stressful environments (Stachowicz 2001). Mycorrhizal associations can have positive, neutral, or negative effects on plants, depending on the balance of costs and benefits to the host (Smith and Smith 1996, Johnson et al. 1997). Mycorrhizae can increase plant growth over nonmycorrhizal plants under strenuous abiotic conditions such as water stress (Stahl et al. 1998, Mathur and Vyas 2000), salinity (Ruiz-Lozano and Azcón 2000), acid pH (Clark et al. 1999)

and elevated metals (Leyval et al. 1997, Meharg and Cairney 2000), but we know little about how AM effects shift across varying levels of these stressors.

Revegetation of Hardrock Mine Wastes

Among the most challenging disturbed systems in need of restoration are vast quantities of mine waste materials scattered throughout mountains and river systems of the world (Dudka and Adriano 1997, Tordoff et al. 2000). The characteristic low pH, low nutrient content, and extremely high metal levels of most mine tailings result in exposed areas where plants cannot naturally establish and microbial populations are depressed (Visser 1985, Noyd et al. 1995, Moynahan et al. 2002). Mining-related disturbance can reduce or eliminate AM abundance, diversity, growth, and function (Jasper et al. 1989, Leyval et al. 1997, Del Val et al. 1999, Meharg and Cairney 2000). Strategies to reclaim acidic mine wastes, including neutralization by liming, organic matter incorporation, and seeding can improve revegetation, but more effective and reliable strategies are needed (Tordoff et al. 2000).

Advances in revegetation techniques often arise from important findings in plant sciences combined with practical experience. Seed mixes for mine revegetation projects generally consist of species that have demonstrated the ability to grow on disturbed sites in past projects and experiments, or on naturally harsh soils (Thornburg 1982, Powell 1988, Munshower 1994). Unfortunately, this work has been conducted in the absence of the mycorrhizal fungal symbionts that occur with plants growing in natural conditions. This may result in a bias

against mycorrhizal-dependent plant species, including many desirable native plants. Because mycorrhizal communities in mine-disturbed sites are usually depressed or absent (Jasper 1989, Leyval et al. 1997, Moynahan et al. 2002), and because AM may improve plant growth, plant community dynamics, and soil structure, AM inoculation may be important to the success of mine waste revegetation projects.

Reintroduction of mycorrhizae, bacteria, and other soil microorganisms by application of commercial inoculum or inoculum collected from uncontaminated soil systems has become more common in revegetation designs (Allen 1989, Norland 1993, Pflieger et al. 1994, St. John 1998, Amaranthus et al. 1999, Tordoff et al. 2000). The scientific literature supports two alternative inoculation strategies. First, AM inoculum from metals-contaminated sites may be pre-conditioned to metals-stress, resulting in greater benefits to the host plants (Leyval et al. 1997, Meharg and Cairney 2000). Conversely, inoculum from uncontaminated soil may be more diverse than that from a metals-contaminated soil (Del Val et al. 1999), and diverse AM fungal communities have been shown to result in more productive and diverse plant communities (Van der Heijden et al. 1998). Field inoculation experiments are not well-documented in the scientific literature, but some studies have shown increased plant production on mine tailings inoculated with AM fungi from vegetated tailings (Noyd et al. 1996) and anecdotal evidence of increased plant survival and diversity on disturbed soil inoculated with commercial AM fungi (St. John 1995).

Scope of Dissertation

The minimal biotic activity in mine waste-disturbed lands provides an excellent system to examine belowground ecological interactions as soil components are reintroduced. Figure 1 presents a simplified conceptual framework for my dissertation research, and depicts the four soil system components (plants, abiotic soil, AM fungi, and other soil microorganisms) that were manipulated to promote plant growth in mine wastes. Traditional approaches to mine revegetation include manipulation of the abiotic soil and plant species, while effects of AM fungi and other soil microorganisms are often overlooked.

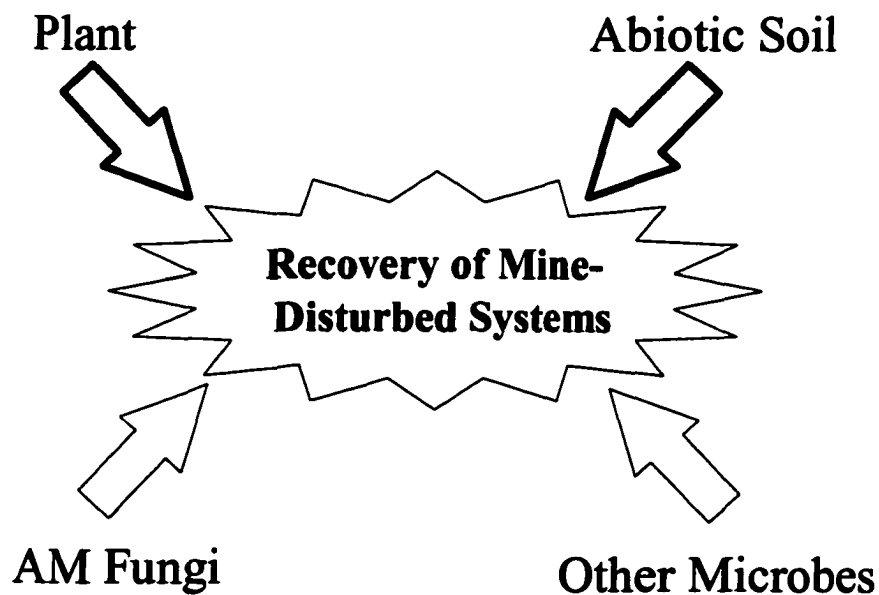


Figure 1. Model of soil ecosystem components contributing to recovery of mine-disturbed systems. Bold arrows emphasize that plants and abiotic soil are usually the only factors manipulated and monitored in reclamation efforts.

My dissertation describes a series of greenhouse and field studies designed to systematically investigate plant growth effects of AM in combination with each of the other soil components. The first study, described in Chapter 2, examined individual and combined effects of AM inoculum source and other microbial community (MC) inoculum source on growth of tufted hairgrass (*Deschampsia cespitosa*), an acid- and metal-resistant native plant, grown in mine tailings. Inoculum sources included AM and MC from a metals-contaminated site and from an uncontaminated site. Mechanisms of plant growth effects were explored with plant tissue analysis of phosphorus and metal levels. Improving our understanding of individual and combined effects of soil microorganisms on plants in severely disturbed soils may contribute to a better understanding of how disturbed systems can be successfully reconstructed through restoration practices.

Chapter 3 investigates effects of AM inoculation across an abiotic gradient, with pH ranging from 3.4 to 7.4, and decreasing metals concentrations, as affected by no, low, medium and high levels of lime amendment. Mycorrhizal effects on tufted hairgrass were compared with AM from metals-contaminated and uncontaminated sources. This work assessed the importance of positive interactions along abiotic gradients and provides information relevant to restoration practitioners regarding AM effects in mine tailings treated with lime. This chapter extends ecological theory of positive interactions developed in plant communities to the consideration of positive plant-microbe interactions.

Chapter 4 describes research testing effects of AM from metals-contaminated and uncontaminated sources on six native plant species, with varying tolerances to abiotic stress. Understanding how different plant species respond to AM inoculation is particularly important to restoration managers, who often select stress-tolerant revegetation species based on past performance without AM colonization. Re-evaluating plant species with their natural AM symbioses may expand the pool of desirable revegetation species and lead to more productive and diverse plant communities.

The field study presented in Chapter 5 examines effects of compost addition and AM inoculum source on three species of container-grown plants in three coversoil substrates. Effects of AM and compost were demonstrated in a realistic mine reclamation setting, providing crucial support for findings observed in greenhouse studies. It is especially important for AM research to include field studies, since the correlation between field and greenhouse studies is often ambiguous.

Finally, Chapter 6 summarizes my internship, sponsored by the National Science Foundation-funded Training Within Environmental Biology (Training WEB) Program at The University of Montana and the U.S. Environmental Protection Agency. The internship was designed to facilitate communication between restoration practitioners and basic science researchers, with an overarching goal of promoting the incorporation soil ecology principles into restoration strategies. Through workshops and technical advising, I was able to transfer ecological knowledge to revegetation professionals, develop ideas for

**improving communication between basic scientists and restoration managers,
and gain a better understanding of the role of science in natural resource
management.**

References:

Allen, E.B. 1989. The restoration of disturbed arid landscapes with special reference to mycorrhizal fungi. *Journal of Arid Environments* 17:279-286.

Amaranthus, M.P., E. Cazares, and D.A. Perry. 1999. The role of soil microorganisms in restoration. In *Proceedings: Pacific Northwest forest and rangeland soil organism symposium*. R.T. Meurisse, W.G. Ypsilantis, and C. Seybold, eds., pp. 179-189, General Technical Report PNW-GTR-461. USDA Forest Service Pacific Northwest Research Station, Portland, Oregon.

Bertness, M.D. and R.M. Callaway. 1994. Positive interactions in communities. *Trends in Ecology and Evolution* 9:191-193.

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.J., J.K. Frederickson, and L.F. Elliot. 1992. Microbial ecology of the rhizosphere. In *Soil Microbial Ecology*. F.B. Metting, Jr. ed., pp. 27-63, Marcel Dekker, Inc. New York.

Bradshaw, A.D. 1987. Restoration: An acid test for ecology. In *Restoration ecology: A synthetic approach to ecological research*. W.R. Jordan III, M.E. Gilpin, and J.D. Aber, eds., pp. 23-30, Cambridge University Press, Cambridge, UK.

Brooker, R.W. and T.V. Callaghan. 1998. The balance between positive and negative plant interactions and its relationship to environmental gradients: a model. *Oikos* 81:196-207.

Callaway, R.M. 1997. Positive interactions in plant communities and the individualistic-continuum concept. *Oecologia* 112:143-149.

Clark, R.B., S.K. Zeto, and R.W. Zobel. 1999. Arbuscular mycorrhizal fungal isolate effectiveness on growth and root colonization of *Panicum virgatum* in acidic soil. *Soil Biology & Biochemistry* 31:1757-1763.

Connell, J.H. and R.D. Slatyer. 1977. Mechanisms of succession in natural communities and their role in community stability and organization. *American Naturalist* 111:1119-1144.

Del Val, C., J.M. Barea, and C. Azcon-Aguilar. 1999. Diversity of arbuscular mycorrhizal fungus populations in heavy-metal-contaminated soils. *Applied and Environmental Microbiology* 65:718-723.

Dudka, S. and D.C. Adriano. 1997. Environmental impacts of metal ore mining and processing: a review. *Journal of Environmental Quality* 26:590-602.

Jasper, D.A., L.K. Abbott, and A.D. Robson. 1989. The loss of VA mycorrhizal infectivity during bauxite mining may limit the growth of *Acacia pulchella* R.Br. *Australian Journal of Botany* 37:33-42.

Johnson, N.C., J.H. Graham, and F.A. Smith. 1997. Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phytologist* 135:575-585.

Leyval, C., K. Turnau, and K. Haselwandter. 1997. Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. *Mycorrhiza* 7:139-153.

Martin, F.M., S. Perotto, and P. Bonfante. 2001. Mycorrhizal fungi: a fungal community at the interface between soil and roots. In *The rhizosphere - biochemistry and organic substances at the soil-plant interface*. R. Pinton, Z. Varanini, and P. Nannipieri, eds., pp. 263-296, Marcel Dekker, Inc. New York.

Mathur, N. and A. Vyas. 2000. Influence of arbuscular mycorrhizae on biomass production, nutrient uptake and physiological changes in *Ziziphus mauritiana* Lam. under water stress. *Journal of Arid Environments* 45:191-195.

Meharg, A.A. and J.W.G. Cairney. 2000. Co-evolution of mycorrhizal symbionts and their hosts to metal-contaminated environments. *Advances in Ecological Research* 30:69-112.

Moynahan, O.S., C.A. Zabinski, and J.E. Gannon. 2002. Microbial community structure and carbon-utilization diversity in a mine tailings revegetation study. *Restoration Ecology* 10:77-87.

Munshower, F.F. 1994. *Practical Handbook of Disturbed Land Revegetation*, Lewis Publishers, Inc. Boca Raton, Florida. 140pp.

Newsham, K.K., A.H. Fitter, and A.R. Watkinson. 1995. Multi-functionality and biodiversity in arbuscular mycorrhizas. *Trends in Ecology and Evolution* 10:407-411.

Norland, M.R. 1993. *Soil factors affecting mycorrhizal use in surface mine reclamation*. Information circular 9345, United States Bureau of Mines, Pittsburg, PA.

Noyd, R.K., F.L. Pflieger, M.R. Norland, and M.J. Sadowsky. 1995. Native prairie grasses and microbial community responses to reclamation of taconite iron ore tailing. *Canadian Journal of Botany* 73:1645-1654.

Noyd, R.K., F.L. Pflieger, and M.R. Norland. 1996. Field responses to added organic matter, arbuscular mycorrhizal fungi, and fertilizer in reclamation of taconite iron ore tailing. *Plant and Soil* 179:89-97.

Pflieger, F.L., E.L. Stewart, and R.K. Noyd. 1994. Role of VAM fungi in mine land revegetation. In *Mycorrhizae and Plant Health*. F.L. Pflieger and R.G. Linderman, eds., pp. 47-82, The American Phytopathological Society, St. Paul, Minnesota.

Powell, J.L. 1988. Revegetation options. In *Reclamation of Surface-Mined Lands*. L.L. Hossner, ed., pp. 49-91, CRC Press, Inc. Boca Raton, Florida.

Pyke, D.A. and S. Archer. 1991. Plant-plant interactions affecting plant establishment and persistence in revegetated rangeland. *Journal of Range Management* 44:550-557.

Ruiz-Lozano, J.M. and R. Azcon. 2000. Symbiotic efficiency and infectivity of an autochthonous arbuscular mycorrhizal *Glomus* sp. from saline soils and *Glomus deserticola* under salinity. *Mycorrhiza* 10:137-143.

Smith, F.A. and S.E. Smith. 1996. Mutualism and parasitism: diversity in function and structure in the "arbuscular" (VA) mycorrhizal symbiosis. *Advances in Botanical Research* 22:1-43.

St. John, T.V. 1993. Benefits of mycorrhizae in revegetation and restoration. In *Interface between ecology and land development in California*. J.E. Keeley, ed., pp. 269-272, Southern California Academy of Sciences, Los Angeles.

St. John, T.V. 1998. Mycorrhizal inoculation in habitat restoration. *Land and Water* September/October:17-19.

Stachowicz, J.J. 2001. Mutualism, facilitation, and the structure of ecological communities. *BioScience* 51:235-246.

Stahl, P.D., G.E. Schuman, S.M. Frost, and S.E. Williams. 1998. Arbuscular mycorrhizae and water stress tolerance of Wyoming big sagebrush seedlings. *Soil Science Society of America Journal* 62:1309-1313.

Thornburg, A.A. 1982. *Plant Materials for Use on Surface-Mined Lands in Arid and Semiarid Regions*, Soil Conservation Service, United States Department of Agriculture, Washington, DC. 88pp.

Tordoff, G.M., A.J.M. Baker, and A.J. Willis. 2000. Current approaches to the revegetation and reclamation of metalliferous mine wastes. *Chemosphere* 41:219-228.

Van der Heijden, M.G.A., J.N. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A. Wiemken, and I.R. Sanders. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability, and productivity. *Nature* 396:69-72.

Visser, S. 1985. Management of microbial processes in surface mined land reclamation in western Canada. In *Soil reclamation processes- Microbiological analyses and applications*. R.L. Tate and D.A. Klein, eds., pp. 203-241, Marcel Dekker, Inc. New York.

Walker, L.R. and F.S. Chapin. 1987. Interactions among processes controlling successional change. *Oikos* 50:131-135.

Young, T.P., J.M. Chase, and R.T. Huddleston. 2001. Community succession and assembly - comparing, contrasting and combining paradigms in the context of ecological restoration. *Ecological Restoration* 19:5-18.

CHAPTER 2

Plant Growth Effects Due to Arbuscular Mycorrhizal and Microbial Inoculum From Metals-Contaminated and Uncontaminated Sources

ABSTRACT

Ecological restoration of severely disturbed sites requires soil microorganisms that can directly and indirectly affect plant and soil development. *Deschampsia cespitosa* (tufted hairgrass) was grown in mine tailings with microbial treatments in a 2-way complete factorial design, testing effects of arbuscular mycorrhizal (AM) inoculum sources and other microbial community (MC) inoculum sources on plant biomass. Arbuscular mycorrhizal fungi and MC were added separately and in combination to host plants, to assess AM x MC interactions. The sources of AM and MC were 1) sterilized inoculum, 2) inoculum from a metals-contaminated site, and 3) inoculum from an uncontaminated site. Both AM and MC treatments significantly affected plant biomass, and the source of inoculum was significant for both. Plants grown with AM from metals-contaminated soil had 67% greater biomass, while biomass of plants growing with uncontaminated soil AM was not statistically different than the non-mycorrhizal control plants. The only significant difference caused by MC inoculation was a negative effect, in which the MC from metals-contaminated soil reduced AM fungal colonization and eliminated the positive effect of the AM from metals-contaminated soil. Biomass patterns corresponded with phosphorus uptake by plants, but not with tissue metal (Cd, Cu, Fe, Pb and Zn) levels, indicating that benefits of AM from metals-contaminated soils to the plant were probably general mycorrhizal benefits, rather than direct metal-resistance conferred by AM. Elucidating individual and combined effects of soil microorganisms on plants in severely disturbed soils will

contribute to a better understanding of how disturbed systems can be successfully reconstructed through restoration practices.

INTRODUCTION

Awareness of complex soil ecological interactions has changed ecologists' understanding of plant community development and function. Such an understanding is critical for the field of restoration ecology, where methods are being explored to reintroduce missing or impoverished system components, including the crucial microbial drivers of soil ecology. This is especially true for the reclamation of hardrock mine waste deposits, such as milled tailings and waste rock. The characteristic low pH, low nutrient content, and extremely high metal levels of most mine tailings result in exposed areas where plants cannot naturally establish and microbial populations are depressed (Visser 1985, Noyd et al. 1995, Moynahan et al. 2002). Heavy metal contamination can have profound negative effects on microbial biomass (Brookes and McGrath 1984), microbial community structure (Pennanen et al. 1996, Bååth et al. 1998), and microbial processes such as carbon and nitrogen mineralization (Doelman et al. 1986), general organic matter turnover rates (Chandler and Brookes 1991), nitrogen fixation (Brookes et al. 1986, Lorenz et al. 1992), and the ability to metabolize a variety of carbon sources (Knight et al. 1997, Kelly and Tate 1998, Dobler et al. 2000). Elevated metal levels and mine-related disturbance also can result in depressed AM colonization rates, inhibited AM function, and reduced

fungus species diversity (Danielson 1985, Jasper et al. 1989, Leyval et al. 1997, Del Val et al. 1999, Meharg and Cairney 2000).

Reintroduction of mycorrhizae, bacteria, and other soil microorganisms by application of commercial inoculum or inoculum collected from uncontaminated soil systems has become more common in revegetation designs (Allen 1989, Norland 1993, Pflieger et al. 1994, St. John 1998, Amaranthus et al. 1999, Tordoff et al. 2000). Field inoculation experiments are not well-documented in the scientific literature, but some studies have shown increased plant production on mine tailings inoculated with AM fungi from vegetated tailings (Noyd et al. 1996) and anecdotal evidence of increased plant survival and diversity on disturbed soil inoculated with commercial AM fungi (St. John 1995).

Microbial reintroduction to reclamation sites is additionally complex because of numerous, poorly-understood microbial interactions. For example, mycorrhizal fungi may depend on "mycorrhization helper bacteria" to facilitate plant root colonization (Garbaye 1994, Hodge 2000). Plant biomass, AM colonization levels, and N-uptake can be improved by simultaneous inoculation with nitrogen-fixing bacteria and plant-growth-promoting rhizobacteria (Requena et al. 1997). The specificity of microbial interactions is not well known, and evidence is mixed as to whether AM inoculation may be more effective when accompanied by microbes from the same soil source (Shetty et al. 1994, Requena et al. 1997).

The importance of AM inoculum source for plants growing in mine wastes has been suggested by studies comparing AM fungi from metals-contaminated

sites versus uncontaminated sites. AM fungal strains isolated from Cd- and Zn-contaminated soils have shown varying levels of metals resistance, resulting in improved fungal functioning, increased colonization, and improved plant growth response when compared to isolates from uncontaminated soils (Gildon and Tinker 1983, Shetty et al. 1995, Weissenhorn et al. 1994, Leyval et al. 1997, Meharg and Cairney 2000). Other studies have found no difference, or decreased plant growth with AM from contaminated sources (Shetty et al. 1994, Enkhtuya et al. 2000), although both studies noted that these effects were observed in moderately contaminated soils, while all plants showed extremely poor growth in mine tailings or severely contaminated soils. Shetty et al. (1995) later noted that AM fungi from contaminated soils resulted in greater plant biomass when grown under high Zn conditions, while AM from uncontaminated soil resulted in greater plant biomass when grown under lower Zn concentrations.

The mechanisms for AM-induced plant benefits in metals-contaminated environments are not well known (Leyval et al. 1997, Meharg and Cairney 2000). It has been proposed that AM enhance plant metal resistance by preventing metal toxicity to host plants through reduced metal exposure with mechanisms such as binding metals to external AM hyphae, active efflux or limited influx, restricted transport, or sequestration of metals in AM tissues (Leyval et al. 1997). Research demonstrating altered metal translocation in mycorrhizal plants (Dehn and Schüepp 1989, Hetrick et al. 1994, Shetty et al. 1995, Leyval et al. 1997), and metal-binding properties of AM (Galli et al. 1994, Joner et al. 2000, Cuenca et al. 2001) has provided support for the hypothesis of AM-conferred metal

resistance. Alternatively, AM benefits to plants growing in metals-contaminated soils may be due to general AM benefits, including enhanced P uptake, improved water relations and pathogen resistance, which are made possible in contaminated soils when metal-resistant AM fungi are present (Meharg and Cairney 2000).

While previous research has demonstrated metals-resistance of fungal species isolates, it is important to note that metals adaptation of AM inocula also may occur as shifts in fungal species composition. Field inoculation methods often include a mixture of AM fungal species, especially when whole soil inoculum is added, underscoring the importance of considering the role of community-level changes. The scientific literature supports two alternative inoculation strategies. First, AM inoculum from metals-contaminated sites may be pre-conditioned to metals-stress, resulting in greater benefits to the host plants (Leyval et al. 1997, Meharg and Cairney 2000). Conversely, inoculum from uncontaminated soil may be more diverse than that from a metals-contaminated site (Del Val et al. 1999), and diverse AM fungal communities have been shown to result in more productive and diverse plant communities (Van der Heijden et al. 1998).

Our research builds on previous studies with the following objectives: i) to measure AM and other microbial community inoculation effects on plant growth in mine tailings, ii) to determine whether growth effects vary with inoculum source (inocula from contaminated vs. uncontaminated soils), iii) to assess AM inoculum effects when combined with their native associated soil microbes versus the

alternate microbial inoculum source, and iv) to elucidate mechanisms of AM-enhanced plant growth (altered metal uptake and/or enhanced phosphorus acquisition). We compared the effects of AM and other microbial community inocula from a metals-contaminated soil, where soil microbes may have adjusted to metal-stress, to inocula from an uncontaminated soil, which may provide a more diverse microbial community. These effects were measured on a metals-resistant host plant, *Deschampsia cespitosa*, growing in a mine tailings substrate with high metals concentrations, low pH, and low nutrient availability.

METHODS

Experimental Design/Soil chemical characterization

Response of *Deschampsia cespitosa* L. (Beauv.) (tufted hairgrass), was tested in mine tailings with a pH of 3.5, high metal levels, and low nutrient content (Table 1). *Deschampsia cespitosa* is often tolerant of acidic, elevated-metal conditions (Von Frenckell-Insam and Hutchinson 1993, Munshower 1994), and can naturally colonize mine waste deposits. Tailings material was collected from an abandoned mine tailings deposit in southwest Montana (46°20'N, 112°13'W), homogenized thoroughly, sieved through a 5 mm mesh, and distributed into pots (15 cm dia).

Effects of mycorrhizal and other microbial inoculation were examined with a two-way complete factorial (3 x 3) randomized design. The two factors examined were arbuscular mycorrhizal (AM) inoculum source and other microbial community (MC) inoculum source. Each factor (AM and MC) was assessed with

three different treatments: 1) autoclaved inoculum, 2) inoculum from a metals-contaminated site, and 3) inoculum from an uncontaminated site. This design (3 AM treatments x 3 MC treatments) resulted in nine treatment combinations, which were tested with fourteen replicates each.

Inoculation

The metals-contaminated inoculum was collected from a mine tailings revegetation project in southwestern Montana that had lime-neutralized tailings, high metal levels, and plants with high mycorrhizal colonization levels (Moynahan et al. 2002). The uncontaminated inoculum was from a bluebunch wheatgrass (*Pseudoroegneria spicata*), rough fescue (*Festuca scabrella*), Idaho fescue (*Festuca idahoensis*)-dominated grassland in western Montana with no metal contamination, and high mycorrhizal colonization rates.

AM inoculum was prepared by thoroughly washing plant roots from each site, and cutting roots into 1 cm fragments. The negative control was a 1:1 mix of the two inoculum sources, autoclaved twice at 121°C for 15 min, with 24 h between sessions in the autoclave. Two g of the appropriate inoculum was added as a continuous layer approximately 2 cm below the surface of the tailings growth substrate.

The MC inoculum was prepared by passing a soil slurry (1:4 by weight, soil:water) through a series of filters (250µm, 25µm, 11µm). This process excludes mycorrhizal propagules, while soil bacteria, non-mycorrhizal spores, and other microorganisms pass through the filters. The negative control was a

1:1 mix of the two inoculum sources, autoclaved twice at 121°C for 15 min, with 24 h between sessions in the autoclave. Twenty-five ml of the appropriate microbial suspension was added to each pot.

Plant Cultivation and Harvest

Deschampsia cespitosa germinants were thinned to a single plant per pot. Plants were watered to field capacity every one-two days, and nutrients were added every three weeks with 50 ml of 1/8-strength modified Hoagland's solution (minus P) during the first five months of growth, and 50 ml of 1/4-strength Hoagland's solution during the last two months of growth. After seven months, plants were harvested and washed thoroughly. Roots and shoots were dried for three days at 60°C and weighed.

Mycorrhizal Colonization Measurements

A subsample of roots was collected from each plant after biomass measurements were taken to determine mycorrhizal colonization levels. Plant roots were cleared in 2.5% KOH for 48 h, acidified in 3% HCl for 12 h, and stained with Trypan blue for 12 h (modification of Phillips and Hayman 1970). The percentage of roots colonized by mycorrhizal fungi was determined by the magnified intersections method of McGonigle et al. (1990).

Plant Tissue Analysis

Subsamples of AM- and MC-treated plants were selected for tissue metal and nutrient analyses. To compare AM treatments, five samples of each AM source treatment were selected from plants that received no MC inoculum. To explore effects of MC, five plants from each MC treatment were selected from plants that had AM inoculum from the metals-contaminated soil.

Whole root and shoot samples were washed thoroughly in deionized water, dried, and ground using a ball mill (Spex Industries, Inc.). Plant samples were digested with a double acid, peroxide, hot-block digest, according to E.P.A. Method 3050B, modified to ¼ volume (Edgell 1988). Analytes, including As, Cd, Cu, Fe, Pb, Zn, and P were quantified by inductively coupled plasma atomic emission spectrometry (ICP-AES) analysis of plant digests using E.P.A. Method 200.7 (Jarrell-Ash ICP-AES 800 instrument) (Martin et al. 1994). The QA/QC results are summarized in Appendix A. Analytes were compared for metal and tissue P concentrations (mg kg⁻¹), and plant total P content (concentration x biomass).

Soil Analysis

For pH measurements, 10 g of soil were combined with 20 ml of deionized water, allowed to equilibrate for 5 min, and measured with a pH probe (Accumet Basic, Fisher Scientific). For the other geochemical analyses, samples were dried, sieved through a 2 mm mesh, and ground using a ball mill (Spex

Industries, Inc.). Total C and N levels were determined by analyzing 0.25-0.5 g with an elemental analyzer (CE Instruments elemental analyzer).

Metals and other relevant elements were determined by ICP-AES analysis for both digestible and water-extractable fractions of all samples. Soil samples to be analyzed for total metals were digested with a double acid, peroxide, hot-block digest, according to E.P.A. Method 3050B, modified to ¼ volume (Edgell 1988). Water extractable metals were determined by combining 1 g of sieved, unground sample with 40 ml of milli-Q water. The resulting slurries were mixed on a shaker table for 12 h and cleared by centrifugation for 20 min at 3500 rpm. Supernatants were then filtered through 0.45 µm membrane and preserved with 80 µl HNO₃. Analytes, including Cd, Cu, Fe, Pb, Zn, and P were quantified by ICP-AES of soil digests and extracts using E.P.A. Method 200.7 (Jarrell-Ash ICP-AES 800 instrument) (Martin et al. 1994). The QA/QC results are summarized in Appendix A.

Statistical Analysis

Treatment differences in plant biomass were detected with a two-way analysis of variance (ANOVA, $\alpha=0.05$), followed by Tukey's tests for multiple comparisons (SPSS 10.0.5). Plant tissue metals differences were compared by multivariate analysis of covariance (MANCOVA), using tissue biomass as a covariate and least squares differences of estimated marginal means for post-hoc comparisons. Plant phosphorus concentration and plant phosphorus content were analyzed with analysis of covariance (ANCOVA), using plant biomass as a

covariate and least squares differences of estimated marginal means for post-hoc comparisons.

RESULTS

Plant Growth Effects

Total plant biomass was significantly affected by both AM source and MC source (2-way ANOVA, AM: $F_{2,80}=4.935$, $P=0.010$; MC: $F_{2,80}=4.773$, $P=0.011$). The interaction term was not statistically significant ($F_{4,80}= 1.144$, $P=0.342$), but the observed power for the interaction test was very low (power=0.344). The AM from metals-contaminated soil resulted in significantly greater total plant biomass than plants without AM inoculation and those with AM from uncontaminated soil (Figure 1), except for plants treated with MC from the metals-contaminated soil. While the MC treatments did not improve plant growth over controls, the MC inoculum from metals-contaminated soil reduced total biomass of plants inoculated with AM from the metals-contaminated soils (compared to the same mycorrhizal treatment with no MC and MC from uncontaminated soil).

Similar patterns were observed when analyzing root biomass and shoot biomass separately. There were no significant differences between AM and MC treatments for biomass allocation in terms of root-mass-ratio (2-way ANOVA, AM: $F_{2,80}=0.300$, $P=0.741$; MC: $F_{2,80}=0.113$, $P=0.894$, AM x MC: $F_{4,80}=0.050$, $P=0.995$).

Mycorrhizal colonization

Mycorrhizal colonization levels were low across all mycorrhizal treatments. Precise colonization rates could not be determined because many mycorrhizal structures were observed, but not countable because they appeared to be degrading and/or were not clearly attached to hyphae. Conservative figures, quantifying only clear, intact AM structures revealed the following average colonization rates: non-mycorrhizal treatment had 0% colonization, the AM from uncontaminated soil had 2% colonization, the AM from a metals-contaminated site had 7% colonization, although the plants with AM from the metals-contaminated site grown with MC from the metals-contaminated site had only 2% colonization.

Plant Tissue Analysis

MANCOVA indicated that neither AM nor MC significantly affected overall metal concentrations (Cd, Cu, Fe, Pb, Zn) in root and shoot tissues (Table 2). While the overall effect was not statistically significant, the subsequent univariate analyses revealed that both AM treatments resulted in significantly higher Zn concentrations in roots than the non-mycorrhizal plants (Table 2). This pattern was also significant for overall plant Zn content (univariate ANOVA, $F_{2,9}=4.627$, $P=0.042$). There was a mildly significant effect of AM on shoot Pb concentration in which both mycorrhizal inoculum sources resulted in higher shoot Pb levels than was observed in their non-mycorrhizal counterparts. MC source had a mildly significant effect on shoot Cd concentration, with the MC from metals-

contaminated soil resulting in higher shoot-Cd concentrations than MC from uncontaminated soil and the sterilized MC control. There was no statistically significant difference between AM or MC treatments in root-to-shoot ratios of metals concentrations (AM: $F_{2,10}=1.177$, $P=0.389$; MC: $F_{2,13}=1.362$, $P=0.273$).

AM inoculum source significantly affected P concentration in shoots ($F_{2,10}=4.420$, $P=0.042$), but not in roots ($F_{2,9}=2.485$, $P=0.138$). Total P content of plants was significantly altered by AM treatment ($F_{2,9}=9.967$, $P=0.005$), with the AM from metals-contaminated soil resulting in significantly greater P than the AM from uncontaminated soils and the non-mycorrhizal controls (Figure 2). The MC treatments did not significantly affect P concentrations or contents of plants.

DISCUSSION

In this study, both AM and MC affected plant biomass of the relatively metal-resistant *D. cespitosa* growing in mine tailings, and in both cases the inoculum source determined plant biomass. The effects of AM ranged from neutral to positive, depending on the source of inoculum. Biomass of plants growing with the uncontaminated soil AM was not statistically different than the non-mycorrhizal control plants, while AM from metals-contaminated soil increased plant biomass by a mean of 67%. The only difference caused by MC inoculation was a negative effect, in which the MC from metals-contaminated soil eliminated the positive effect of the AM from metals-contaminated soil.

While the source of fungal inoculum may not be very important in some situations (Van der Heijden and Kuyper 2001), it may be important in extreme

conditions, such as highly metals-contaminated environments (Shetty et al. 1995, Leyval et al. 1997, Meharg and Cairney 2000). Some studies have reported that AM source does not improve plant growth in moderately metals-contaminated soils, but the effect could not be adequately assessed in extremely harsh soils because all plants grew poorly or died (Shetty et al. 1994, Enkhtuya et al. 2001). Our research allows examination of AM source effects on plants growing directly in metals-contaminated mine tailings because the plant host is naturally resistant to acidic, metal-elevated conditions. The increased plant growth with the AM community from metals-contaminated soil indicates that AM source is important for certain plant species growing in harsh conditions of acidity and elevated metal levels, although it is not clear whether the effect was due to adaptations of individual fungal species or differences in AM fungal species present.

This research also examined the nature of interactions between AM fungi and other rhizosphere microorganisms. While no effect of MC source was observed when combined with no AM and uncontaminated soil AM, the MC from the metals-contaminated soil actually reduced plant biomass, relative to no MC and uncontaminated soil MC, when combined with AM from metals-contaminated soil. Likewise, Shetty et al. (1994) saw no growth improvement of mycorrhizal plants in contaminated soil with additions of microbial soil suspensions from contaminated or uncontaminated sources. The MC from the metals-contaminated soil reduced AM colonization levels to 2%, from 7% observed with no MC and MC from uncontaminated soil, which corresponded with the reduction in plant biomass in the presence of MC from contaminated soil. The negative

effects of the MC from the metals-contaminated environment may be due to the presence of a pathogenic microorganism in the microbial suspension that negatively affected the plant directly by inhibiting growth, or indirectly by inhibiting mycorrhizal establishment. The fact that the negative effects of metals-contaminated soil MC were only observed when plants were grown with AM from metals-contaminated soil, and that AM colonization rates of plants grown with AM from metals-contaminated soil decreased when MC from metals-contaminated soil was added, suggests that the negative effects of metals-contaminated soil MC is via inhibition of mycorrhizal colonization.

AM-enhanced plant growth in metals-contaminated soils may be due to metal resistance conferred to the plant through reduced plant metal toxicity, or due to general mycorrhizae benefits, such as improved nutrient uptake (Meharg and Cairney 2000). Our research showed plants with AM from metals-contaminated soil had dramatically increased biomass, coupled with increases in plant tissue P. There were no corresponding significant decreases in plant tissue metal levels. On the contrary, tissue Zn levels increased in mycorrhizal plants, as has been observed in other studies (Shetty et al. 1994, Leyval et al. 1997), suggesting that benefits of AM from metals-contaminated soil is not due to decreased metal uptake. The biomass, P, and metals patterns in plant tissues presented here indicate that, while AM from metals-contaminated soil show a greater resistance to elevated metals than the uncontaminated soil AM community, the plant growth effect is due to fungal resistance that allows AM to maintain normal mycorrhizal functions under these harsh conditions. Benefits to

the plant appeared to be general mycorrhizal effects, notably P uptake, rather than reduced metal toxicity that would be reflected in decreased plant tissue metal levels. These findings support Meharg and Cairney's (2000) estimation that in highly metalliferous conditions the mycorrhizal association performs its usual ecological role, but does not confer enhanced metal resistance to the host.

Findings of this study hold promise for the difficult task of revegetating severely metals-contaminated and mine-disturbed systems. This study demonstrates that AM can improve plant growth in metals-contaminated mine wastes, but that the effect may depend on the source of the AM inoculum. While many new AM inoculum products are available to restoration planners, they should be screened for efficacy in the disturbed soil being restored. Even if efforts are made to provide metals-resistant AM communities, loss of environmental acclimation or adaptation can occur during culturing of bulk inoculum (Enkhtuya et al. 2000). Effects observed in this study may provide insight to improving revegetation success, but these findings should be tested in the field and with a variety of plant species and growth substrates. This research improves our understanding of soil ecology in severely disturbed environments and may enhance our ability to reintroduce soil biological components to create functional restored ecosystems.

ACKNOWLEDGEMENTS

This work was supported by the NSF-funded Training Within Environmental Biology program at the University of Montana (NSF-GRT #9553611 to P. Kukuk, C. Brewer, and F. Allendorf) and the Montana Agricultural Experiment Station. Thanks to the Murdock Environmental Biogeochemistry Lab for analytical assistance, and Bob Wintergerst of the Deerlodge National Forest for assistance in selecting and obtaining tailings materials.

REFERENCES

- Allen, M.F. 1989. Mycorrhizae and rehabilitation of disturbed arid soils: processes and practices. *Arid Soil Research* 3:229-241.
- Amaranthus, M.P., E. Cazares, and D.A. Perry. 1999. The role of soil microorganisms in restoration. In *Proceedings: Pacific Northwest forest and rangeland soil organism symposium*. R.T. Meurisse, W.G. Ypsilantis, and C. Seybold, eds., pp. 179-189, General Technical Report PNW-GTR-461. USDA Forest Service Pacific Northwest Research Station, Portland, Oregon.
- Bååth, E., M. Diaz-Ravina, Å. Frostegård, and C.D. Campbell. 1998. Effect of metal-rich sludge amendments on the soil microbial community. *Applied and Environmental Microbiology* 64:238-245.
- Brookes, P.C., S.P. McGrath, and C. Heijnen. 1986. Metal residues in soils previously treated with sewage-sludge and their effects on growth and nitrogen fixation by blue-green algae. *Soil Biology & Biochemistry* 18:345-353.
- Brookes, P.C. and S.P. McGrath. 1984. Effects of metal toxicity on the size of the soil microbial biomass. *Journal of Soil Science* 35:341-346.
- Chandler, K. and P.C. Brookes. 1991. Effects of heavy metals from past application of sewage sludge on microbial biomass and organic matter accumulation in a sandy loam and silty loam U.K. soil. *Soil Biology & Biochemistry* 23:927-932.
- Cuenca, G., Z. De Andrade, and E. Meneses. 2001. The presence of aluminum in arbuscular mycorrhizas of *Clusia multiflora* exposed to increased acidity. *Plant and Soil* 231:233-241.
- Danielson, R.M. 1985. Mycorrhizae and reclamation of stressed terrestrial environments. In *Soil reclamation processes- Microbiological analyses and applications*. R.L. Tate and D.A. Klein, eds., pp. 173-201, Marcel Dekker, Inc. New York.
- Dehn, B. and H. Schüepp. 1989. Influence of VA mycorrhizae on the uptake and distribution of heavy metals in plants. *Agriculture, Ecosystems and Environment* 29:79-83.
- Del Val, C., J.M. Barea, and C. Azcon-Aguilar. 1999. Diversity of arbuscular mycorrhizal fungus populations in heavy-metal-contaminated soils. *Applied and Environmental Microbiology* 65:718-723.

Dobler, R., M. Saner, and R. Bachofen. 2000. Population changes of soil microbial communities induced by hydrocarbon and heavy metal contamination. *Bioremediation Journal* 4:41-56.

Doelman, P., V. Jensen, A. Kjoeller, and L.H. Soerenson. 1986. Resistance of soil microbial communities to heavy metals. *Microbial Communities in Soil* 33:369-384.

Edgell, K. 1988. USEPA method study 37-SW-846 method 3050 acid digestion of sediments, sludges, and soils. EPA contract No. 68-03-3254, November 1988.

Enkhtuya, B., J. Rydlova, and M. Vosatka. 2000. Effectiveness of indigenous and non-indigenous isolates of arbuscular mycorrhizal fungi in soils from degraded ecosystems and man-made habitats. *Applied Soil Ecology* 14:201-211.

Galli, U., H. Schuepp, and C. Brunold. 1994. Heavy metal binding by mycorrhizal fungi. *Physiologia Plantarum* 92:364-368.

Garbaye, J. 1994. Helper bacteria: a new dimension to mycorrhizal symbiosis. *New Phytologist* 128:197-210

Gildon, A. and P.B. Tinker. 1983. Interactions of vesicular-arbuscular mycorrhizal infection and heavy metals in plants: I. The effects of heavy metals on the development of vesicular-arbuscular mycorrhizas. *New Phytologist* 95:247-261.

Hetrick, B.A.D., G.W.T. Wilson, and D.A.H. Figge. 1994. The influence of mycorrhizal symbiosis and fertilizer amendments on establishment of vegetation in heavy metal mine spoil. *Environmental Pollution* 86:171-179.

Hodge, A. 2000. Microbial ecology of the arbuscular mycorrhiza. *FEMS Microbiology Ecology* 32:91-96.

Jasper, D.A., L.K. Abbott, and A.D. Robson. 1989. The loss of VA mycorrhizal infectivity during bauxite mining may limit the growth of *Acacia pulchella* R.Br. *Australian Journal of Botany* 37:33-42.

Joner, E.J., R. Briones, and C. Leyval. 2000. Metal-binding capacity of arbuscular mycorrhizal mycelium. *Plant and Soil* 226:227-234.

Kelly, J.J. and R.L. Tate, III. 1998. Effects of heavy metal contamination and remediation on soil microbial communities in the vicinity of a zinc smelter. *Journal of Environmental Quality* 27:609-617.

Knight, B.P., S.P. McGrath, and A.M. Chaudri. 1997. Biomass carbon measurements and substrate utilization patterns of microbial populations from

soils amended with cadmium, copper, or zinc. *Applied and Environmental Microbiology* 63:39-43.

Leyval, C., K. Turnau, and K. Haselwandter. 1997. Effect of heavy metal pollution on mycorrhizal colonization and function: Physiological, ecological and applied aspects. *Mycorrhiza* 7:139-153.

Lorenz, S.E., S.P. McGrath, and K.E. Giller. 1992. Assessment of free-living nitrogen fixation activity as a biological indicator of heavy metal toxicity in soil. *Soil Biology & Biochemistry* 24:601-606.

Martin, T.D., C.A. Brockhoff, J.T. Creed, and EMMC Methods Workgroup. 1994. Method 200.7, Revision 4.4, Determination of Metals and trace elements in water and wastes by inductively coupled plasma atomic emission spectrometry. In *Methods for the determination of metals in environmental samples*. EPA/600/R-94/111. Environmental Protection Agency, Cincinnati, OH.

McGonigle, T.P., M.H. Miller, D.G. Evans, G.L. Fairchild, and J.A. Swan. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist* 115:495-501.

Meharg, A.A. and J.W.G. Cairney. 2000. Co-evolution of mycorrhizal symbionts and their hosts to metal-contaminated environments. *Advances in Ecological Research* 30:69-112.

Moynahan, O.S., C.A. Zabinski, and J.E. Gannon. 2002. Microbial community structure and carbon-utilization diversity in a mine tailings revegetation study. *Restoration Ecology* 10:77-87.

Munshower, F.F. 1994. *Practical Handbook of Disturbed Land Revegetation*, Lewis Publishers, Inc. Boca Raton, Florida. 140pp.

Norland, M.R. 1993. *Soil factors affecting mycorrhizal use in surface mine reclamation*. Information circular 9345, United States Bureau of Mines, Pittsburgh, PA.

Noyd, R.K., F.L. Pflieger, M.R. Norland, and M.J. Sadowsky. 1995. Native prairie grasses and microbial community responses to reclamation of taconite iron ore tailing. *Canadian Journal of Botany* 73:1645-1654.

Noyd, R.K., F.L. Pflieger, and M.R. Norland. 1996. Field responses to added organic matter, arbuscular mycorrhizal fungi, and fertilizer in reclamation of taconite iron ore tailing. *Plant and Soil* 179:89-97.

Pennanen, T., Å. Frostegård, H. Fritze, and E. Bååth. 1996. Phospholipid fatty acid composition and heavy metal tolerance of soil microbial communities along

two heavy metal-polluted gradients in coniferous forests. *Applied and Environmental Microbiology* 62:420-428.

Pfleger, F.L., E.L. Stewart, and R.K. Noyd. 1994. Role of VAM fungi in mine land revegetation. In *Mycorrhizae and Plant Health*. F.L. Pfleger and R.G. Linderman, eds., pp. 47-82, The American Phytopathological Society, St. Paul, Minnesota.

Phillips, J.M. and D.S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* 55:158-161.

Requena, N., I. Jimenez, M. Toro, and J.M. Barea. 1997. Interactions between plant-growth-promoting rhizobacteria (PGPR), arbuscular mycorrhizal fungi and *Rhizobium* spp. in the rhizosphere of *Anthyllis cytisoides*, a model legume for revegetation in mediterranean semi-arid ecosystems. *New Phytologist* 136:667-677.

Shetty, K.G., B.A.D. Hetrick, D.A.H. Figge, and A.P. Schwab. 1994. Effects of mycorrhizae and other soil microbes on revegetation of heavy metal contaminated mine spoil. *Environmental Pollution* 86:181-188

Shetty, K.G., B.A.D. Hetrick, and A.P. Schwab. 1995. Effects of mycorrhizae and fertilizer amendments on zinc tolerance of plants. *Environmental Pollution* 88:307-314.

St. John, T.V. 1995. Specially-modified land imprinter inoculates soil with mycorrhizal fungi (California). *Restoration & Management Notes* 14, 84-85.

St. John, T.V. 1998. Mycorrhizal inoculation in habitat restoration. *Land and Water* September/October:17-19.

Tordoff, G.M., A.J.M. Baker, and A.J. Willis. 2000. Current approaches to the revegetation and reclamation of metalliferous mine wastes. *Chemosphere* 41:219-228.

Van der Heijden, M.G.A., J.N. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A. Wiemken, and I.R. Sanders. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability, and productivity. *Nature* 396:69-72.

Van der Heijden, E.W. and T.W. Kuyper. 2001. Does origin of mycorrhizal plant or mycorrhizal fungus influence effectiveness of the mycorrhizal symbiosis? *Plant and Soil* 230:161-174.

Visser, S. 1985. Management of microbial processes in surface mined land reclamation in western Canada. In *Soil reclamation processes- Microbiological analyses and applications*. R.L. Tate and D.A. Klein, eds., pp. 203-241, Marcel Dekker, Inc. New York.

Von Frenckell-Insam, B.A.K. and T.C. Hutchinson. 1993. Occurrence of heavy metal tolerance and co-tolerance in *Deschampsia cespitosa* (L.) Beauv. from European and Canadian populations. *Canadian Journal Of Botany* 125:555-564.

Weissenhorn, I., A. Glashoff, C. Leyval, and J. Berthelin. 1994. Differential tolerance to Cd and Zn of arbuscular mycorrhizal (AM) fungal spores isolated from heavy metal-polluted and unpolluted soils. *Plant and Soil* 167:189-196.

Table 1. Geochemical properties of mine tailings growth substrate. Mean values (n=3) are mg kg⁻¹, unless otherwise noted.

	pH	N	C	As	Cd	Cu	Fe	Pb	Zn	P
Total Analytes*	3.42	<0.08%	<0.75%	244	3	118	29,290	3158	370	478
Water-extractable Analytes				0.03	0.6	6.1	4.3	0.8	73	0.6

*Total analytes detected with ICP-AES represent total digestible fractions

Table 2. Metal concentrations in *Deschampsia cespitosa* root and shoot plant tissues cultivated in mine tailings inoculated with AM and MC sources.

Factor Effect	Overall Significance of MANCOVA		Inoculum Treatment	Mean Metal Concentration (mg kg ⁻¹)				
	F	P-value		Cd	Cu	Fe	Pb	Zn
AM source on root concentrations	1.364	0.317	No AM	23a	127a	11742a	1025a	344a
			Metals AM	22a	180a	11295a	1153a	607b
			Uncont. AM	27a	262a	9952a	1204a	618b
AM source on Shoot concentrations	1.543	0.236	No AM	4a	14a	726a	67a	244a
			Metals AM	4a	15a	1174a	112b	290a
			Uncont. AM	5a	18a	1236a	120b	380a
MC source on root concentration	0.850	0.592	No MC	22a	180a	11296a	1154a	608a
			Metals MC	41a	226a	13501a	1241a	628a
			Uncont. MC	22a	195a	11210a	1000a	542a
MC source on Shoot concentrations	1.884	0.125	No MC	4a	15a	1174a	112a	290a
			Metals MC	11b	17a	821a	82a	477a
			Uncont. MC	5a	12a	1083a	104a	205a

Test statistics are according to MANCOVA using Wilks' lambda. Univariate ANCOVAs comparing inoculum treatment effects were subsequently conducted on plant tissue metal concentrations. Letters signify mean metal concentrations (5 replicates) that are not significantly different according to LSD multiple comparisons ($P=0.10$).

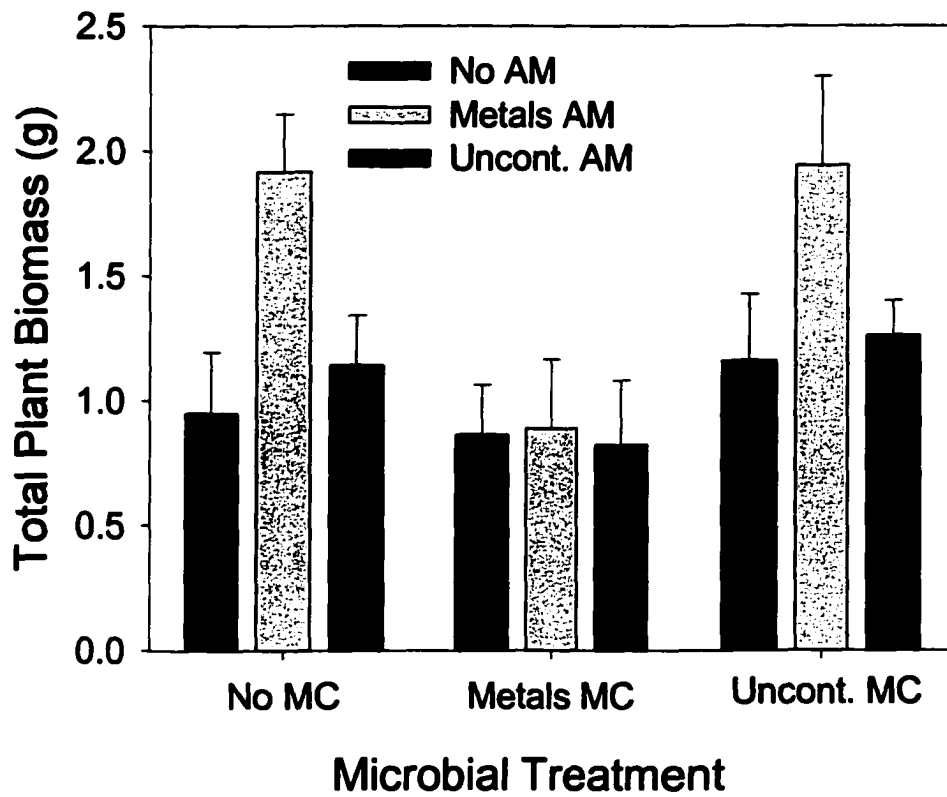


Figure 1. Total biomass of plants grown with all combinations of AM and MC inoculum treatments. Error bars represent standard error of the mean.

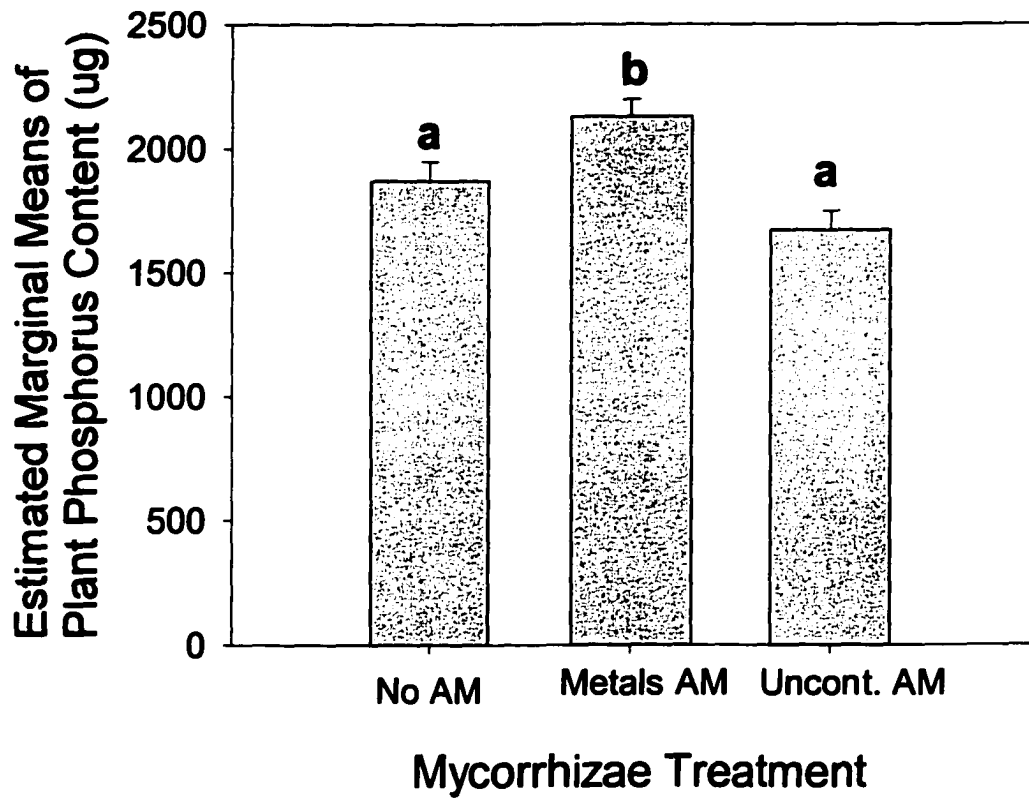


Figure 2. Estimated marginal means of total phosphorus content in plants grown with different AM inoculum sources. Error bars represent standard error of the mean. Letters above bars represent statistically significant different means.

CHAPTER 3

Mycorrhizal Dependency of *Deschampsia cespitosa* Across a pH- and Metals-Induced Stress Gradient

ABSTRACT

Lessons from plant community ecology, such as the importance of soil microorganisms and positive interactions, can guide strategies for rehabilitation of disturbed lands. The experiment reported in this chapter examines how arbuscular mycorrhizae (AM) help plants grow in mine wastes across a gradient of pH and metals concentrations. *Deschampsia cespitosa* (tufted hairgrass) was grown in mine tailings in a complete factorial design, testing effects of AM inoculum sources and lime (CaCO_3) addition. The sources of AM were 1) sterilized inoculum, 2) inoculum from a metals-contaminated site, and 3) inoculum from an uncontaminated site. The AM treatments were tested in unamended mine tailings and tailings amended with low, medium and high lime levels. Lime additions significantly increased pH (from pH 3.5 in the non-limed tailings to 7.4 in the high lime treatment) and decreased soil metal levels, such that nonmycorrhizal plants were smallest in the non-limed tailings and largest with the medium lime treatment. Overall, AM from metals-contaminated soil increased biomass over the nonmycorrhizal treatment by 24%, while the AM from uncontaminated soil was not different from either of the other AM treatments, although the magnitude of AM effects varied with lime treatment. Calculated plant-perceived stress was highest in the non-limed tailings and lowest in the medium lime treatment. Mycorrhizal dependency, which reflects the proportion of mycorrhizal plant biomass that can be attributed to the AM association, varied between lime levels. Differences in mycorrhizal dependency mirrored patterns of plant-perceived stress, except in the non-limed tailings, where there was no

significant effect of AM. Shifts in plant mycorrhizal dependency along this stress gradient may be due to changing cost:benefit ratios, as the symbiosis responds to abiotic factors related to pH and soil geochemistry. This study indicates that AM symbioses can promote plant growth in mine wastes, and that the nature of the symbiosis will shift depending on AM source and environmental stress level.

INTRODUCTION

Two research areas that have received increased attention in recent plant ecology literature may be particularly important to the development of restoration practices for disturbed lands. The first is the effects of soil microorganisms on plant community composition and dynamics. The second is the role of interspecific positive interactions between plants in stressful environments. This study examines the changing nature of plant-microbe interactions across an abiotic stress gradient.

Plant community dynamics often depend on associations with soil microorganisms (Bolton et al. 1992, Bever et al. 1997, Van der Heijden et al. 1998). Soil and rhizosphere microbial communities that rely on plant carbon inputs can provide a variety of benefits to individual plants and plant communities (Bolton et al. 1992, Martin et al. 2001). Among the best-documented plant-microbe mutualisms are associations between plant roots and mycorrhizal fungi, such as arbuscular mycorrhizae (AM). In exchange for fixed carbon from the plant, the fungal symbiont can provide the plant with increased nutrient uptake (especially phosphorus), improved water status, pathogen protection, and

possibly enhanced metal-resistance (Newsham et al. 1995, Martin et al. 2001). The magnitude of the benefit conferred to the plant, termed mycorrhizal dependency, may vary with each combination of plant host, fungal symbiont, and environmental conditions (Plenchette et al. 1983, Johnson et al. 1997).

The abundance and diversity of plant species are determined by a balance of ecological processes that may shift with different environmental gradients and successional stages (Connell and Slatyer 1977, Walker and Chapin 1987, Bertness and Callaway 1994, Stachowicz 2001). While facilitation and competition co-occur in plant communities, their relative importance in structuring communities may shift under different conditions (Bertness and Callaway 1994, Callaway 1997, Brooker and Callaghan 1998). Bertness and Callaway (1994) proposed a model in which competition is more important in communities in low stress environments, while positive interactions are more common in communities with high physical stress and/or high consumer pressure. The balance between plant competition and facilitation may vary with environmental severity, and the importance of positive interactions probably has been underestimated since, historically, most ecological research has been conducted in the context of relatively benign conditions (Brooker and Callaghan 1998, Mulder et al. 2001). Because disturbed lands present a variety of co-occurring stressors to plant communities, it may be especially important to include facilitative interactions as part of ecological restoration strategies (Pyke and Archer 1991, Young et al. 2001).

While these studies focused on positive interactions between plants, the undeniable dependence of plant communities on soil microbes suggests that positive interactions with microorganisms also may be especially important in abiotically stressful environments (Stachowicz 2001). Mycorrhizal associations can have positive, neutral, or negative effects on plants, depending on the balance of costs and benefits to the host (Smith and Smith 1996, Johnson et al. 1997). Our current understanding of abiotic influences on mycorrhizal dependency has come primarily from studies of mycorrhizal plants under varying nutrient status and light levels. Fertilization lessens plant needs for AM-obtained nutrients, which can result in either decreased AM colonization, or decreased mycorrhizal benefit, hence lower mycorrhizal dependency (Johnson et al. 1997). Mycorrhizal benefit also can decrease under low light conditions, because the host plant has less excess photosynthate to allocate to the AM fungus (Bethlenfalvay and Pacovsky 1983, Tester and Smith 1985). While nutrients and light directly affect the cost:benefit ratio of the symbiosis, AM affect plant growth under the influence of a variety of other environmental stressors, which may affect AM dependence differently. Mycorrhizae can increase plant growth over nonmycorrhizal plants under strenuous abiotic conditions such as water stress (Stahl et al. 1998, Mathur and Vyas 2000), salinity (Ruiz-Lozano and Azcón 2000), acid pH (Clark et al. 1999) and elevated metals (Leyval et al. 1997, Meharg and Cairney 2000), but we know very little about how mycorrhizal dependency shifts across varying levels of these stressors.

In addition to environmental conditions, mycorrhizal dependency is determined by plant and fungal symbiont characteristics (Johnson et al. 1997, Van der Heijden et al. 2001). Host plant response to the same fungal isolate can vary between different plant species (Plenchette et al. 1983) or genotypes of the same species (Bryla and Koide 1998). Mycorrhizal dependency of a single plant species can vary with different individual fungal species (Mathur and Vyas 2000), isolates of the same species of fungus (Gildon and Tinker 1983), and with mycorrhizal communities (Van der Heijden et al. 1998, Chapter 2). Mycorrhizal fungal community composition can shift with changes in the soil environment, such as fertilization or disturbance (Johnson 1993, Del Val et al. 1999). Other research has shown that AM fungi adapted to high stress conditions, such as metal contamination, can result in greater plant growth response in metals-elevated soils (Gildon and Tinker 1983, Leyval et al. 1997, Meharg and Cairney 2000), although the mechanism for this effect is yet unclear. Because disturbed environments often are characterized by unique abiotic characteristics, it is important to understand effects of mycorrhizal source on host plant growth with different levels of abiotic stressors.

Among the most challenging disturbed systems in need of restoration are vast quantities of mine waste materials scattered throughout mountains and river systems of the world (Dudka and Adriano 1997, Tordoff et al. 2000). Hardrock mine wastes, often characterized by extreme acidity, elevated metal levels, and low fertility, can be hazardous to human and general ecological health. Strategies to reclaim acidic mine wastes, such as neutralization by liming,

organic matter incorporation, and seeding can improve revegetation, but more effective and reliable strategies are needed (Tordoff et al. 2000). An understanding of positive plant-microbe interactions across pH and metals gradients may improve our ability to re-establish functional plant communities on these severely disturbed lands.

Our research examines the importance of positive plant-microbe interactions across an abiotic stress gradient. The stress gradient was created by amending acidic, metal-rich mine tailings with lime, which neutralized acidity and reduced metal-availability. The objectives of this study were i) to investigate AM inoculum source effects on plant growth in mine wastes, and ii) to examine mycorrhizal dependency of plants as pH- and metals-induced stress levels, manipulated by lime addition, varied. We hypothesize that mycorrhizal dependency will increase as plant-perceived stress increases, and that AM from the metals-contaminated soil will be more beneficial than AM from uncontaminated soil.

METHODS

Experimental Design

Effects of lime amendment and AM source on *Deschampsia cespitosa* L. (Beauv.) (tufted hairgrass) were examined with a greenhouse experiment utilizing a two-way complete factorial (4 x 3) randomized design. Lime effects were assessed at four levels: no lime, low lime addition, medium lime addition, and high lime addition. Mycorrhizal source effects were measured across three

treatments: autoclaved inoculum (nonmycorrhizal), inoculum from a metals-contaminated site, and inoculum from an uncontaminated site. This design (4 lime levels x 3 AM treatments) resulted in 12 treatment combinations, with 15 replicates each.

Mine tailings, the milled material remaining after ore is processed, were collected from an abandoned tailings deposit in southwestern Montana (46°20'N, 112°13'W), homogenized thoroughly, and sieved through a 5 mm mesh. Response of *D. cespitosa*, was tested in unamended tailings materials with pH of 3.5, and tailings amended with 0.2%, 0.6%, or 3.0% lime (CaCO₃, % dry wt) (Table 1). *Deschampsia cespitosa* was used in this experiment because it is tolerant of acidic and elevated-metal conditions (Von Frenckell-Insam & Hutchinson 1993, Munshower 1994).

Inoculation with AM Fungal Communities

The metals-contaminated inoculum was collected from a mine tailings revegetation project in southwestern Montana that had neutralized tailings, high metal levels (Table 1), and mycorrhizal plants. The uncontaminated inoculum was from a bluebunch wheatgrass (*Pseudoroegneria spicata*) and Idaho fescue (*Festuca idahoensis*) dominated grassland in western Montana with no metal contamination (Table 1), and mycorrhizal plants.

Mycorrhizal inoculation was achieved by combining soil from each inoculum source, sterilized soil from the alternate inoculum source, and nonmycorrhizal tailings growth substrate, to minimize physical and chemical

differences between inocula. The metals-contaminated soil AM treatment contained 10 ml metals-contaminated soil, 10 ml sterilized uncontaminated soil, and 10 ml tailings. The uncontaminated soil AM treatment contained 10 ml uncontaminated soil, 10 ml sterilized metals-contaminated soil, and 10 ml tailings. The nonmycorrhizal treatment contained 10 ml sterilized metals-contaminated soil, 10 ml sterilized uncontaminated soil, and 10 ml tailings. The sterilized soils were autoclaved twice at 121° C for 15 min, with 24 h between sessions in the autoclave. The inoculum mixtures were added to pots and covered with 1 cm of sterilized silica sand.

To minimize differences in the non-mycorrhizal microbial community, a microbial sievate prepared from both inoculum sources was added to all pots. The microbial sievate was prepared by passing a soil slurry (1:1:8 by weight, metals-contaminated soil:uncontaminated soil:deionized water) through a series of filters (250µm, 25µm, 11µm). The filtration excludes mycorrhizal propagules, while soil bacteria, nonmycorrhizal spores, and other microorganisms pass through the filters. Twenty-five ml of the appropriate microbial suspension was added to each pot.

Plant Cultivation and Harvest

Deschampsia cespitosa was grown from seed in 6 cm dia x 25 cm deep pots, and germinants were thinned to a single plant per pot. Plants were watered to field capacity every 2-3 days, and nutrients were added every 2 weeks with 30 ml of ¼-strength modified Hoagland's solution (minus P) during the first 3 months

of growth, and 30 ml of ½-strength Hoagland's solution (low P) during the last 2 months of growth. Pots were randomly arranged on the greenhouse bench and moved every week to minimize microsite effects. After 5 months of growth, plants were harvested and washed thoroughly. Roots and shoots were dried for 72 h at 60°C, and biomass was recorded.

Mycorrhizal Colonization Measurements

A subsample of roots was collected from five replicates per treatment after biomass measurements to determine mycorrhizal colonization levels. Plant roots were cleared in 2.5% KOH for 48 h, acidified in 3% HCl for 12 h, and stained with Trypan blue for 12 h (variation of Phillips and Hayman 1970). The percent of root colonized by mycorrhizal fungi was determined by the magnified intersections method (McGonigle et al. 1990).

Soil Analysis

For pH measurements, 10 g of soil was combined with 10 ml of deionized water, allowed to equilibrate for 5 min, and measured with a pH probe (Accumet Basic, Fisher Scientific). For the other geochemical analyses, samples were dried, sieved through a 2 mm mesh, and ground using a ball mill (Spex Industries, Inc.). Total C and N levels were determined by analyzing 0.25-0.5 g with an elemental analyzer (CE Instruments elemental analyzer).

Concentrations of metals and other ecologically relevant elements were determined by inductively coupled plasma atomic emission spectrometry (ICP-

AES) analysis for both digestible and water-extractable fractions of all samples. Soil samples to be analyzed for total recoverable metals were digested with a double acid, peroxide, hot-block digest, according to E.P.A. Method 3050B, modified to ¼ volume (Edgell 1988). Water-extractable metals were determined by combining 1 g of sieved, unground sample with 40 ml of milli-Q water. Slurries were mixed on a shaker table for 12 h and cleared by centrifugation for 20 min at 3500 rpm. The resulting supernatants were filtered through 0.45 µm membrane and preserved with 80 µl HNO₃. Analytes, including Cd, Cu, Fe, Pb, Zn, and P were quantified by ICP-AES analysis of soil extracts and digests using E.P.A. Method 200.7 (Jarrell-Ash ICP-AES 800 instrument) (Martin et al. 1994). The QA/QC results are summarized in Appendix A.

Calculations and Statistical Analysis

Plant-perceived stress relative to this experiment was calculated for each soil treatment as the mean biomass of nonmycorrhizal plants growing under optimal conditions (medium lime addition) and the mean biomass of nonmycorrhizal plants at each lime level. Mycorrhizal dependency was calculated as described by Plenchette et al. (1983):

$$\text{Mycorrhizal dependency} = 100 * ((\text{mycorrhizal plant dry mass} - \text{nonmycorrhizal plant dry mass}) / \text{mycorrhizal plant dry mass}).$$

Plant biomass treatment differences were detected with a two-way analysis of variance (ANOVA, $\alpha=0.05$), followed by Tukey's test for multiple

comparisons of overall factor effects, and contrasts of AM treatments within each lime level, and lime levels within the nonmycorrhizal treatment (SPSS 10.0).

Treatment effects on AM colonization for all AM-inoculated plants were determined with two-way ANOVA, followed by Tukey's test for multiple comparisons, and contrasts comparing AM treatments within each lime level, and lime levels within the nonmycorrhizal AM treatment (SPSS 10.0).

Nonmycorrhizal control plants, all of which had 0% colonization, were omitted from analyses comparing treatment effects on AM colonization.

RESULTS

Soil pH and Plant Stress Gradient

Increased lime addition to tailings resulted in increased pH, and decreased metal levels to below the practical quantifiable limit (Table 1). The soil amendments resulted in statistically significant changes in plant biomass, such that the biomass increased with each increased lime addition, but decreased with the greatest lime addition (Figure 1). Plant-perceived abiotic stress, which estimates the stress levels of non-mycorrhizal plants in each lime treatment, was evaluated relative to the most favorable soil condition observed, and is depicted by the dotted line in Figure 1.

Mycorrhizal colonization

Mycorrhizal colonization levels were affected by AM source, but not significantly affected by lime level (Table 2; 2-way ANOVA, AM: $F_{1,33}=15.415$,

$P < 0.001$; Lime: $F_{3,33} = 2.245$, $P = 0.101$). The only significant differences in AM colonization levels were observed with the metals-contaminated AM treatment in the high limed tailings, which resulted in higher AM colonization levels than uncontaminated soil AM, and higher AM colonization than other lime treatments. All plants in the nonmycorrhizal treatment had 0% colonization levels in root samples.

Plant Growth Effects

Total plant biomass was significantly affected by both lime addition and AM inoculation (2-way ANOVA, Lime: $F_{3,140} = 16.737$, $P < 0.001$; AM: $F_{2,140} = 7.802$, $P = 0.001$). Averaged across AM source treatments, plants growing in non-limed tailings were significantly smaller than plants growing in any of the limed treatments. Within the nonmycorrhizal plants, plant biomass significantly increased with low and medium lime treatments, but decreased in the high lime treatment such that the high lime treatment was equivalent to the low lime treatment (Figure 1, $F_{3,140} = 5.917$, $P = 0.001$).

AM inoculation significantly increased plant biomass (Figure 2). Overall, AM inoculum from metals-contaminated soil resulted in 24% greater biomass than the nonmycorrhizal plants (Tukey's HSD: $P < 0.001$). Plants with uncontaminated soil AM had an intermediate mean biomass that was not significantly different than nonmycorrhizal plants (Tukey's HSD: $P = 0.311$), but was lower than plants with AM from metals-contaminated soils (Tukey's HSD: $P = 0.040$). Individual contrasts indicate that the degree to which plants benefited

from AM inoculation varied between lime treatments ($F_{8,140}=2.495$, $P=0.015$) (Figure 2).

Similar statistical differences were observed when treatment comparisons were based on shoot biomass (2-way ANOVA, Lime: $F_{3,140}=17.289$, $P<0.001$; AM: $F_{2,140}=6.246$, $P=0.003$) and root biomass (2-way ANOVA, Lime: $F_{3,140}=12.549$, $P<0.001$; AM: $F_{2,140}=8.870$, $P<0.001$) (Table 2). There was a statistically significant effect of AM treatment on root mass ratio, but not lime level (2-way ANOVA, Lime: $F_{3,140}=1.366$, $P=0.256$; AM: $F_{2,140}=4.536$, $P=0.012$) (Table 2). Metals-contaminated soil AM resulted in slightly greater root mass ratio than the uncontaminated soil AM (Tukey's HSD: $P=0.005$), and the nonmycorrhizal treatment yielded intermediate values that were not statistically different from either metals-contaminated AM (Tukey's HSD: $P=0.411$) or uncontaminated soil AM (Tukey's HSD: $P=0.162$).

Mycorrhizal Dependency and Plant Stress Trends

Mycorrhizal dependency reflects the proportion of mycorrhizal plant response that can be attributed to the mycorrhizal association, and is plotted on Figure 3 along with plant-perceived stress. Mycorrhizal dependency varied across lime treatments and was lowest in the relatively favorable conditions of the medium lime treatment (Figure 3), for which there were no significant differences between mycorrhizal plants and nonmycorrhizal plants (Figure 2). The pattern of lime effects on mycorrhizal dependency was similar between the two AM sources, although the mycorrhizal dependency was greater with the AM

from metals-contaminated soil (Figure 3). Plant mycorrhizal dependency mirrored plant-perceived stress across the three lime treatments. In the most stressful conditions of the non-limed tailings, mycorrhizal dependency decreased, illustrating the reduced benefit of AM in extremely stressful environments.

DISCUSSION

Lime treatments successfully decreased plant-perceived abiotic stress in mine tailings, although the improvement did not correlate directly with increased lime addition. The non-limed tailings presented the most stressful conditions, with the lowest pH and highest metal levels, resulting in the lowest mean plant biomass. Plant biomass increased with low and medium lime additions, which corresponded with increasing pH and decreasing metal levels. With high lime addition, however, pH increased moderately and metal levels were below detection, the same as the medium lime tailings treatment. Plant biomass decreased with high lime addition relative to the medium lime addition, possibly due to insolubility of essential nutrients at higher pH (Stevenson 1986), although most nutrient levels measured were below instrument detection levels for both treatments (Table 1).

Stress levels perceived by the fungal symbiont are more difficult to assess, but mycorrhizal colonization levels reflect fungal activity coupled with plant response. AM colonization levels were not significantly affected by lime treatment or AM source, except for the higher colonization of AM from metals-contaminated soil in the high lime treatment.

Ecological theory of positive interactions for plants predicts that the importance of mutualism will increase with increasingly stressful conditions (Bertness and Callaway 1994, Mulder et al. 2001). If models for plant-plant positive interactions hold true for plant-microbe interactions, mycorrhizal dependency may be positively correlated with stress. More specifically, mycorrhizal dependency would be highest in the non-limed tailings, moderate in the low-limed tailings, lowest in the medium-limed tailings, and moderate again in the high-limed tailings. Our study shows that mycorrhizal dependency trends with both AM sources mirror plant stress level, except in the extremely harsh conditions of the non-limed tailings. Previous research of plant-plant interactions under extremely stressful conditions indicates that above a certain threshold of stress the potential role of positive interactions can be overshadowed by near-lethal stress levels of individual plants (Bertness and Callaway 1994).

Shifts in AM plant growth effects under different abiotic conditions may be due to changes in the balance between AM costs and benefits to the plant (Smith and Smith 1996, Johnson et al. 1997). The lower-than-expected mycorrhizal dependency in the non-limed tailings suggests that the relative carbon costs are high and/or the AM benefits to the plant are low. AM colonization and fungal structures were equivalent to levels in other lime treatments, indicating that the fungus may be functioning similarly in the plant (Johnson et al. 1997). Extraradical hyphal function, however, could be inhibited by high metal concentrations and reduce benefits of the symbiosis to the host plant (Leyval et al. 1997, Meharg and Cairney 2000). Low pH and high metals can negatively

affect photosynthesis (Reddy and Prasad 1990, Barón et al. 1995, Lambers et al. 1998), which can result in reduced biomass, as was observed in this study for the nonmycorrhizal plants. This reduction in photosynthesis for growth, combined with equal fungal colonization, results in greater carbon cost per benefit gained from the fungus. Under stressful conditions plant roots may exude greater amounts of carbon (Anderson 1992), which generally leads to higher AM colonization levels, but also may represent a greater carbon loss from the plant.

In the low lime treatment, plants experience moderate stress with AM colonization levels equivalent to other soil treatments. Mycorrhizal dependency is highest under these conditions. This suggests that the benefits of mycorrhizae to the host plant are greatest, relative to the carbon costs of supporting the AM fungus.

The medium lime treatment represents the most benign conditions in the study, with neutralized acidity and metal levels below detection. Under these circumstances, the plant biomass was largest and roots were always colonized by AM. Although still below instrument detection limits, phosphorus would be most available for plant uptake at pH 7.1 (Stevenson 1986), so the fungus provides less of an advantage and represents a greater overall cost to the plant. In the medium lime treatment, plant biomass was not significantly different in the nonmycorrhizal plants than with either AM inoculum. The AM in these conditions may be at an inflection point, where the plant costs balance with fungal benefits, resulting in no net gain or loss in plant biomass.

Increased plant stress with the high lime treatment may be due to lower plant-available nutrient levels. The high lime addition provides an excess of CaCO_3 and raised the pH to 7.4, where phosphorus is likely to occur as insoluble CaPO_4 (Stevenson 1986). In this situation, AM would provide the important benefit of increased nutrient access, especially phosphorus, for plants. This possibility may explain the increased mycorrhizal dependency in the high lime treatment.

Plants can benefit from AM associations in high-stress environments, such as the mine wastes investigated in this study. The degree of benefit will vary with abiotic conditions, as well as with fungal and plant species. Prior research has shown that AM from metals-contaminated soils can provide greater benefit to plants than AM from uncontaminated soils (Gildon and Tinker 1983, Leyval et al 1997, Meharg and Cairney 2000). Alternatively, mycorrhizal communities from uncontaminated environments may be more diverse (Del Val et al. 1999), and diverse AM fungal communities may lead to more productive and diverse plant communities (Van der Heijden et al. 1998). If AM from metals-contaminated soils in this study were acclimated or adapted to specific metals contamination, we would expect it to be most effective in the non-limed and low-limed tailings, while the uncontaminated soil AM would be most effective in the medium and high lime treatments in which pH was neutralized and metals were below detection. In all cases of mycorrhizal dependency, however, AM from metals-contaminated soil was most effective. This suggests that the AM fungal community from metals-contaminated soil is actually more adapted to general stress conditions or

functions in a more plant-productive way, rather than possessing metals resistance.

While this study showed no AM benefit for *D. cespitosa* in benign growth substrate, effects might be different under field conditions without watering and fertilization, and for plant species with less inherent acid- and metals-resistance. Our findings provide insight to how plants function with increasing stress, and add to the growing evidence that microbial associations can be essential for plant success in disturbed environments. Including microbial symbioses in disturbed land restoration designs can provide an important advantage to revegetation.

This research provides evidence that, like plant-plant interactions, plant-AM fungal interactions may be more important to plant growth in increasingly stressful environments, except when extreme stress levels are detrimental to plant growth. Further, the data suggest that the reason for this pattern is a shift in cost:benefit ratio that changes with geochemical alterations in the soil. This study supports previous work indicating that carbon costs, balanced with nutrient benefits, determine AM function (Smith and Smith 1996, Johnson et al. 1997), but also demonstrates that the form of the costs and benefits can depend on the nature of the abiotic stress. Instead of just nutrient and light deficiencies, plants in this study were responding to changing levels of severe acidity, metal toxicity effects to plants and fungal symbionts, potential carbon losses from stress-induced exudation, and nutrient deficiencies indirectly caused by geochemical transformations. While correlative data and geochemical principles help explain potential mechanisms for AM function with increasing lime additions, interactions

between individual stressors and symbionts under different lime levels should be explored with more controlled experimentation. This research provides evidence that the importance of AM symbioses may grow with increasing abiotic stress, which expands our understanding of soil ecology and provides important information to guide restoration of disturbed environments.

ACKNOWLEDGEMENTS

This work was supported by the NSF-funded Training Within Environmental Biology program at the University of Montana (NSF-GRT #9553611 to P. Kukuk, C. Brewer, and F. Allendorf). Thanks to Sara Barth and Kendra Hinxman for field and laboratory assistance.

REFERENCES

- Barón, M., J.B. Arellano, and J.L. Gorge. 1995. Copper and photosystem II: a controversial relationship. *Physiologia Plantarum* 94:174-180.
- Bertness, M.D., and R.M. Callaway. 1994. Positive interactions in communities. *Trends in Ecology and Evolution* 9:191-193.
- Bethlenfalvay, G.J., and R.S. Pacovsky. 1983. Light effects in mycorrhizal soybeans. *Plant Physiology* 73:969-972.
- 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.J., J.K. Frederickson, and L.F. Elliot. 1992. Microbial ecology of the rhizosphere. Pages 27-63 in Metting, editor. *Soil Microbial Ecology*. Marcel Dekker, Inc. New York.
- Brooker, R.W., and T.V. Callaghan. 1998. The balance between positive and negative plant interactions and its relationship to environmental gradients: a model. *Oikos* 81:196-207.
- Bryla, D.R., and R.T. Koide. 1998. Mycorrhizal response of two tomato genotypes relates to their ability to acquire and utilize phosphorus. *Annals of Botany* 82:849-857.
- Callaway, R.M. 1997. Positive interactions in plant communities and the individualistic-continuum concept. *Oecologia* 112:143-149.
- Clark, R.B., S.K. Zeto, and R.W. Zobel. 1999. Arbuscular mycorrhizal fungal isolate effectiveness on growth and root colonization of *Panicum virgatum* in acidic soil. *Soil Biology & Biochemistry* 31:1757-1763.
- Connell, J.H., and R.D. Slatyer. 1977. Mechanisms of succession in natural communities and their role in community stability and organization. *American Naturalist* 111:1119-1144.
- Del Val, C., J.M. Barea, and C. Azcon-Aguilar. 1999. Diversity of arbuscular mycorrhizal fungus populations in heavy-metal-contaminated soils. *Applied and Environmental Microbiology* 65:718-723.
- Dudka, S., and D.C. Adriano. 1997. Environmental impacts of metal ore mining and processing: a review. *Journal of Environmental Quality* 26:590-602.

Edgell, K. 1988. USEPA method study 37-SW-846 method 3050 acid digestion of sediments, sludges, and soils. EPA contract No. 68-03-3254, November 1988.

Gildon, A., and P.B. Tinker. 1983. Interactions of vesicular-arbuscular mycorrhizal infection and heavy metals in plants: I. The effects of heavy metals on the development of vesicular-arbuscular mycorrhizas. *New Phytologist* 95:247-261.

Johnson, N.C. 1993. Can fertilization of soil select less mutualistic mycorrhizae? *Ecological Applications* 3:749-757.

Johnson, N.C., J.H. Graham, and F.A. Smith. 1997. Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phytologist* 135:575-585.

Leyval, C., K. Turnau, and K. Haselwandter. 1997. Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. *Mycorrhiza* 7:139-153.

Martin, F.M., S. Perotto, and P. Bonfante. 2001. Mycorrhizal fungi: A fungal community at the interface between soil and roots. Pages 263-296 in R. Pinton, Z. Varanini, and P. Nannipieri, editors. *The Rhizosphere - Biochemistry and Organic Substances at the Soil-Plant Interface*. Marcel Dekker, Inc. New York.

Martin, T.D., C.A. Brockhoff, J.T. Creed, and EMMC Methods Workgroup. 1994. Method 200.7, Revision 4.4, Determination of Metals and trace elements in water and wastes by inductively coupled plasma atomic emission spectrometry. In *Methods for the determination of metals in environmental samples*. EPA/600/R-94/111. Environmental Protection Agency, Cincinnati, OH.

Mathur, N., and A. Vyas. 2000. Influence of arbuscular mycorrhizae on biomass production, nutrient uptake and physiological changes in *Ziziphus mauritiana* Lam. under water stress. *Journal of Arid Environments* 45:191-195.

McGonigle, T.P., M.H. Miller, D.G. Evans, G.L. Fairchild, and J.A. Swan. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist* 115:495-501.

Meharg, A.A., and J.W.G. Cairney. 2000. Co-evolution of mycorrhizal symbionts and their hosts to metal-contaminated environments. *Advances in Ecological Research* 30:69-112.

Mulder, C.P.H., D.D. Uliassi, and D.F. Doak. 2001. Physical stress and diversity-productivity relationships: The role of positive interactions. *Proceedings of the National Academy of Sciences* 98:6704-6708.

Munshower, F.F. 1994. *Practical handbook of disturbed land revegetation*. Boca Raton, Florida: Lewis Publishers, Inc. 265 pp.

Newsham, K.K., A.H. Fitter, and A.R. Watkinson. 1995. Multi-functionality and biodiversity in arbuscular mycorrhizas. *Trends in Ecology and Evolution* 10:407-411.

Phillips, J.M., and D.S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* 55:158-161.

Plenchette, C., J.A. Fortin, and V. Furlan. 1983. Growth responses of several plant species to mycorrhizae in a soil of moderate P-fertility. I. Mycorrhizal dependency under field conditions. *Plant and Soil* 70:199-209.

Pyke, D.A., and S. Archer. 1991. Plant-plant interactions affecting plant establishment and persistence in revegetated rangeland. *Journal of Range Management* 44:550-557.

Reddy, G.N., and M.N.V. Prasad. 1990. Heavy metal-binding proteins/peptides: occurrence, structure, synthesis and functions. A review. *Environmental and Experimental Botany* 30:251-264.

Ruiz-Lozano, J.M., and R. Azcon. 2000. Symbiotic efficiency and infectivity of an autochthonous arbuscular mycorrhizal *Glomus* sp. from saline soils and *Glomus deserticola* under salinity. *Mycorrhiza* 10:137-143.

Smith, F.A., and S.E. Smith. 1996. Mutualism and parasitism: diversity in function and structure in the "arbuscular" (VA) mycorrhizal symbiosis. *Advances in Botanical Research* 22:1-43.

Stachowicz, J.J. 2001. Mutualism, facilitation, and the structure of ecological communities. *BioScience* 51:235-246.

Stahl, P.D., G.E. Schuman, S.M. Frost, and S.E. Williams. 1998. Arbuscular mycorrhizae and water stress tolerance of Wyoming big sagebrush seedlings. *Soil Science Society of America Journal* 62:1309-1313.

Stevenson, F.J. 1986. *Cycles of Soil*. New York: John Wiley & Sons, Inc. 380pp.

Tester, M., and F.A. Smith. 1985. Phosphate inflow into *Trifolium subterraneum* L.: Effects of photon irradiance and mycorrhizal infection. *Soil Biology & Biochemistry* 17:807-810.

Tordoff, G.M., A.J.M. Baker, and A.J. Willis. 2000. Current approaches to the revegetation and reclamation of metalliferous mine wastes. *Chemosphere* 41:219-228.

Van der Heijden, M.G.A., J.N. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A. Wiemken, and I.R. Sanders. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability, and productivity. *Nature* 396:69-72.

Van der Heijden, E.W., and T.W. Kuyper. 2001. Does origin of mycorrhizal plant or mycorrhizal fungus influence effectiveness of the mycorrhizal symbiosis? *Plant and Soil* 230:161-174.

Von Frenckell-Insam, B.A.K., and T.C. Hutchinson. 1993. Occurrence of heavy metal tolerance and co-tolerance in *Deschampsia cespitosa* (L.) Beauv. from European and Canadian populations. *Canadian Journal Of Botany* 125:555-564.

Walker, L.R., and F.S. Chapin. 1987. Interactions among processes controlling successional change. *Oikos* 50:131-135.

Young, T.P., J.M. Chase, and R.T. Huddleston. 2001. Community succession and assembly - comparing, contrasting and combining paradigms in the context of ecological restoration. *Ecological Restoration* 19:5-18.

Table 1. Geochemical characteristics of AM inoculum source soils and tailings growth substrate from each lime treatment. Values represent means of 3 replicate samples. Values that were below instrument detection limits are listed as less than the practical quantifiable limit for that analyte.

Sample	pH	N	C	As	Cd	Cu	Fe	Pb	Zn	P
(mg kg ⁻¹)										
<u>AM Inoculum Soils</u>										
Metals-Contaminated Inoculum Source, Water-extractable metals	7.8			2.23	0.04	1.09	3.4	<0.4	<0.04	2.7
Uncontaminated Soil Inoculum Source, Water-extractable metals	6.1			<0.2	<0.04	<0.12	21.6	<0.4	<0.04	10.5
<u>Plant Growth Substrates</u>										
No Lime Total Digestible Metals	3.4	<0.08%	<0.75%	244	3	118	29,290	3158	370	478
No Lime Water-extractable Metals	3.4			<0.2	0.22	3.19	4.41	26.21	36.22	<0.4
Low Lime Water-extractable Metals	4.6			<0.2	0.38	0.34	3.14	7.97	33.51	<0.4
Medium Lime Water-extractable Metals	7.1			<0.2	<0.04	<0.12	3.91	<0.4	<0.04	<0.4
High Lime Water-extractable Metals	7.4			<0.2	<0.04	<0.12	2.36	<0.4	<0.04	<0.4

Table 2. Mean AM colonization levels and plant growth characteristics of all treatments. AM values show average colonization rates (% colonized intersections) of total AM structures, arbuscules (Arb) and vesicles (Ves).

Treatment	Total AM Colonization (%)	Arb (%)	Ves (%)	Shoot biomass (g)	Root Biomass (g)	Root Mass Ratio
No Lime						
No AM	0	0	0	0.355	0.255	0.388
Metals AM	24	4.8	2.3	0.453	0.314	0.399
Uncont. AM	13	3.8	0.6	0.438	0.250	0.345
Low Lime						
No AM	0	0	0	0.506	0.330	0.385
Metals AM	16	2.4	1.9	0.671	0.525	0.432
Uncont. AM	10	0.8	3.6	0.633	0.427	0.406
Medium Lime						
No AM	0	0	0	0.605	0.425	0.414
Metals AM	16	4.2	1.0	0.681	0.471	0.408
Uncont. AM	3	0.8	0.4	0.600	0.350	0.361
High Lime						
No AM	0	0	0	0.529	0.333	0.391
Metals AM	43	6.8	6.9	0.603	0.427	0.408
Uncont. AM	5	0.8	1.2	0.582	0.386	0.381

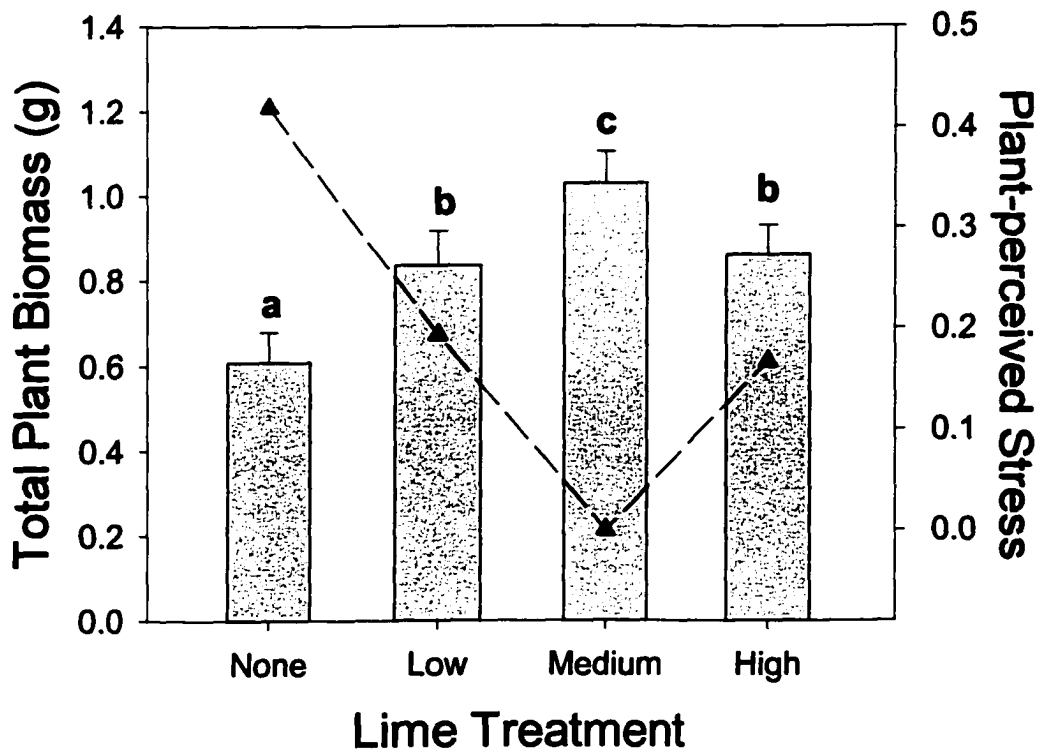


Figure 1. Total biomass of nonmycorrhizal plants across lime treatments. The dashed line represents plant-perceived stress level for each soil treatment, calculated relative to the medium lime treatment. Error bars represent standard error of the mean. Letters above bars designate statistically significant different means.

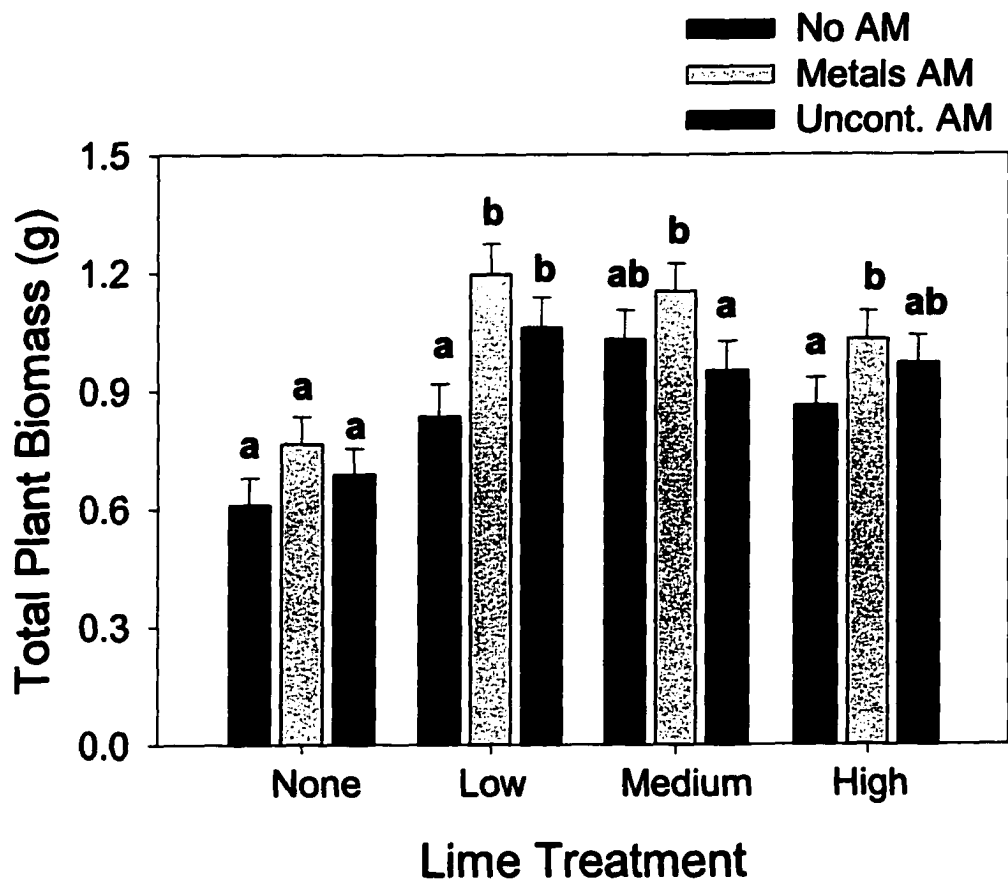


Figure 2. Total biomass of plants for all lime and AM treatments. Error bars represent standard error of the mean. Letters above bars designate statistically significant different means between AM treatments within each lime level.

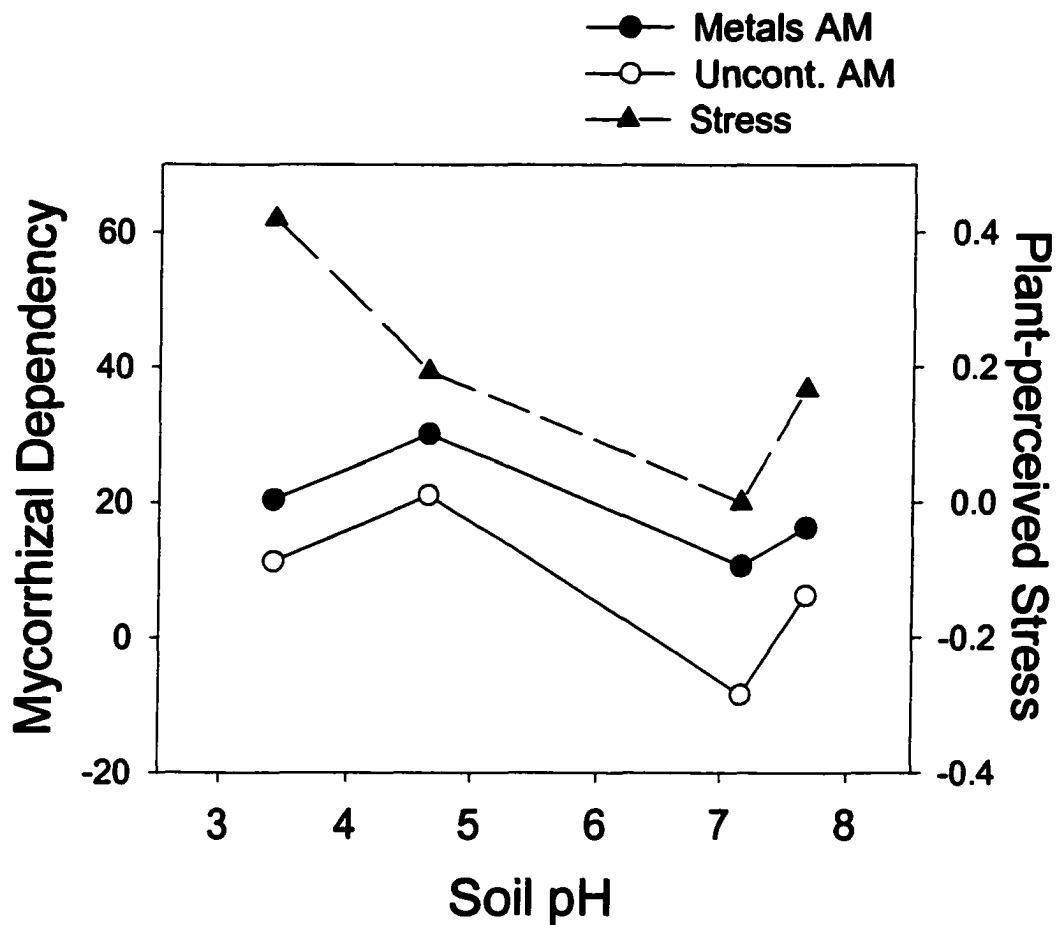


Figure 3. Plant-perceived stress and mycorrhizal dependency versus soil pH. Calculations for stress and mycorrhizal dependency are described in the Methods section. Because these values are derived from treatment means, variance cannot be estimated. Patterns are intended to display overall data trends to compare with theory, rather than to represent statistically significant treatment differences.

CHAPTER 4

Mycorrhizal Source Effects on Six Native Plants with Potential for Mine Tailings Revegetation

ABSTRACT

Soil microorganisms, such as arbuscular mycorrhizal fungi, have important effects on plant communities, and should be incorporated into ecological restoration of disturbed lands. Effects of three arbuscular mycorrhizal (AM) sources were evaluated with six native plant species growing in limed mine tailings. Sources of AM included 1) sterilized inoculum, 2) inoculum from a metals-contaminated site, and 3) inoculum from an uncontaminated site. Plant species were selected for their sensitivity to metal-contamination and potential use for mine revegetation. Tufted hairgrass (*Deschampsia cespitosa*) and yarrow (*Achillea millefolium*) are often used in mine waste revegetation because of their tolerance of soil acidity and elevated metals. Bluebunch wheatgrass (*Pseudoroegneria spicata*), rough fescue (*Festuca scabrella*), blue flax (*Linum lewisii*), and narrow-leaved purple coneflower (*Echinacea angustifolia*) are generally more sensitive to harsh soil conditions and are not widely used for mine revegetation. Biomass of yarrow, tufted hairgrass, and bluebunch wheatgrass was not affected by AM source. Metals-contaminated soil AM increased biomass of rough fescue (53%), blue flax (283%), and purple coneflower (798%) relative to nonmycorrhizal plants. Uncontaminated soil AM increased biomass of blue flax (262%) and purple coneflower (646%), but not rough fescue. Root mass ratios of blue flax and purple coneflower, the most mycorrhizal-dependent species, were greater in mycorrhizal than nonmycorrhizal plants. This study shows that AM effects vary with plant species and AM inoculum source, and exposes the bias of selection of revegetation species toward plants that are not

mycorrhizal-dependent. Re-evaluating plant species with their natural AM symbioses will expand the pool of desirable revegetation species and lead to more productive and diverse plant communities.

INTRODUCTION

Advances in revegetation techniques often arise from important findings in in plant sciences. For example, an active research area in plant ecology focuses on mycorrhizal influences on plant growth and community development. Mycorrhizae are naturally occurring symbioses that occur with 90% of all plant families (Sylvia 1994). While there are several types of mycorrhizae, this research focuses on arbuscular mycorrhizae (AM, sometimes known as VAM). AM are an often-mutualistic symbiosis between a plant root and a specialized fungus in which the fungus forms structures inside plant cortical cells and extends outside the root into the soil. The fungus benefits from a direct source of carbon supplied by the plant, while the plant potentially receives many benefits such as increased nutrient uptake, improved water status, pathogen protection, and possibly enhanced metal resistance (Bolton et al. 1992, Newsham 1995, Martin et al. 2001).

Individual plants can benefit from AM through improved plant growth and reproduction (Lu and Koide 1994, Martin et al. 2001), which may potentially alter plant community dynamics (Miller and Allen 1992, Hartnett et al. 1993, Van der Heijden et al. 1998). In a mine restoration context, mycorrhizal inoculation can result in increased plant survival and growth, and higher levels of community

productivity (Call and Davies 1988, Pflieger et al. 1994, Noyd et al. 1996, St. John 1998).

While AM may be very important for recovering plant communities, numbers of mycorrhizal propagules can be substantially reduced with mine-related disturbance systems, sometimes as much as 100% (Moorman and Reeves 1979, Jasper et al. 1989, Gould et al. 1996, Jennings et al. 2000). AM abundance and diversity also can be reduced in metal-contaminated systems (Allen et al. 1987, Del Val et al. 1999, Moynahan et al. 2002). Greenhouse and laboratory studies show that AM spore germination, hyphal penetration and root colonization can be inhibited by elevated metal concentrations, but that some AM fungal species may be resistant to these conditions (Gildon and Tinker 1983, Weissenhorn et al. 1994, Leyval et al. 1997, Meharg and Cairney 2000).

The nature and potential benefit of mycorrhizal symbioses depends on the plant and fungal species, as well as the soil environment (Johnson et al. 1992, Johnson et al. 1997, Van der Heijden et al. 2001). Plant responses to AM vary between plant species (Plenchette et al. 1983) and genotypes (Bryla and Koide 1998), and between fungal species (Mathur and Vyas 2000) and fungal species isolates (Gildon and Tinker 1983). Fungal species adapted to elevated metal levels result in increased plant growth in metal-contaminated soils relative to AM from uncontaminated soils (Gildon and Tinker 1983, Meharg and Cairney 2000, Chapter 2), suggesting the use of AM fungi from metal-contaminated soils for mine waste revegetation. Alternatively, AM fungal communities from uncontaminated soil may be more diverse (Del Val et al. 1999), and AM fungal

diversity has been linked to increased plant community productivity and diversity (Van der Heijden et al. 1998). Clearly, more research is needed to elucidate patterns of plant host and fungal specificity in metals-contaminated soils, which may be used to inform restoration decisions regarding plant species selection and AM inoculation.

Metal ore mining processes create hazardous wastes that impact environments world-wide (Moore and Luoma 1990, Dudka and Adriano 1997, Tordoff et al. 2000). Whether these mine wastes are removed to a repository, capped, or treated in place, the disturbed ground must be reclaimed and revegetated. Seed mixes for mine revegetation projects are generally comprised of species that have demonstrated the ability to grow on disturbed sites in past projects and experiments, or on naturally harsh soils (Thornburg 1982, Powell 1988, Munshower 1994b). Unfortunately, this work has been conducted in the absence of the mycorrhizal fungal symbionts that occur with plants growing in natural conditions. This results in a bias against mycorrhizal-dependent plant species, including many desirable native plants. Because mycorrhizal communities in mine-disturbed sites are usually depressed or absent (Jasper 1989, Leyval et al. 1997, Moynahan et al. 2002), and because AM may improve plant growth and plant community dynamics, AM inoculation may be important to the success of mine waste revegetation projects.

This study examines mycorrhizal dependence of six native plant species that have potential for mine revegetation in Montana, two of which are considered relatively resistant to metals and stressful conditions, and four of

which are more sensitive and are not generally used for mine revegetation. Tufted hairgrass (*Deschampsia cespitosa*) and yarrow (*Achillea millefolium*) have been shown to be relatively tolerant of acidity, elevated metals, and other environmental stress (Von Frenckell-Insam and Hutchinson 1993, Munshower 1994a, Munshower 1995, Marty 2001) and are recommended for use in revegetation seed mixes (Neuman et al. 1993, Munshower 1994b, Munshower 1995). Bluebunch wheatgrass (*Pseudoroegneria spicata*), rough fescue (*Festuca scabrella*), blue flax (*Linum lewisii*), and narrow-leaved purple coneflower (*Echinacea angustifolia*) are considered to be more sensitive to harsh soils because of poor performance in greenhouse experiments (Neuman et al. 1993, Munshower 1995, Marty 2001), absence in metals-disturbed sites compared to reference sites (Lipton et al. 1993, Lipton et al. 1995), poor success in revegetation projects (Munshower and Blodgett 1989, Schafer et al. 1993), or documented environmental limitations (Smoliak et al. 1990, Munshower 1994a, Munshower 1995, Marty 2001). Although there are mixed reports of stress-tolerant ecotypes and revegetation potential for bluebunch wheatgrass and blue flax (Munshower and Blodgett 1989, Munshower 1995, Marty 2001), they are categorized as sensitive in this study because the seed sources used were not metal-adapted, and these species are not widely used in mine revegetation.

These plant species have not previously been evaluated for mine revegetation potential with mycorrhizal symbionts, as would occur in a natural setting. In this study, mycorrhizal effects on these plant species were tested with three AM treatments, including no AM, AM from metals-contaminated soil, and

AM from uncontaminated soil. The objectives of this research were i) to determine whether the growth of metals-resistant and/or metals-sensitive native plants growing in neutralized mine tailings can be enhanced with AM, and ii) to determine whether different sources of AM vary in their effects on plant growth.

METHODS

Experimental Design/Soil Chemical Characterization

Effects of AM sources were examined in separate greenhouse experiments for each of six plant species native to western Montana: tufted hairgrass, yarrow, bluebunch wheatgrass, rough fescue, blue flax, and purple coneflower. Mycorrhizal source effects were assessed with three treatments: 1) autoclaved inoculum, 2) inoculum from a metals-contaminated site, and 3) inoculum from an uncontaminated site. These treatments were tested with a complete randomized block experiment, with 11 replicates of each treatment.

Plants were grown in mine tailings, the milled material remaining after ore is processed, collected from an abandoned tailings deposit in southwestern Montana (46°20'N, 112°13'W). Tailings were homogenized thoroughly, sieved through a 5 mm mesh, and amended with 3% lime (CaCO₃ by dry wt; Montana Limestone Co., Warren MT) to increase the pH from 3.5 in unamended tailings to 7.4.

Inoculation with AM Fungal Communities

The metals-contaminated inoculum was collected from a mine tailings revegetation project in southwestern Montana that had neutralized tailings, high metal levels (Table 1), and mycorrhizal plants. The uncontaminated inoculum was from a bluebunch wheatgrass- and Idaho fescue (*Festuca idahoensis*)-dominated grassland in western Montana with no metal contamination (Table 1), and mycorrhizal plants.

Mycorrhizal inoculation was achieved by combining live soil from each inoculum source, sterilized soil from the alternate inoculum source, and tailings growth substrate that was known to be nonmycorrhizal, to minimize physical and chemical differences between inocula. The metals-contaminated soil AM treatment contained 10 ml metals-contaminated soil, 10 ml sterilized uncontaminated soil, and 10 ml tailings. The uncontaminated soil AM treatment contained 10 ml uncontaminated soil, 10 ml sterilized metals-contaminated soil, and 10 ml tailings. The nonmycorrhizal treatment contained 10 ml sterilized metals-contaminated soil, 10 ml sterilized uncontaminated soil, and 10 ml tailings. The sterilized soils were autoclaved twice at 121° C for 15 min, with 24 h between sessions in the autoclave. The inoculum mixtures were added to pots and covered with 1 cm sterilized silica sand.

To minimize differences in the non-mycorrhizal microbial community, a microbial sievate from both inoculum sources was added to all pots. The microbial sievate was prepared by passing a soil slurry (1:1:8 by weight, metals-contaminated soil:uncontaminated soil:deionized water) through a series of filters

(250 μ m, 25 μ m, 11 μ m). The filtration excludes mycorrhizal propagules, while soil bacteria, nonmycorrhizal spores, and other microorganisms pass through the filters. Twenty-five ml of the appropriate microbial suspension was added to each pot.

Plant Cultivation and Harvest

Plants were seeded into 6 cm dia x 25 cm deep pots, and germinants were thinned to a single plant per pot. Plants were watered to field capacity every 2-3 days, and nutrients were added every 2 weeks with 30 ml $\frac{1}{4}$ -strength modified Hoagland's solution (minus P) during the first 3 months of growth, and 30 ml $\frac{1}{2}$ -strength Hoagland's solution (low P) during the last 2 months of growth. Pots were randomly arranged on the greenhouse bench and moved every week to minimize microsite effects. After 5 months of growth, plants were harvested and washed thoroughly. Roots and shoots were dried for 72 h at 60°C, and biomass was recorded.

Mycorrhizal Colonization Measurements

A subsample of roots was collected from five replicates per treatment after biomass measurements to determine mycorrhizal colonization levels. Plant roots were cleared in 2.5% KOH for 48 h, acidified in 3% HCl for 12 h, and stained with Trypan blue for 12 h (variation of Phillips and Hayman 1970). The percentage of roots colonized by mycorrhizal fungi was determined by the magnified intersections method (McGonigle et al. 1990).

Soil Analysis

For pH measurements, 10 g of soil was combined with 10 ml of deionized water, allowed to equilibrate for 5 min, and measured with a pH probe (Accumet Basic, Fisher Scientific). For the other geochemical analyses, samples were dried, sieved through a 2 mm mesh, and ground using a ball mill (Spex Industries, Inc.). Total C and N levels were determined by analyzing 0.25-0.5 g with an elemental analyzer (CE Instruments elemental analyzer).

Concentrations of metals and other ecologically relevant elements were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) analysis for total digestible, DTPA-extractable, and water-extractable fractions of all samples. Soil samples analyzed for total recoverable metals were digested with a double acid, peroxide, hot-block digest, according to E.P.A. Method 3050B, modified to $\frac{1}{4}$ volume (Edgell 1988). Water extractable elements were determined by combining 1 g of sieved, unground sample with 40 ml milli-Q water. Slurries were mixed on a shaker table for 12 h and centrifuged for 20 min at 3500 rpm. Supernatants were filtered through 0.45 μm membrane and preserved with 80 μl HNO_3 . DTPA-extractable fractions were extracted and analyzed by the Soil Analytical Lab at Montana State University. For DTPA extractions, 10 g soil and 20 ml DTPA solution (0.005 M DTPA, 0.01M CaCl_2) were combined, shaken for 2 h, and filtered through a Whatman #40 filter. Analytes, including As, Cd, Cu, Fe, Pb, Zn, and P were quantified by ICP-AES analysis of soil extracts and digests using E.P.A. Method 200.7 (Jarrell-Ash ICP-

AES 800 instrument) (Martin et al. 1994). The QA/QC results are summarized in Appendix A.

Calculations and Statistical Analysis

Root mass ratio was calculated as root biomass / total biomass. Mycorrhizal treatment differences were tested with a 1-way analysis of variance (ANOVA) on each species ($\alpha=0.05$), followed by Tukey's tests for multiple comparisons (SPSS 10.0). To meet assumptions of the analyses, blue flax total biomass data were square-transformed and purple coneflower total biomass data were natural log-transformed prior to statistical analyses.

RESULTS

Mine tailings plant growth substrate

The unamended mine tailings had extremely high concentrations of total digestible and DTPA-extractable metals, and generally low nutrient levels (Table 1). After neutralization with lime, DTPA-extractable metals were significantly lower, although some metals, especially Cu and Pb, were still elevated.

Mycorrhizal colonization

Mycorrhizal colonization levels varied between AM inoculum sources and between plant species (Table 2). The AM from metals-contaminated soil resulted in higher colonization levels than AM from uncontaminated soil across species, except purple coneflower which had comparable AM colonization levels with both

AM inocula. Mycorrhizal colonization levels varied widely across species, with rough fescue showing the lowest AM colonization and purple coneflower showing the highest. All plants in the nonmycorrhizal treatment had 0% colonization levels in root samples.

Plant Growth Effects

Biomass of tufted hairgrass and yarrow, the more stress-tolerant species, was not significantly different between AM treatments (Figure 1). While the general trends indicated that AM from metal-contaminated sites resulted in slightly greater biomass, this difference was not statistically significant for either tufted hairgrass ($F_{2,42}=0.081$, $P=0.992$) or yarrow ($F_{2,24}=0.943$, $P=0.403$).

Three of the four sensitive species showed significant increases in total biomass when grown with AM (Figure 1). Blue flax biomass increased significantly with both AM inoculum sources ($F_{2,30}=16.019$, $P<0.001$), but the AM from the metals-contaminated source resulted in a greater response, with 283% increase over the mean biomass of non-mycorrhizal plants. Purple coneflower biomass also drastically increased with both AM treatments ($F_{2,21}=50.019$, $P<0.001$), with the metals AM producing a 798% increase in mean total biomass over non-mycorrhizal plants. Rough fescue showed a mildly significant increase ($F_{2,29}=2.691$, $P=0.085$) with the AM from metals-contaminated soil, resulting in a 53% increase in mean plant biomass relative to nonmycorrhizal plants. AM from uncontaminated soil did not affect biomass of rough fescue relative to nonmycorrhizal plants. Bluebunch wheatgrass grown with the metals AM also

had a higher mean total biomass than the non-mycorrhizal control and the uncontaminated soil AM, but the increase was not statistically significant ($F_{2,30}=2.097$, $P=0.140$).

Root mass ratio, calculated as root biomass / total biomass, reflects the biomass allocation of individual plants within each treatment group. Mycorrhizal treatments did not alter root mass ratios of tufted hairgrass ($F_{2,42}=1.031$, $P=0.366$), yarrow ($F_{2,24}=1.421$, $P=0.261$), or rough fescue ($F_{2,29}=0.191$, $P=0.827$) (Figure 2). Both AM source treatments resulted in significantly lower root mass ratios than non-mycorrhizal controls in blue flax ($F_{2,30}=6.450$, $P=0.005$), and purple coneflower ($F_{2,21}=7.066$, $P=0.005$). For bluebunch wheatgrass, AM from uncontaminated soil resulted in lower root mass ratios relative to AM from metals-contaminated soil ($F_{2,30}=7.709$, $P=0.036$), but neither AM treatment was significantly different from the non-mycorrhizal control.

DISCUSSION

In previous studies (Chapters 2 and 3), I have shown that AM can positively influence plant growth in metals-contaminated substrates, and that AM from metals-contaminated soil results in greater host plant response than AM from uncontaminated soil. This study tests the generality of previous findings across host plant species. Mycorrhizal plant growth effects differed for individual plant species with varying levels of stress resistance. This is especially relevant for disturbed land restoration, where reassembly of functional plant communities

depends on the presence of soil ecosystem components and interactions between soil biota and plant communities.

In this study, plants with known metals and stress resistance (tufted hairgrass and yarrow), which are included as recommended species for mine revegetation, were not significantly affected by AM inocula. Conversely, plant species that are not known for metal-tolerance (blue flax, rough fescue and purple coneflower) showed increased plant biomass when inoculated with AM from one or both of the AM sources. Bluebunch wheatgrass, which is not widely used in mine revegetation, but has some metal-resistant varieties (Marty 2001), showed a tendency of increased biomass with AM from metals-contaminated soil, but this trend was not statistically significant.

The plant species with greatest mycorrhizal effects (blue flax and purple coneflower) showed lower root mass ratios when mycorrhizal, indicating relatively less biomass allocation to root tissue. Inhibited root growth is a primary symptom of metal toxicity in plants, and results in reduced water and nutrient uptake capabilities (Lambers et al. 1998). These root mass ratio patterns suggest that a mechanism for AM benefit to blue flax and purple coneflower may be that the fungus effectively extends the root function, so that the plant can acquire soil resources for biomass production without having to rely on root growth in toxic soils.

Interestingly, differences between AM source effects on plant biomass varied between host plant species. In all of the sensitive species, AM from metals-contaminated soils increased plant growth relative to non-mycorrhizal

plants. The AM from uncontaminated soil did not affect biomass of bluebunch wheatgrass or rough fescue, but greatly increased the biomass of blue flax and purple coneflower. The neutralization of the tailings in this study greatly reduced DTPA-extractable metal levels, but some metals (such as Cu and Pb) were still elevated. Further, DTPA-extraction is intended to estimate plant-available analytes, but it is impossible to determine metal levels that actually affect plant growth. Previous research has indicated that AM fungal species can be adapted to metals-contaminated conditions (Gildon and Tinker 1983, Leyval et al. 1997, Meharg and Cairney 2000). This study, which compares fungal communities rather than isolates, indicates that some differential effects between inoculum sources may be due to plant and fungal symbiont compatibility, rather than environmental adaptation.

It is important to note that the nature of the mycorrhizal dependency can change with environmental conditions (Smith and Smith 1996, Johnson et al. 1997). For example, tufted hairgrass, which is not AM-dependent in neutralized tailings of this experiment, shows significant mycorrhizal dependency when growing with metals-contaminated soil AM when pH and metals stress is increased (Chapters 2 and 3). Likewise, AM effects may vary in field conditions where plants are subject to a variety of other environmental stressors.

These findings have three important implications for restoration management. First, plant species lists for mine revegetation that were developed without AM are biased to select for plant species that are not mycorrhizal dependent. Second, when selecting species for seed mixes, it is important to consider that

many desirable species may be better candidates than suspected, as long as appropriate mycorrhizae are present. Third, chances for maximizing species richness from diverse seed mixes may be increased by inoculating with appropriate mycorrhizae. While AM effects will vary with plant types and species, AM inoculum sources, and environmental factors associated with revegetation projects, this study demonstrates the importance of including this crucial component of plant-soil systems as part of restoration designs.

ACKNOWLEDGEMENTS

This work was supported by the NSF-funded Training Within Environmental Biology program at the University of Montana (NSF-GRT #9553611 to P. Kukuk, C. Brewer, and F. Allendorf). Thanks to Sara Barth and Kendra Hinxman for field and laboratory assistance, and to Wind River Seed for providing native plant seeds and propagation advice.

REFERENCES

- Allen, E.B., J.C. Chambers, K.F. Connor, M.F. Allen, and R.W. Brown. 1987. Natural reestablishment of mycorrhizae in disturbed alpine ecosystems. *Arctic and Alpine Research* 19:11-20.
- Bolton, H.J., J.K. Frederickson, and L.F. Elliot. 1992. Microbial ecology of the rhizosphere. In *Soil Microbial Ecology*. F.B. Metting, Jr. ed., pp. 27-63, Marcel Dekker, Inc. New York.
- Bryla, D.R. and R.T. Koide. 1998. Mycorrhizal response of two tomato genotypes relates to their ability to acquire and utilize phosphorus. *Annals of Botany* 82:849-857.
- Call, C.A. and F.T. Davies. 1988. Effects of vesicular-arbuscular mycorrhizae on survival and growth of perennial grasses in lignite overburden in Texas. *Agriculture, Ecosystems & Environment* 24:395-405.
- Del Val, C., J.M. Barea, and C. Azcon-Aguilar. 1999. Diversity of arbuscular mycorrhizal fungus populations in heavy-metal-contaminated soils. *Applied and Environmental Microbiology* 65:718-723.
- Dudka, S. and D.C. Adriano. 1997. Environmental impacts of metal ore mining and processing: a review. *Journal of Environmental Quality* 26:590-602.
- Edgell, K. 1988. USEPA method study 37-SW-846 method 3050 acid digestion of sediments, sludges, and soils. EPA contract No. 68-03-3254, November 1988. (Abstract)
- Gildon, A. and P.B. Tinker. 1983. Interactions of vesicular-arbuscular mycorrhizal infection and heavy metals in plants: I. The effects of heavy metals on the development of vesicular-arbuscular mycorrhizas. *New Phytologist* 95:247-261.
- Gould, A.B., J.W. Hendrix, and R.S. Ferriss. 1996. Relationship of mycorrhizal activity to time following reclamation of surface mine land in western Kentucky. I. Propagule and spore population densities. *Canadian Journal of Botany* 74:247-261
- Hartnett, D.C., B.A.D. Hetrick, G.W.T. Wilson, and D.J. Gibson. 1993. Mycorrhizal influence on intra- and interspecific neighbour interactions among co-occurring prairie grasses. *Journal of Ecology* 81:787-795.
- Jasper, D.A., L.K. Abbott, and A.D. Robson. 1989. The loss of VA mycorrhizal infectivity during bauxite mining may limit the growth of *Acacia pulchella* R.Br. *Australian Journal of Botany* 37:33-42.

Jennings, S.R. and J.M. Krueger. 2000. Metal mine tailing reprocessing for enhanced vegetation establishment: clean tailing technology. In *Proceedings of the 2000 Billings Land Reclamation Symposium*. pp. 544-553, Reclamation Research Unit, Montana State University, Bozeman, Montana.

Johnson, N.C., D. Tilman, and D. Wedin. 1992. Plant and soil controls on mycorrhizal fungal communities. *Ecology* 73:2034-2042.

Johnson, N.C., J.H. Graham, and F.A. Smith. 1997. Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phytologist* 135:575-585.

Lambers, H., F.S. Chapin, and T.L. Pons. 1998. *Plant Physiological Ecology*, Springer-Verlag New York Inc. New York. 540pp.

Leyval, C., K. Turnau, and K. Haselwandter. 1997. Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. *Mycorrhiza* 7:139-153.

Lipton, J., H. Galbraith, K. LeJeune, and B. RCG/Hagler, Inc. 1993. *Terrestrial Resources Injury Assessment Report - Upper Clark Fork River Basin*, RCG/Hagler, Bailly, Inc.

Lipton, J., D. Cacula, Montana Natural Resource Damage Litigation Program, and B. RCG/Hagler, Inc. 1995. *Terrestrial Resources Injury Assessment Report - Upper Clark Fork River NPL Sites. Appendices A - E*, RCG/Hagler, Bailly, Inc.

Lu, X. and R.T. Koide. 1994. The effects of mycorrhizal infection on components of plant growth and reproduction. *New Phytologist* 128:211-218.

Martin, F.M., S. Perotto, and P. Bonfante. 2001. Mycorrhizal fungi: A fungal community at the interface between soil and roots. In *The rhizosphere - biochemistry and organic substances at the soil-plant interface*. R. Pinton, Z. Varanini, and P. Nannipieri, eds., pp. 263-296, Marcel Dekker, Inc. New York.

Martin, T.D., C.A. Brockhoff, J.T. Creed, and EMMC Methods Workgroup. 1994. Method 200.7, Revision 4.4, Determination of Metals and trace elements in water and wastes by inductively coupled plasma atomic emission spectrometry. In *Methods for the determination of metals in environmental samples*. EPA/600/R-94/111. Environmental Protection Agency, Cincinnati, OH.

Marty, L. 2001. Performance of plants grown in low pH and heavy metal contaminated soil under greenhouse conditions. In *Development of Acid/Heavy Metal-tolerant Cultivars Project Biennial Report*. April 1-September 30, 2001:7-27.

- Mathur, N. and A. Vyas. 2000. Influence of arbuscular mycorrhizae on biomass production, nutrient uptake and physiological changes in *Ziziphus mauritiana* Lam. under water stress. *Journal of Arid Environments* 45:191-195.
- McGonigle, T.P., M.H. Miller, D.G. Evans, G.L. Fairchild, and J.A. Swan. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist* 115:495-501.
- Meharg, A.A. and J.W.G. Cairney. 2000. Co-evolution of mycorrhizal symbionts and their hosts to metal-contaminated environments. *Advances in Ecological Research* 30:69-112.
- Miller, S.L. and E.B. Allen. 1992. Mycorrhizae, nutrient translocation, and interactions between plants. In *Mycorrhizal Functioning*. M.F. Allen, ed., pp. 301-332, Chapman & Hall, Inc. London.
- Moore, J.N. and S.N. Luoma. 1990. Hazardous wastes from large-scale metal extraction. *Environmental Science and Technology* 24:1278-1285
- Moorman, T. and B.F. Reeves. 1979. The role of endomycorrhizae in revegetation practices in the semi-arid west. II. A bioassay to determine the effect of land disturbance on endomycorrhizal populations. *American Journal Botany* 66:14-18.
- Moynahan, O.S., C.A. Zabinski, and J.E. Gannon. 2002. Microbial community structure and carbon-utilization diversity in a mine tailings revegetation study. *Restoration Ecology* 10:77-87.
- Munshower, F.F. 1994a. *Perennial Grasses for Revegetation of Disturbed Lands in the Northern Great Plains and the Intermountain Region*, Reclamation Research Unit, Montana State University, Bozeman, Montana. 138pp.
- Munshower, F.F. 1994b. *Practical Handbook of Disturbed Land Revegetation*, Lewis Publishers, Inc. Boca Raton, Florida. 140pp.
- Munshower, F.F. 1995. *Forbs, Shrubs and Trees for Revegetation of Disturbed Lands in the Northern Great Plains and Adjacent Areas*, Reclamation Research Unit, Montana State University, Bozeman, Montana. 179pp.
- Munshower, F.F. and S.D. Blodgett. 1989. *Plant Species for Seeding on Toxic Minesoils in Arid Regions and Characterization of the Acid Soils in Which These Species Naturally Occur*, Reclamation Research Unit, Montana State University, Bozeman, Montana. 101pp.
- Neuman, D.R., F.F. Munshower, D.J. Dollhopf, S.R. Jennings, W.M. Schafer, and J.D. Goering. 1993. Streambank tailings revegetation. In *Planning,*

Rehabilitation and Treatment of Disturbed Lands. Sixth Billings Symposium. pp. 1-16, Reclamation Research Unit, Montana State University, Bozeman, Montana.

Newsham, K.K., A.H. Fitter, and A.R. Watkinson. 1995. Multi-functionality and biodiversity in arbuscular mycorrhizas. *Trends in Ecology and Evolution* 10:407-411.

Noyd, R.K., F.L. Pflieger, and M.R. Norland. 1996. Field responses to added organic matter, arbuscular mycorrhizal fungi, and fertilizer in reclamation of taconite iron ore tailing. *Plant and Soil* 179:89-97.

Pflieger, F.L., E.L. Stewart, and R.K. Noyd. 1994. Role of VAM fungi in mine land revegetation. In *Mycorrhizae and Plant Health*. F.L. Pflieger and R.G. Linderman, eds., pp. 47-82, The American Phytopathological Society, St. Paul, Minnesota.

Phillips, J.M. and D.S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* 55:158-161.

Plenchette, C., J.A. Fortin, and V. Furlan. 1983. Growth responses of several plant species to mycorrhizae in a soil of moderate P-fertility. I. Mycorrhizal dependency under field conditions. *Plant and Soil* 70:199-209.

Powell, J.L. 1988. Revegetation options. In *Reclamation of Surface-Mined Lands*. L.L. Hossner, ed., pp. 49-91, CRC Press, Inc. Boca Raton, Florida.

Schafer and Associates and Reclamation Research Unit. 1993. *Streambank Tailings and Revegetation Studies Final Report. SBC-STARS-III--D-R0-013193. November 10, 1993.*

Smith, F.A. and S.E. Smith. 1996. Mutualism and parasitism: diversity in function and structure in the "arbuscular" (VA) mycorrhizal symbiosis. *Advances in Botanical Research* 22:1-43.

Smoliak, S., R.L. Ditterline, J.C. Scheetz, L.K. Holzworth, J.R. Sims, L.E. Wiesner, D.E. Baldrige, and G.L. Tibke. 1990. Plant species. In *Montana Interagency Plant Materials Handbook*. D.E. Baldrige and R.G. Lohmiller, eds., pp. 8-169, Montana State University Extension Service, Bozeman, Montana.

St. John, T.V. 1998. Mycorrhizal inoculation in habitat restoration. *Land and Water* September/October:17-19.

Sylvia, D.M. 1994. Vesicular-arbuscular mycorrhizal fungi. In *Methods of Soil Analysis, Part 2. Microbiological and biochemical properties*. pp. 351-378, Soil Science Society of America, Madison, WI.

Thornburg, A.A. 1982. *Plant Materials for Use on Surface-Mined Lands in Arid and Semiarid Regions*, Soil Conservation Service, United States Department of Agriculture, Washington, DC. 88pp.

Tordoff, G.M., A.J.M. Baker, and A.J. Willis. 2000. Current approaches to the revegetation and reclamation of metalliferous mine wastes. *Chemosphere* 41:219-228.

Van der Heijden, M.G.A., J.N. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A. Wiemken, and I.R. Sanders. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability, and productivity. *Nature* 396:69-72.

Van der Heijden, E.W. and T.W. Kuyper. 2001. Does origin of mycorrhizal plant or mycorrhizal fungus influence effectiveness of the mycorrhizal symbiosis? *Plant and Soil* 230:161-174.

Von Frenckell-Insam, B.A.K. and T.C. Hutchinson. 1993. Occurrence of heavy metal tolerance and co-tolerance in *Deschampsia cespitosa* (L.) Beauv. from European and Canadian populations. *Canadian Journal Of Botany* 125:555-564.

Weissenhorn, I., A. Glashoff, C. Leyval, and J. Berthelin. 1994. Differential tolerance to Cd and Zn of arbuscular mycorrhizal (AM) fungal spores isolated from heavy metal-polluted and unpolluted soils. *Plant and Soil* 167:189-196.

Table 1. Geochemical characteristics of mine tailings growth substrate and AM inoculum source soils. Values represent means of 3 replicate samples. Values that were below instrument detection limits are listed as less than the practical quantifiable limit for the analyte.

Sample	pH	N	C	As	Cd	Cu	Fe	Pb	Zn	P
(mg kg ⁻¹)										
<u>Plant Growth Substrate</u>										
Total Digestible analytes (unlimed tailings)	3.42	<0.08%	<0.75%	244	3	118	29,290	3158	370	478
DTPA-extractable analytes (limed tailings)	7.40			0.1	0.1	0.7	1.8	111.5	6.6	<0.4*
<u>AM Inoculum Sources</u>										
Metals-Contaminated Inoculum DTPA-extractable analytes	7.8			0.1	0.2	62.1	2.9	3.9	31.7	2.7*
Uncontaminated Soil Inoculum DTPA-extractable analytes	6.1			<0.1	.07	2.3	20	0.7	2.9	10.5*

*Extractable P was determined by water extraction.

Table 2. Mean AM colonization and plant growth characteristics of all treatments. AM values show average colonization rates (percent colonized intersections) of total AM structures, arbuscules (Arb) and vesicles (Ves).

Treatment		Total AM Colonization (%)	Arb (%)	Ves (%)	Shoot biomass (g)	Root Biomass (g)
Tufted						
Hairgrass	No AM	0	0	0	0.535	0.339
	Metals AM	42	7	7	0.539	0.375
	Uncont. AM	5	1	1	0.527	0.339
Yarrow						
Yarrow	No AM	0	0	0	0.230	0.175
	Metals AM	59	14	13	0.306	0.183
	Uncont. AM	27	5	1	0.190	0.138
Bluebunch						
Wheatgrass	No AM	0	0	0	0.415	0.371
	Metals AM	29	5	1	0.448	0.452
	Uncont. AM	14	6	0	0.431	0.321
Blue Flax						
Blue Flax	No AM	0	0	0	0.084	0.116
	Metals AM	52	19	3	0.379	0.387
	Uncont. AM	40	20	2	0.356	0.368
Rough						
Fescue	No AM	0	0	0	0.171	0.120
	Metals AM	17	3	1	0.296	0.148
	Uncont. AM	6	0	1	0.220	0.118
Purple						
Coneflower	No AM	0	0	0	0.021	0.043
	Metals AM	93	51	19	0.233	0.338
	Uncont. AM	94	45	48	0.216	0.258

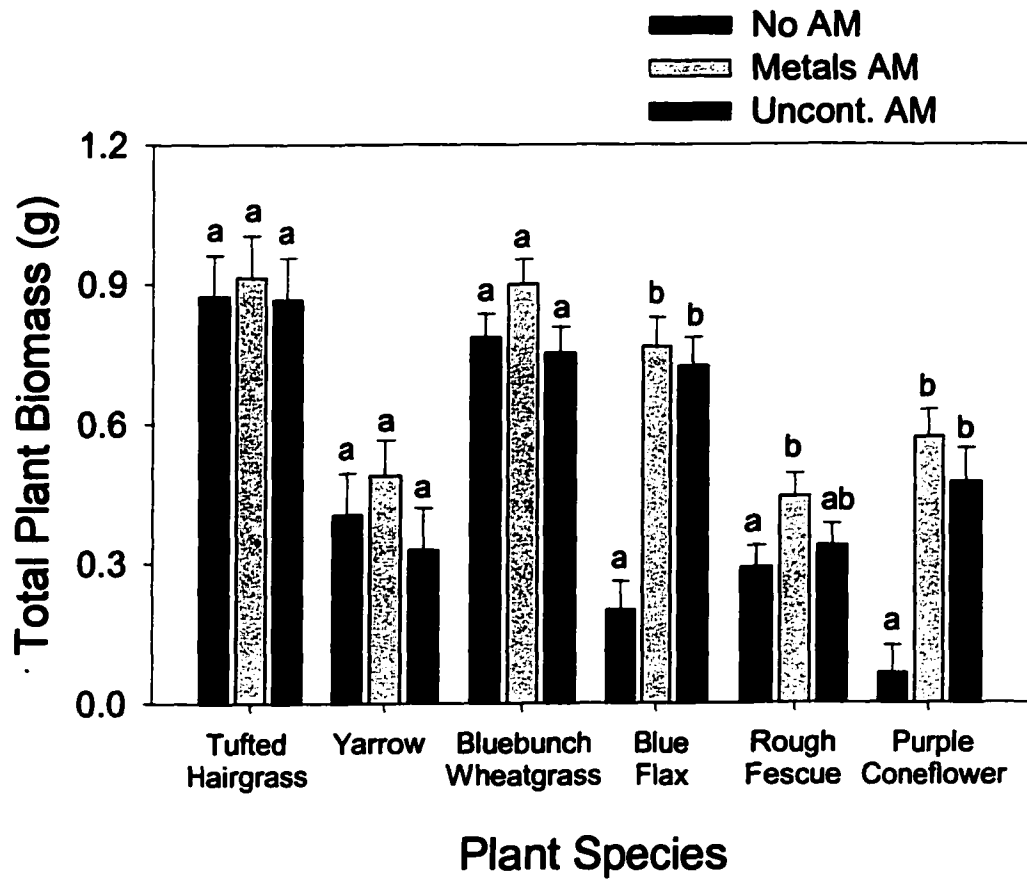


Figure 1. Total plant biomass. Error bars represent standard error of the mean. Letters above bars designate statistically significant different means between AM treatments, within each plant species.

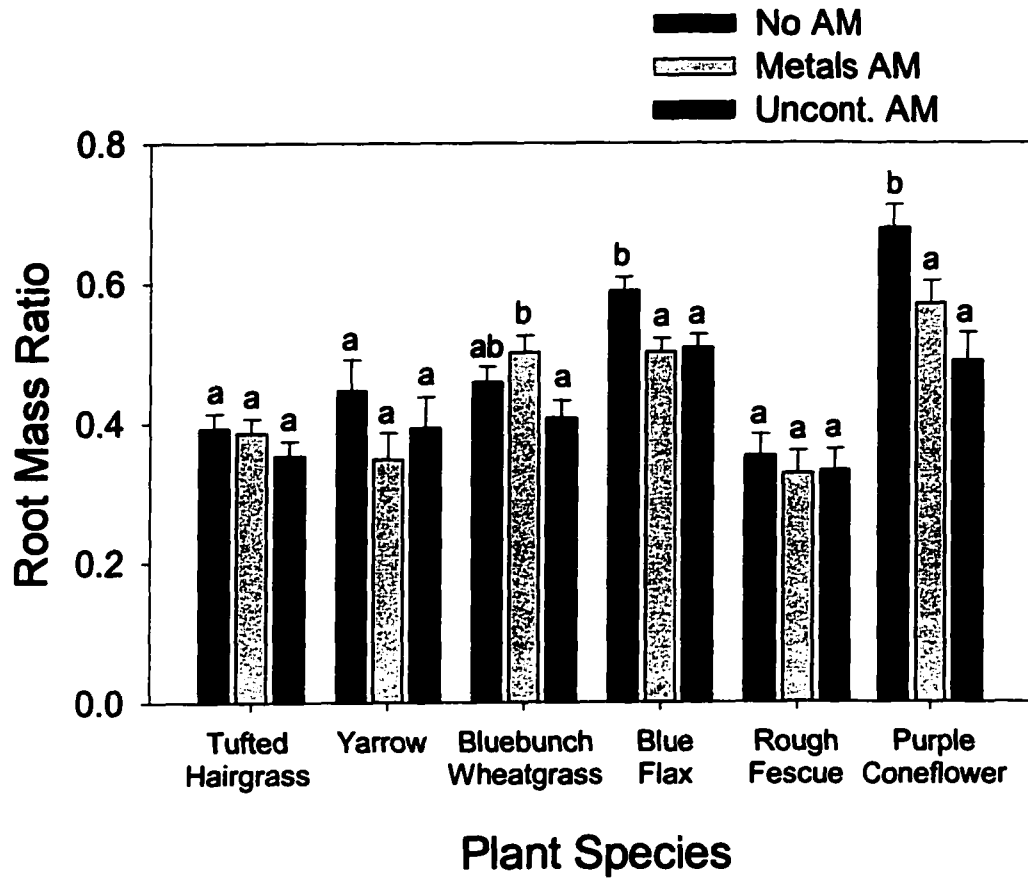


Figure 2. Root mass ratios of plants with AM treatments in each species. Error bars represent standard error of the mean. Letters above bars designate statistically significant different means between AM treatments, within each plant species.

CHAPTER 5

Effects of Compost and Arbuscular Mycorrhizal Inocula on Simple Native Plant Communities Transplanted for Mine Waste Revegetation

ABSTRACT

Restoration of disturbed lands can be enhanced by ecological amendments such as compost addition and mycorrhizal inoculation, but documentation of their individual and combined effects on plant growth in the field is rare. This study examines the effects of compost addition and arbuscular mycorrhizal (AM) source on three plant species in three coversoil substrates. Inoculated transplants of tufted hairgrass (*Deschampsia cespitosa*), bluebunch wheatgrass (*Pseudoroegneria spicata*), and yarrow (*Achillea millefolium*) were planted into coversoil substrates (overburden, low-sulfide ore tailings, and reprocessed tailings) with and without compost. The AM treatments included 1) sterilized inoculum, 2) inoculum from a metals-contaminated site, and 3) inoculum from an uncontaminated site. Coversoil substrates with and without compost addition varied in moisture, pH, nutrient levels, metal levels, and microbial numbers. After two years of growth, plants growing in compost-amended substrates had 84% greater biomass, with a 111% increase in number of flowering stalks. Overall, AM from metals-contaminated soil increased plant biomass by 19% and number of flowering stalks by 20% over nonmycorrhizal plants, while plant growth with AM from uncontaminated soils was not different from nonmycorrhizal plants. Similar trends occurred within each plant species, although differences were less conspicuous and not statistically significant. This research improves our understanding of ecological mine restoration and demonstrates that revegetation benefits most from a combination of compost addition and appropriate AM inoculation.

INTRODUCTION

Ecological restoration uses ecological knowledge to guide rehabilitation of disturbed lands (Jordan et al. 1987, Young et al. 2001). Plant ecologists have become increasingly aware that plant community dynamics often depend on the abiotic and biotic soil environment. Plant communities are shaped by environmental gradients as individual plant species balance resource acquisition with physiological limits to environmental stress (Lambers et al. 1998). Soil biota control plant communities in a variety of ways, ranging from general functions such as organic matter turnover to very specific symbioses (Brussaard 1997, Martin et al. 2001). While plant-microbe interactions range from positive to negative, soil microorganisms can potentially enhance plant growth by increasing nutrient availability, providing pathogen protection, and improving soil structure (Bolton et al. 1992, Martin et al. 2001). Understanding effects of biotic and abiotic soil factors on plants will improve our ability to manage disturbed sites and successfully rehabilitate plant-soil systems.

Among the most challenging systems to restore are environments disturbed by hardrock mining. Historic and active mining has resulted in billions of tons of metal-laden mine wastes deposited in many regions of the world (Moore and Luoma 1990, Dudka and Adriano 1997, Tordoff et al. 2000). Accelerated by weathering, many sulfidic mine wastes can acidify and leach toxic metals into surface, subsurface and groundwater systems, becoming hazardous to microbes, plants, animals, and humans (Moore and Luoma 1990, Dudka and

Adriano 1997). Revegetation is an important component of mine waste rehabilitation, to stabilize sediments and immobilize heavy metals. Establishing plant communities in such complex and harsh environments requires a solid understanding of the primary ecosystem components involved with revegetation, including soil, microorganisms, and plants.

Reclamation protocols for potentially hazardous mine wastes often include covering waste materials with benign soils to prevent contaminant exposure and entry into the environment, and to provide suitable growth media for revegetation (Smith 1988, Harris et al. 1996). While healthy topsoil is desirable for revegetation, the magnitude of many mine reclamation projects, the shortage of topsoil sources, and the disturbance associated with removing topsoil often result in the use of alternative coversoil to cover wastes and to provide adequate plant growth substrate (Munshower 1994, Jennings and Krueger 2000). Acceptable coversoil may exist at the mine site, such as overburden found between the root zone and ore, which is removed prior to mining and saved for subsequent reclamation of the site. Alternative coversoils include milled tailings material, either from a low-sulfide ore which has minimal potential to form acids and release metals, or high-sulfide tailings that are reprocessed to remove sulfidic pyrite materials, leaving tailings that are relatively environmentally stable (Jennings and Krueger 2000). Because coversoils can vary substantially, it is important to examine the effect of soil amendments on revegetation across a variety of substrates types that are used for mine reclamation.

Revegetation can be limited in some coversoils because substrates are often relatively biologically inert, infertile, and physically inhospitable to plant life. Organic matter amendments, such as compost, are important for increasing soil organic carbon, introducing soil microorganisms, and improving soil chemical and physical characteristics (Tester 1990, Schoenholtz et al. 1992, Moynahan et al. 2000). Noyd et al. (1995, 1996, 1997) found that composted yard waste added to taconite iron ore tailings increased plant cover, microbial properties, and accrual of organic matter in soil. Organic additions result in increased plant cover (DeLuca and Lynch 1997, Meikle et al. 1999, Noyd et al. 1996), reduced soil bulk density (Schoenholtz et al. 1992, Tester 1990), lowered penetration resistance (Tester 1990), increased water content (Schoenholtz et al. 1992, Tester 1990), increased total and mineralizable N (Schoenholtz et al. 1992), decreased exchangeable metal levels (DeLuca and Lynch 1997) and altered pH (DeLuca and Lynch 1997, Tester 1990) in a variety of soil types. Such soil alterations can lead to improved conditions for soil biota and plant root growth.

Another complementary strategy for enhancing plant growth in inhospitable soils is inoculation with specialized microorganisms, such as mycorrhizal fungi (Pfleger et al. 1994, Khan et al. 2000, Tordoff et al. 2000). Arbuscular mycorrhizae (AM, sometimes also known as VAM) are symbiotic plant-fungal interactions that occur naturally in 90% off all plant families (Sylvia 1994). In exchange for increased nutrient uptake, the plant provides the fungus with its sole source of carbon. In several settings with metal-contaminated soils, mycorrhizal plants have exhibited enhanced growth and metal tolerance

compared to nonmycorrhizal plants (Gildon and Tinker 1983, Leyval et al. 1997, Meharg and Cairney 2000, Chapter 2). AM may be especially important for plant success under generally stressful conditions, as is often the case with mine-related disturbance (Johnson et al. 1997, Chapter 4). Field studies testing AM inoculation for mine revegetation are rare, but previous research indicates that AM inoculation resulted in increased plant production on mine tailings (Noyd et al. 1996), AM-inoculated legumes showed increased growth and survival over non-inoculated plants (Lambert and Cole 1980), and growth and development of container plants were enhanced with AM (Call and Davies 1988).

Although AM may be very important to plant success, mycorrhizal propagule density and colonization are often very low in mine spoils, even after reclamation (Jasper et al. 1989, Gould et al. 1996, Moynahan et al. 2002). Arbuscular mycorrhizal inoculum can be added to soils before seeding, or container plants can be inoculated in the nursery before transplanting (Pfleger et al. 1994, St. John 1990). While both approaches have an important role in revegetation, this study focuses on AM effects on inoculated transplants. A variety of inoculum sources are available for revegetation, ranging from native topsoil to commercial products (Norland 1993, St. John 1998), but little is known about how different AM inocula affect vegetation responses.

Most of the evidence indicating that AM may be important for revegetation has been conducted in the greenhouse, where light, nutrient, water, and temperature conditions differ from field environments. Since all of these environmental factors may affect AM function (Bethlenfalvay and Pacovsky 1983,

Bledsoe 1992, Johnson et al. 1997), greenhouse results may not accurately predict field responses. Also, most studies examine individually-grown plant responses to AM inoculation, rather than examining multiple species' responses in a plant community context. While individual plant effects are important, different species may exhibit diverse responses to inoculation and plant interactions can affect overall community effects (Miller and Allen 1992, Pfleger et al. 1994, Chapter 3).

Finally, many of these studies examine effects of single AM fungal species, while evidence indicates that AM fungal communities function differently than single species, and that different sources of AM fungi can affect plants differently (Van der Heijden et al. 1998, Meharg and Cairney 2000, Chapter 3). This suggests that plant community response should be examined with AM fungal communities from multiple sources. To expand our understanding of AM contributions to revegetation success, we must assess the function of AM in field conditions as they interact with different plant species, fungal sources and soil conditions.

Successful reclamation of disturbed sites requires attention to many interacting physical, chemical, and biological components of disturbed ecosystems. This research examines how compost and AM inoculation affect biomass and flower production of transplanted container plants in simple communities in several coversoil substrates. The objectives of this study were i) to test the effects of compost addition and AM inoculation on growth of transplanted simple plant communities in three coversoil growth substrates, ii) to

determine whether different AM inoculum sources (AM from metals-contaminated soil vs. AM from uncontaminated soil) affect plant growth differently, and iii) to determine whether compost and AM effects vary with different growth substrates and plant species.

METHODS

Site Description and Experimental Design

The study site was on waste areas of an active copper mine in southwest Montana (46°1'N, 112°30'W). The research area contained three geographically separate coversoil substrate types, including low-sulfide ore tailings, reprocessed tailings, and overburden substrate. The low-sulfide ore tailings were freshly milled tailings material from ore with known low sulfide content. The reprocessed tailings were produced from milled tailings, consisting of several mined ore grades, that were reprocessed with spiral separators to remove additional sulfides. The overburden material was a coversoil from the mine's stockpiles that is generally used by the mine for revegetation.

Using coversoil substrate areas as blocks, a blocked split-split-plot experiment was used to test the effects of compost addition and AM on plant growth of simple plant communities constructed with three native plant species. In fall of 1999, one test area, approximately 15m x 15m, was established at each of the three substrate deposits. Each area was divided into eight plots, which were randomly assigned a compost treatment (plus or minus compost). Compost was added at the rate of 90 dry tonnes/ha, incorporated to a depth of

15 cm to achieve 2% organic carbon, and all plots were fertilized (155 kg/ha 16-16-16 N-P-K). All plots were scarified, although due to technical difficulties the overburden and reprocessed tailings plots were disked while the low-sulfide ore tailings plots were roto-tilled. Within each of the 24 plots, three 1-m² subplots were randomly assigned an AM treatment. Each subplot received nine transplants, three each of three species, which had been propagated with the appropriate AM treatment. The 9 plants were randomly placed and evenly spaced within the 1m² subplots, with 0.25 m between neighboring plants. After transplanting, plants were covered with protective straw erosion control blankets in October 1999, which were removed in April 2000.

Plant Propagation

Plants were grown in the greenhouse for seven months prior to transplanting. Species include tufted hairgrass (*Deschampsia cespitosa*), bluebunch wheatgrass (*Pseudoroegneria spicata*) and yarrow (*Achillea millefolium*). Each plant species was grown with one of the following mycorrhizal inocula: 1) sterilized mycorrhizal inoculum, 2) inoculum from a metals-contaminated soil, or 3) inoculum from an uncontaminated soil. The plants were grown in autoclaved silica sand in 115 ml pots, with inoculum added as a layer of soil-sand mix in each pot, as described in the next section. Plants were grown in a greenhouse, thinned to one plant per pot, rotated bi-weekly in trays, and given dilute nutrients (10 ml 1/8-strength Hoagland's solution) every three weeks, or as needed. To minimize AM treatment effects before transplanting, the

nonmycorrhizal plants were given ¼-strength P during the third month of growth to compensate for nutrient imbalances due to mycorrhizal differences. A subsample of four transplants from each species-AM treatment combination was randomly selected at the time of planting, and shoot and root biomass and AM colonization levels were measured.

AM Inoculation

The metals-contaminated inoculum was collected from a mine tailings revegetation project in southwestern Montana that had neutralized tailings, high metal levels, and plants with high mycorrhizal colonization levels. The undisturbed inoculum was from a bluebunch wheatgrass (*Pseudoroegneria spicata*), Idaho fescue (*Festuca idahoensis*)-dominated grassland in western Montana with no metal contamination, and high mycorrhizal colonization rates. Inoculum soils were homogenized and sieved, using a 2 mm mesh.

AM inoculum was a combination of live soil from each inoculum source with sterilized soil from the alternate inoculum source, to minimize physical and chemical differences between inoculum source soils. The metals-contaminated soil AM treatment contained 5 ml metals-contaminated soil, 5 ml sterilized uncontaminated soil, and 5 ml sand. The uncontaminated soil AM treatment contained 5 ml uncontaminated soil, 5 ml sterilized metals-contaminated soil, and 5 ml sand. The nonmycorrhizal treatment contained 5 ml sterilized metals-contaminated soil, 5 ml sterilized uncontaminated soil, and 5 ml sand. The sterilized sand and soils were autoclaved twice at 121° C for 15 minutes, with 24

hours between autoclavings. The inoculum mixtures were added to pots and covered with 1 cm sterilized silica sand.

To isolate the effect of AM fungi from overall soil microbial effects, a microbial sievate prepared from both inoculum sources was added to all pots. The microbial sievate was prepared by passing a soil slurry (1:1:8 by weight, metals-contaminated soil:uncontaminated soil:deionized water) through a series of filters (250µm, 25µm, 11µm). This process excludes mycorrhizal propagules, while soil bacteria, nonmycorrhizal spores, and other microorganisms pass through the filters. Five ml of the appropriate microbial wash was added to each pot.

Soil Characterization

Sampling Method. Soils were characterized at the whole plot level, except for pH measurements, which were made at the subplot level to confirm homogeneity within plots. Soil samples were collected from each of the 24 research plots in October, 1999. Eight cores (2.2cm x 10cm) were taken from four areas across each compost treatment plot, extracted using a sterilized soil probe (Forestry Suppliers, Inc., Jackson, MS), and aseptically composited for one sample. Three soil cores were similarly collected and composited from each subplot for pH measurements. Soil samples were placed in sterile bags and transported on ice to the lab for processing within 24 hours. Samples not processed immediately were dried and stored for future soil analyses.

Soil Geochemical Analysis. Soil moisture contents were determined by gravimetric difference between wet and dry weights of soil samples. Twenty-five g of each sample were dried at 60° C for three days and weighed. For pH, 10 g of soil was combined with 10 ml of deionized water, allowed to equilibrate for 5 min, and measured with a pH probe (Accumet Basic, Fisher Scientific). For other geochemical analyses, samples were dried, sieved using a 2 mm mesh, and ground using a ball mill (Spex Industries, Inc.). Total C and N levels were determined by analyzing 0.25-0.5 g with an elemental analyzer (CE Instruments elemental analyzer). Inorganic carbon was measured by coulometry with acidification (H₂SO₄).

Concentrations of metals and other ecologically relevant elements were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) analysis for both digestible and water-extractable fractions of all samples. Soil samples to be analyzed for total metals were digested with a double acid, peroxide, hot-block digest, according to E.P.A. Method 3050B, modified to ¼ volume (Edgell, 1988). Water extractable metals were determined by combining 1 g of sieved, unground sample with 40 ml milli-Q water. Slurries were mixed on a shaker table for 12 hours and centrifuged for 20 minutes at 3500 rpm. Supernatants were filtered through 0.45 mm membrane and preserved with 80ml HNO₃. Analytes, including Cd, Cu, Fe, Pb, Zn, and P were quantified by ICP-AES analysis of soil extracts and digests using E.P.A. Method 200.7 (Jarrell-Ash ICP-AES 800 instrument) (Martin et al., 1994). The QA/QC results are summarized in Appendix A.

Culturable Bacteria and Fungi. Soil samples were homogenized, diluted 1:10 in 0.1% sodium pyrophosphate (Sigma Chemical Co., St. Louis, MO), and sonicated for 10 min. Heterotrophic bacteria and actinomycetes (as detected by colony and microscopic morphology) were determined on R2A agar (Difco Laboratories) following incubation for 4 d at 25°C. Total fungal counts were determined on Rose Bengal agar (Difco Laboratories) supplemented with 0.1g/L chloramphenicol (Sigma Chemical Co., St. Louis, MO).

Mycorrhizal Infectivity Potential. To determine baseline potential for mycorrhizal colonization, mycorrhizal bioassays were conducted in soils from each treatment plot. Bait plants grown in the test soil were harvested after several weeks and AM colonization levels were used as a measure of relative density of mycorrhizal propagules in that soil (Brundrett et al. 1996). Soil samples, consisting of six composited subsamples, were collected in ziplock bags, homogenized, and placed in 10 cm diameter pots. Each pot was seeded with *D. cespitosa*, the bait plant used to encourage mycorrhizal formation. After ten weeks, plants were harvested and roots were stained (Phillips and Hayman 1970) and examined for mycorrhizal colonization (McGonigle et al. 1990).

Plant Growth Measurements.

Baseline plant measurements (height, number of leaves) and transplant vigor estimates were taken during October 1999, one week after transplanting. Vigor was ranked on an arbitrary scale of 1 to 5, reflecting amount of discoloration, stunted growth, and dead leaf tissue. In August 2001, plants were

measured again (height, basal diameter, number of flowering stalks) and aboveground biomass was collected. Plant biomass samples were dried at 60°C for 4 d and then weighed to determine dry biomass. Root samples were collected from a subsample of plants to confirm AM status at the end of the experiment.

Statistical Analyses.

Soil differences at the whole-plot level, comparing substrate and compost treatments, were determined with 2-way analysis of variance (ANOVA, $\alpha=0.05$) for a randomized block design, followed by Tukey's post-hoc tests for multiple comparisons (SPSS 10.0). Data were transformed as necessary to meet assumptions of the tests. Total C and N were analyzed with non-parametric tests, using Kruskal-Wallis tests to detect substrate effects and Mann-Whitney tests to detect compost effects. Because the tailings soil treatments (treated as blocks) were not replicated, interactions between main effects were not tested, and inference is limited to these test areas.

Plant growth differences were determined with ANOVA for split-split-plot design ($\alpha=0.05$), which examined effects of substrate, compost, AM and plant species on aboveground biomass and number of flowering stalks (SAS 8.0, PROC MIXED). Subsequent contrasts examined effects of compost and AM overall, and compost and AM within each plant species. Because initial plant sizes and vigor were unequal between treatments, initial plant vigor was used as

a covariate in all plant analyses. Biomass and flower stalk count data were square root-transformed to meet assumptions of the statistical tests.

RESULTS

Soil Characteristics

Soil Geochemistry. Soil characteristics varied between substrates and compost treatments (Table 1). Soil moisture was significantly different between substrate types ($F_{2,18}=6.00$, $P=0.009$) but not between compost treatments ($F_{1,18}=2.201$, $P=0.154$). Large variances were associated with each treatment, and the general trend of increased moisture with compost was not significant in any tailings type.

Soil pH measurements were significantly different between substrate types ($F_{2,18}=12.54$, $P<0.001$), as well as compost treatment ($F_{1,18}=13.60$, $P=0.001$) (Table 1). In spite of the significant differences between these factors, the overall pH range was fairly narrow (6.99 to 8.21). In general, composted coversoil substrates had slightly lower pH than non-composted substrates.

Total carbon levels were below detection in all unamended coversoil substrates and were significantly increased with compost addition ($Z=-4.44$, $P<0.001$) (Table 1). Plots with compost had total carbon levels of approximately 2%, and there were no differences between substrates ($\chi^2_2=0.365$, $P=0.833$). Low levels of inorganic carbon were detected in all samples, with differences between substrates ($F_{2,18}=224.99$, $P<0.001$) and compost treatments ($F_{1,18}=5.41$,

$P=0.031$). The low-sulfide ore tailings had the highest inorganic carbon, while the overburden had the lowest.

Both total N and total digestible P were greater in composted plots (total N: $Z=-2.32$, $P=0.020$; total P: $F_{1,18}=65.407$, $P<0.001$), but did not differ between coversoil substrates (Table 1). Water-extractable P was higher in composted plots ($F_{1,18}=1021.33$, $P<0.001$), and higher in overburden plots than in the other two substrates ($F_{2,18}=160.28$, $P<0.001$) (Table 1). There were significant differences in water-extractable K levels between substrate ($F_{2,18}=17.01$, $P<0.001$) and compost ($F_{1,18}=8.404$, $P=0.010$) treatments. Potassium was highest in the low-sulfide ore tailings, lower in the reprocessed tailings, and lowest in the overburden, while compost addition resulted in higher extractable K levels.

Total digestible metals varied greatly between treatments and analytes (Table 1). The overburden plots had the highest levels of all metals, while the reprocessed tailings had higher metal levels than the low-sulfide ore tailings for most elements. Compost did not affect lead levels ($F_{1,18}=0.07$, $P=0.795$), slightly decreased copper ($F_{1,18}=4.06$, $P=0.058$) and iron ($F_{1,18}=3.60$, $P=0.072$), slightly increased arsenic in the overburden plots ($t_{1,6}=2.12$, $P=0.078$), and increased zinc levels in all substrates ($F_{1,18}=17.72$, $P<0.001$).

Culturable Bacteria and Fungi. The abundance of bacteria and fungi, as detected by indirect culturable counts, was affected by both coversoil substrate and compost (Table 2). Substrate type did not have an effect on the number of detectable heterotrophic bacteria ($F_{2,18}=1.292$, $P=0.297$), but compost addition

greatly increased the number of heterotrophic bacteria ($F_{1,18}=114.89$, $P<0.001$). Both substrate ($F_{2,18}=13.77$, $P<0.001$) and compost addition ($F_{1,18}=23.26$, $P<0.001$) influenced the number of actinomycete colonies detected (Table 2). Actinomycete colonies were detected in overburden, while neither low-sulfide ore nor reprocessed tailings samples had detectable ($>10^3$) actinomycetes. All substrate types had higher actinomycete numbers in composted plots compared to non-composted plots. Coversoil substrate strongly influenced the number of culturable fungi detected ($F_{2,18}=8.79$, $P=0.002$), and compost had an even stronger effect ($F_{1,18}=51.83$, $P<0.001$) (Table 2). The overburden and reprocessed substrates had low levels of fungi, while the low-sulfide ore tailings did not yield any detectable fungal colonies. Mean fungal numbers were higher in the composted soils than their non-composted counterparts.

Baseline Mycorrhizal Infectivity Potential. Mycorrhizal infectivity potential assays use bait plants to quantify the potential for mycorrhizal colonization, as determined by the presence of AM propagules and favorable abiotic soil conditions. No mycorrhizal colonization was detected in any of the bioassays conducted on any soil samples (Table 2). This confirms that AM effects in the field were not confounded by pre-existing native AM inoculum.

AM and Compost Effects on Transplant Growth

In spite of efforts to maintain even transplant sizes until planting, baseline transplant sizes varied between AM treatments in a random subsample of plants.

At the time of planting, biomass was different between all AM treatments ($F_{2,27}=17.22$, $P<0.001$) with greatest biomass in nonmycorrhizal plants (mean 0.24 g), followed by plants with AM from uncontaminated soil (mean 0.19 g) and lowest biomass with AM from metals-contaminated soil (mean 0.13 g). This pattern was consistent across plant species. All AM-inoculated plants subsampled prior to planting were mycorrhizal, while all nonmycorrhizal control plants that were subsampled had no sign of AM colonization.

Final transplant growth in the field was evaluated by comparing aboveground biomass and number of flowering stalks. Biomass was affected by substrate type ($F_{2,381}=4.24$, $P=0.015$), compost ($F_{1,381}=163.94$, $P<0.001$), AM ($F_{2,381}=5.07$, $P=0.007$), and plant species ($F_{2,381}=74.48$, $P<0.001$) (Table 3). Both compost addition and AM inoculation increased overall plant biomass across growth substrate types and species (Figure 1a). Overall, compost addition increased aboveground biomass by 84% over plants in non-composted plots. Averaged across both compost treatments, AM from metals contaminated soil increased biomass by 19% over nonmycorrhizal plants ($t=3.13$, $P=0.010$) and 15% over plants with AM from uncontaminated soil ($t=2.48$, $P=0.014$), but biomass of nonmycorrhizal plants and plants with AM from uncontaminated soil did not differ. Mycorrhizae from metals-contaminated soil increased biomass in composted plots, but there were no significant effects of AM in the non-composted plots. The number of flowering stalks produced was highly affected by compost ($F_{1,382}=124.94$, $P<0.001$) and species ($F_{2,382}=21.06$, $P<0.001$), and affected to a lesser degree by AM ($F_{2,382}=2.81$, $P=0.062$) and substrate

($F_{2,382}=4.36$, $P=0.013$). Like biomass, the number of flowering stalks increased by 111% with compost addition (Figure 1b). Averaged across both compost treatments, AM from metals contaminated soil resulted in 20% more flowering stalks than nonmycorrhizal plants ($t=2.34$, $P=0.019$), but there were no differences between uncontaminated soil AM and other AM treatments. While mean number of flowering stalks tended to be higher with AM from metals-contaminated soil, there were no significant differences between AM treatments within each compost level.

While effects of compost and AM were the focus of this study, it is important to note the differences between substrates and individual plant species and how those differences affect compost and AM effects. Plant species differed in average biomass ($F_{2,381}=74.48$, $P<0.001$), with yarrow producing the greatest biomass (overall mean 49 g), followed by bluebunch wheatgrass (overall mean 20 g) and tufted hairgrass (overall mean 14 g). Number of flowering stalks for yarrow (overall mean 27) and bluebunch wheatgrass (overall mean 30) were not significantly different from each other, while tufted hairgrass produced the fewest (overall mean 15; $F_{2,382}=21.06$, $P<0.001$). Averaged across substrates, all species tended to produce higher mean biomass (Figure 2) and flowering stalks (Figure 3) with AM from metals-contaminated soil compared to nonmycorrhizal plants, while the relative measures of plants inoculated with uncontaminated soil AM varied. In general, similar patterns were observed for numbers of flowering stalks (Figure 3). These increases were not statistically significant, probably due to large variances introduced by substrate effects.

Substrate blocks significantly affected plant growth effects, although because this factor was not replicated, it could not be determined whether observed effects were due to the substrate type or confounding effects of location. The low-sulfide ore tailings plots produced greater biomass than the reprocessed tailings and overburden substrate, which were not different from each other ($F_{2,381}=4.24$, $P=0.015$). The low-sulfide ore tailings plants produced more flowers than the reprocessed tailings, while the overburden substrate had intermediate numbers ($F_{2,382}=4.36$, $P=0.013$).

DISCUSSION

The three growth substrates showed many significant biological, geochemical and physical differences. Soil moisture was highly variable within substrate types, but was greater in the low-sulfide ore tailings than the overburden. pH was approximately neutral in all substrates, providing suitable conditions for plant growth. Percent total C and N were below detection in all substrates, while P levels were similar across substrates. Total metal levels were highest in the overburden, followed by the reprocessed tailings. The overburden soil had generally higher baseline microbial characteristics (actinomycetes and fungi) than the low-sulfide ore and reprocessed tailings, although the reprocessed tailings had comparable fungal levels. While these substrates differ, it is difficult to predict which combination of characteristics would be most conducive to plant growth.

The addition of compost improved conditions for plant growth across substrates. The compost greatly increased total soil C, as well as N, P, and K. Compost addition increased heterotrophic bacteria, actinomycetes and fungal populations. These increases in soil fertility and microbial community parameters are conducive to creating functioning soil for successful revegetation.

Plant growth effects after two years improved with both compost addition and AM inoculation. These effects varied with plant species and growth substrate blocks. Aboveground biomass represents productivity of the plants, while number of flowering stalks reflects reproduction investment. Overall plant production and reproductive structures were greatly increased by compost addition, as has been observed in previous research (Noyd et al. 1996, Deluca and Lynch 1997, Johnson 1998). This effect may be due to a variety of changes including improved nutrient availability, water-holding capacity, microbial community, and soil structure (Tester 1990, Schoenholtz et al. 1992, Moynahan 2000).

Previous research has shown that AM associations can increase plant growth and reproductive structures (Lu and Koide 1994, Noyd et al. 1996, Chapters 1, 2, 3). In this study, AM from metals-contaminated soil increased biomass, but not number of flowering stalks in composted plots. In non-composted plots there was not a significant effect of AM on biomass or flowers. AM from uncontaminated soil did not affect plant growth in either situation. Mycorrhizae tend to provide greater benefit to plants under stressful and/or low-fertility conditions, but symbionts may not be able to function if environmental

conditions are too harsh (Johnson et al. 1997). This research indicates that for mine reclamation, where alternative tailings treatments result in varying levels of stress, AM provide the greatest benefit when soils have been ameliorated with compost. Mine revegetation benefits most from the combination of organic soil amendment and mycorrhizal inoculation, supporting results found with AM and compost in taconite iron ore tailings (Noyd et al. 1996) and with ectomycorrhizae and organic amendment in afforestation (Garcia et al. 2000).

When AM and compost effects were examined by species, treatment effects were less conspicuous. This may be partially due to relatively low sample sizes, but it underscores the fact that AM function is determined by the biotic and abiotic environment (Miller and Allen 1992). Previous research comparing the same AM sources used here with six native plant species in the greenhouse showed that yarrow, bluebunch wheatgrass and tufted hairgrass, the three species used in this experiment, were the least mycorrhizal dependent (Chapter 3). It is possible that AM effectiveness may have been more pronounced with a more diverse group of host plant species, including those with greater mycorrhizal dependence. These findings emphasize the importance of examining mycorrhizal function in the context of plant communities, as AM may be affected by biotic interactions that are too complex to measure experimentally.

The AM inoculum from metals-contaminated soil enhanced plant performance over nonmycorrhizal plants, while AM inoculum from uncontaminated soil resulted in slightly higher means that were not significantly different from the other AM treatments. Because these substrates were not

metal-rich, the difference in benefits from AM sources are likely due to differences in general stress tolerance of the AM fungi, or differences in fungal-plant functioning, between the two AM fungal communities. That AM inocula vary in the benefits they provide to host plants emphasizes the necessity for reclamation managers to investigate inoculum sources to confirm that inoculation will help achieve the goals of specific reclamation projects.

It should be noted that this study examines AM and compost effects on growth of container transplants that were 7 months old at planting. Some research indicates that AM effects vary during early plant growth stages (Johnson et al. 1997), which would not be reflected in these data, but may be important for AM inoculation of seeded mine revegetation. Also, AM effects are often inhibited by P fertilization. While the fertilization in this study was moderate, the addition of inorganic P could have limited the observed AM effects.

From a management perspective, it is essential to understand the factors that lead to stabilization of mine wastes, and the conditions under which plants establish and become stable plant communities. The research presented here demonstrates that compost and AM can increase biomass and reproductive structures of simple native plant communities across several coversoil substrate types. By improving our understanding of how plants are affected by interactions with microorganisms and soils, we enhance our ability to design and implement successful restoration of disturbed lands.

ACKNOWLEDGMENTS

This work was supported by the Reclamation Research Unit at Montana State University, the Montana Department of Natural Resources and Conservation, the Montana Department of Environmental Quality and the NSF-funded Training Within Environmental Biology program at the University of Montana (NSF-GRT #9553611 to P. Kukuk, C. Brewer, and F. Allendorf). Thanks to Stuart Jennings, Sara Barth, Marilyn Pratter and Kendra Hinxman for field and laboratory assistance.

REFERENCES

- Bethlenfalvay, G.J. and R.S. Pacovsky. 1983. Light effects in mycorrhizal soybeans. *Plant Physiology* 73:969-972.
- Bledsoe, C.S. 1992. Physiological ecology of ectomycorrhizae: implications for field application. In *Mycorrhizal Functioning*. M.F. Allen, ed., pp. 424-437, Chapman & Hall, Inc. London.
- Bolton, H.J., J.K. Frederickson, and L.F. Elliot. 1992. Microbial ecology of the rhizosphere. In *Soil Microbial Ecology*. F.B. Metting, Jr. ed., pp. 27-63, Marcel Dekker, Inc. New York.
- Brundrett, M., N. Bougher, B. Dell, T. Grove, and N. Malajczuk. 1996. *Working with Mycorrhizas in Forestry and Agriculture*, Australian Center for International Agricultural Research (ACIAR) Monograph 32, Canberra, Australia. 374pp.
- Brussaard, L. et al. 1997. Biodiversity and ecosystem functioning in soil. *Ambio* 26:563-570.
- Call, C.A. and F.T. Davies. 1988. Effects of vesicular-arbuscular mycorrhizae on survival and growth of perennial grasses in lignite overburden in Texas. *Agriculture, Ecosystems & Environment* 24:395-405.
- Deluca, T.H. and E.L. Lynch. 1997. Assessment of amendments in the reclamation of an abandoned mine in Montana. *Intermountain Journal of Sciences* 3:38-46.
- Dudka, S. and D.C. Adriano. 1997. Environmental impacts of metal ore mining and processing: a review. *Journal of Environmental Quality* 26:590-602.
- Edgell, K. 1988. USEPA method study 37-SW-846 method 3050 acid digestion of sediments, sludges, and soils. EPA contract No. 68-03-3254, November 1988.
- Garcia, C., T. Hernandez, A. Roldan, J. Albaladejo, and V. Castillo. 2000. Organic amendment and mycorrhizal inoculation as a practice in afforestation of soils with *Pinus halepensis* Miller: effect on their microbial activity. *Soil Biology & Biochemistry* 32:1173-1181.
- Gildon, A. and P.B. Tinker. 1983. Interactions of vesicular-arbuscular mycorrhizal infection and heavy metals in plants: I. The effects of heavy metals on the development of vesicular-arbuscular mycorrhizas. *New Phytologist* 95:247-261.
- Gould, A.B., J.W. Hendrix, and R.S. Ferriss. 1996. Relationship of mycorrhizal activity to time following reclamation of surface mine land in western Kentucky. I. Propagule and spore population densities. *Canadian Journal of Botany* 74:247-261.

Harris, J.A., P. Birch, and J. Palmer. 1996. *Land Restoration and Reclamation - Principles and Practice*, Addison Wesley Longman Ltd. Essex, England. 230pp.

Jasper, D.A., L.K. Abbott, and A.D. Robson. 1989. The loss of VA mycorrhizal infectivity during bauxite mining may limit the growth of *Acacia pulchella* R.Br. *Australian Journal of Botany* 37:33-42.

Jennings, S.R. and J.M. Krueger. 2000. Metal mine tailing reprocessing for enhanced vegetation establishment: clean tailing technology. In *Proceedings of the 2000 Billings Land Reclamation Symposium*. pp. 544-553, Reclamation Research Unit, Montana State University, Bozeman, Montana.

Johnson, N.C., J.H. Graham, and F.A. Smith. 1997. Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phytologist* 135:575-585.

Johnson, N.C. 1998. Responses of *Salsola kali* and *Panicum virgatum* to mycorrhizal fungi, phosphorus and soil organic matter: implications for reclamation. *Journal of Applied Ecology* 35:86-94.

Jordan III, W.R., M.E. Gilpin, and J.D. Aber. 1987. Restoration ecology: ecological restoration as a technique for basic research. In *Restoration Ecology*. W.R. Jordan III, M.E. Gilpin, and J.D. Aber, eds., pp. 3-22, Cambridge University Press, Cambridge, UK.

Khan, A.G., C. Kuek, T.M. Chaudhry, C.S. Khoo, and W.J. Hayes. 2000. Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. *Chemosphere* 41:197-207.

Lambers, H., F.S. Chapin, and T.L. Pons. 1998. *Plant Physiological Ecology*, Springer-Verlag New York Inc. New York. 540pp.

Lambert, D.H. and H. Cole, Jr. 1980. Effects of mycorrhizae on establishment and performance of forage species in mine spoil. *Agronomy Journal* 72:257-260.

Leyval, C., K. Turnau, and K. Haselwandter. 1997. Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. *Mycorrhiza* 7:139-153.

Lu, X. and R.T. Koide. 1994. The effects of mycorrhizal infection on components of plant growth and reproduction. *New Phytologist* 128:211-218.

Martin, F.M., S. Perotto, and P. Bonfante 2001. Mycorrhizal fungi: a fungal community at the interface between soil and roots. In *The rhizosphere* -

biochemistry and organic substances at the soil-plant interface. R. Pinton, Z. Varanini, and P. Nannipieri, eds., pp. 263-296, Marcel Dekker, Inc. New York.

Martin, T.D., C.A. Brockhoff, J.T. Creed, and EMMC Methods Workgroup. 1994. Method 200.7, Revision 4.4, Determination of Metals and trace elements in water and wastes by inductively coupled plasma atomic emission spectrometry. In *Methods for the determination of metals in environmental samples*. EPA/600/R-94/111. Environmental Protection Agency, Cincinnati, OH.

McGonigle, T.P., M.H. Miller, D.G. Evans, G.L. Fairchild, and J.A. Swan. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist* 115:495-501.

Meharg, A.A. and J.W.G. Cairney. 2000. Co-evolution of mycorrhizal symbionts and their hosts to metal-contaminated environments. *Advances in Ecological Research* 30:69-112.

Meikle, T.W., S. Lu, and J.P. Barta. 1999. Evaluation of nutrient and biological amendments on waste rock. In *Proceedings of the Society for Mine Engineering Annual Meeting*. Denver, CO. March 1-3, 1999.

Miller, S.L. and E.B. Allen. 1992. Mycorrhizae, nutrient translocation, and interactions between plants. In *Mycorrhizal Functioning*. M.F. Allen, ed., pp. 301-332, Chapman & Hall, Inc. London.

Moore, J.N. and S.N. Luoma. 1990. Hazardous wastes from large-scale metal extraction. *Environmental Science and Technology* 24:1278-1285.

Moynahan, O.S., S.R. Jennings, and C.A. Zabinski. 2000. Microbial inoculation potential of organic matter amendments for mine tailings reclamation. In *Proceedings of the 2000 Billings Land Reclamation Symposium*. pp. 645-654, Reclamation Research Unit, Montana State University, Bozeman, Montana.

Moynahan, O.S., C.A. Zabinski, and J.E. Gannon. 2002. Microbial community structure and carbon-utilization diversity in a mine tailings revegetation study. *Restoration Ecology* 10:77-87.

Munshower, F.F. 1994. *Practical Handbook of Disturbed Land Revegetation*, Lewis Publishers, Inc. Boca Raton, Florida. 140pp.

Norland, M.R. 1993. Soil factors affecting mycorrhizal use in surface mine reclamation. Information circular 9345. United States Department of the Interior Bureau of Mines, Pittsburgh, PA, 24 pp.

Noyd, R.K., F.L. Pflieger, M.R. Norland, and M.J. Sadowsky. 1995. Native prairie grasses and microbial community responses to reclamation of taconite iron ore tailing. *Canadian Journal of Botany* 73:1645-1654.

Noyd, R.K., F.L. Pflieger, and M.R. Norland. 1996. Field responses to added organic matter, arbuscular mycorrhizal fungi, and fertilizer in reclamation of taconite iron ore tailing. *Plant and Soil* 179:89-97.

Noyd, R.K., F.L. Pflieger, M.R. Norland, and D.L. Hall. 1997. Native plant productivity and litter decomposition in reclamation of taconite iron ore tailing. *Journal of Environmental Quality* 26:682-687.

Pflieger, F.L., E.L. Stewart, and R.K. Noyd. 1994. Role of VAM fungi in mine land revegetation. In *Mycorrhizae and Plant Health*. F.L. Pflieger and R.G. Linderman, eds., pp. 47-82, The American Phytopathological Society, St. Paul, Minnesota.

Phillips, J.M. and D.S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* 55:158-161.

Schoenholtz, S.H., J.A. Burger, and R.E. Kreh. 1992. Fertilizer and organic amendment effects on mine soil properties and revegetation success. *Soil Science Society of America Journal* 56:1177-1184.

Smith, M.A. 1988. Reclamation and treatment of contaminated land. In *Rehabilitating Damaged Ecosystems - Volume I*. J. Cairns, Jr. ed., pp. 61-90, CRC Press, Inc. Boca Raton, Florida.

St. John, T.V. 1990. Mycorrhizal inoculation of container stock for restoration of self-sufficient vegetation. In *Environmental Restoration: Science and Strategies for Restoring the Earth*. J.J. Berger, ed., pp. 103-112, Island Press, Washington, D.C.

St. John, T.V. 1998. Mycorrhizal inoculation in habitat restoration. *Land and Water* September/October:17-19.

Sylvia, D.M. 1994. Vesicular-arbuscular mycorrhizae. In *Methods of Soil Analysis, Part 2. Microbiological and biochemical properties*. pp. 351-378, Soil Science Society of America, Madison, WI.

Tester, C.F. 1990. Organic amendment effects on physical and chemical properties of a sandy soil. *Soil Science Society of America Journal* 54:827-831.

Tordoff, G.M., A.J.M. Baker, and A.J. Willis. 2000. Current approaches to the revegetation and reclamation of metalliferous mine wastes. *Chemosphere* 41:219-228.

Van der Heijden, M.G.A., J.N. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A. Wiemken, and I.R. Sanders. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability, and productivity. *Nature* 396:69-72.

Young, T.P., J.M. Chase, and R.T. Huddleston. 2001. Community succession and assembly - comparing, contrasting and combining paradigms in the context of ecological restoration. *Ecological Restoration* 19:5-18.

Table 1. Soil geochemical characteristics of substrate and compost plots (whole plots). Values represent means of 4 replicate samples. P and K values represent water-extractable fractions, while As, Cu, Fe, Pb and Zn values represent total digestible levels.

	<u>Low-Sulfide Ore</u>		<u>Overburden</u>		<u>Reprocessed Tailings</u>	
	<u>No Compost</u>	<u>Compost</u>	<u>No Compost</u>	<u>Compost</u>	<u>No Compost</u>	<u>Compost</u>
pH	7.99	7.71	7.66	7.32	7.50	7.20
%Moisture	8.97	9.46	4.08	4.95	6.89	11.04
% Total N	<0.08	0.12	<0.08	0.13	<0.08	0.09
% Total C	<0.75	2.13	<0.75	2.59	<0.75	1.56
P (ppm)	<2	16.51	6.26	37.78	0.58	12.16
K (ppm)	293	330	62	171	165	255
As (ppm)	<10	<10	12.06	13.66	<10	<10
Cu (ppm)	218	230	1111	996	658	634
Fe (ppm)	19,598	18,072	28,318	26,199	20,612	21,361
Pb (ppm)	16	21	117	102	36	43
Zn (ppm)	155	201	274	339	151	197

Table 2. Soil biological characteristics of substrate and compost plots (whole plots). Table values represent means of 4 replicate samples. Colony forming units (CFU) reflect abundance of each microbial group as determined by indirect counts. Mycorrhizal infectivity potential (MIP) represents the average mycorrhizal colonization. BD = Below Detection. Numbers in parentheses indicate the limit of detection.

	<u>Low-Sulfide Ore</u>		<u>Overburden</u>		<u>Reprocessed Tailings</u>	
	<u>No Compost</u>	<u>Compost</u>	<u>No Compost</u>	<u>Compost</u>	<u>No Compost</u>	<u>Compost</u>
Heterotrophic Bacteria (CFU/g)	1.65x10 ⁶	7.40x10 ⁷	6.50x10 ⁵	3.33x10 ⁷	1.52x10 ⁶	5.99x10 ⁵
Actinomycetes (CFU/g)	BD (<10 ³)	1.40x10 ⁵	1.11x10 ⁵	5.83x10 ⁵	BD (<10 ³)	8.00x10 ⁴
Fungi (CFU/g)	BD (<10 ²)	1.12x10 ⁵	2.23x10 ³	1.78x10 ⁵	2.95x10 ³	3.45x10 ⁴
MIP (%)	BD	BD	BD	BD	BD	BD

Table 3. ANOVA results for aboveground biomass and number of flowering stalks of three plant species grown in different coversoil substrates, compost amendments, and AM inocula.

	Biomass (sqrt-transformed)			Number of Flower Stalks (sqrt-transformed)		
	<u>DF</u>	<u>F value</u>	<u>P value</u>	<u>DF</u>	<u>F value</u>	<u>P value</u>
Substrate	2, 381	4.24	0.015	2, 382	4.36	0.013
Compost	1, 381	163.94	<0.001	1, 382	124.94	<0.001
AM	2, 381	5.07	0.007	2, 382	2.81	0.062
Species	2, 381	74.48	<0.001	2, 382	21.06	<0.001
Vigor	1, 381	24.54	<0.001	1, 382	8.61	0.004
Substrate*compost	2, 381	36.39	<0.001	2, 382	29.63	<0.001
Compost*AM	2, 381	0.07	0.930	2, 382	0.01	0.986
AM*Species	4, 381	0.28	0.889	4, 382	0.10	0.981
Compost*species	2, 381	7.24	0.001	2, 382	11.21	<0.001
Substrate*Compost*AM	8, 381	0.91	0.510	8, 382	1.12	0.350
Compost*AM*Species	4, 381	0.63	0.639	4, 382	0.61	0.656

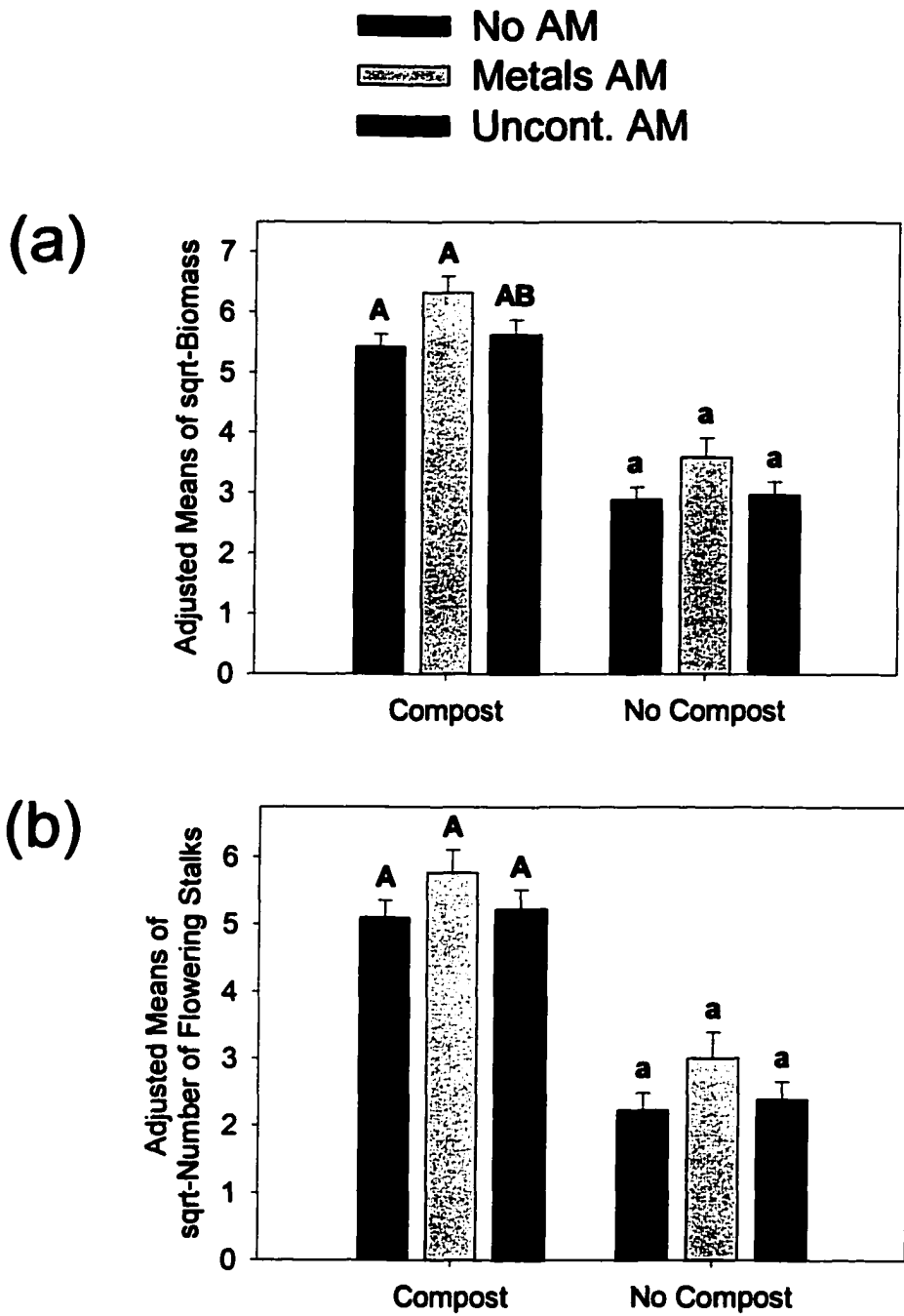


Figure 1. Final transplant growth measurements. Adjusted means reflect a) square root-transformed biomass and b) square root-transformed number of flowering stalks, adjusted for the covariate (initial plant vigor) and the unbalanced split-split-plot design. Error bars represent standard error of the mean. Letters above bars designate statistical significance ($\alpha=0.05$) within each compost treatment.

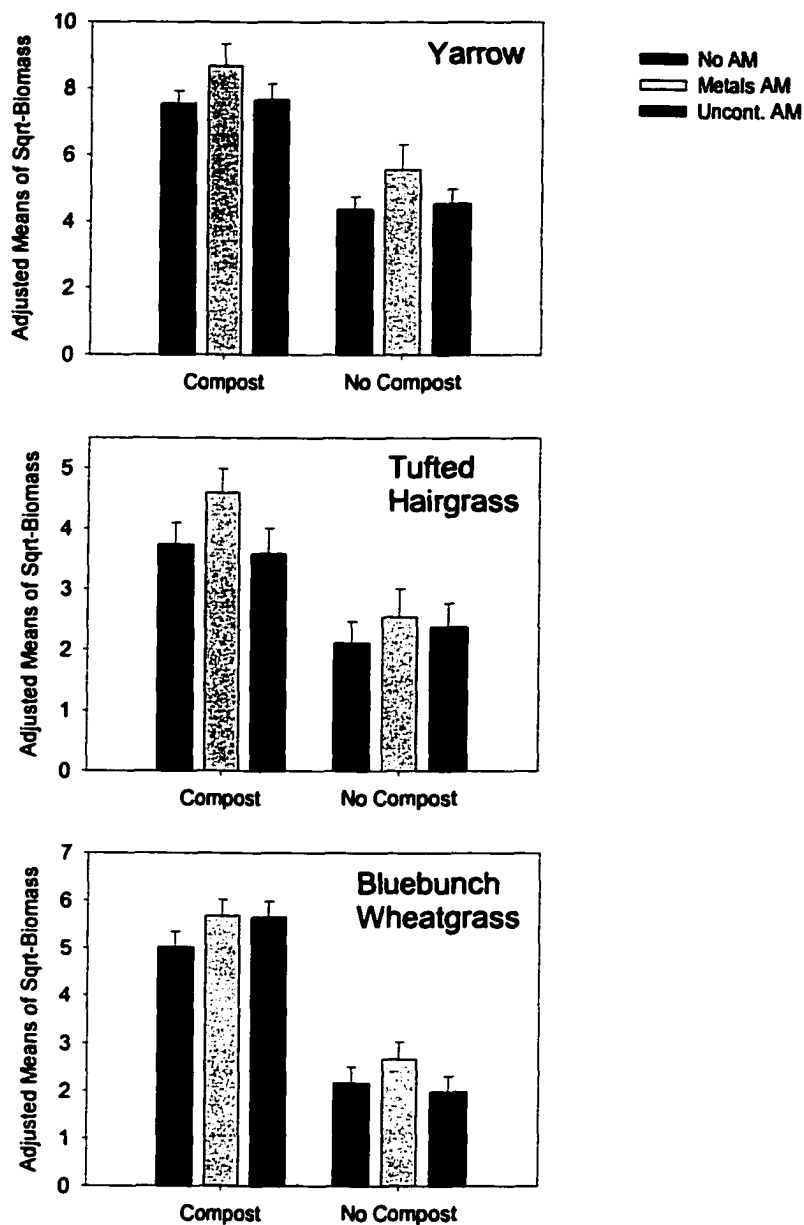


Figure 2. Aboveground biomass, comparing AM effects within compost treatments for each plant species. Adjusted means reflect square root-transformed biomass, adjusted for the covariate (initial plant vigor) and the unbalanced split-split-plot design. Error bars represent standard error of the mean. There were no significant differences between AM treatments within compost treatments.

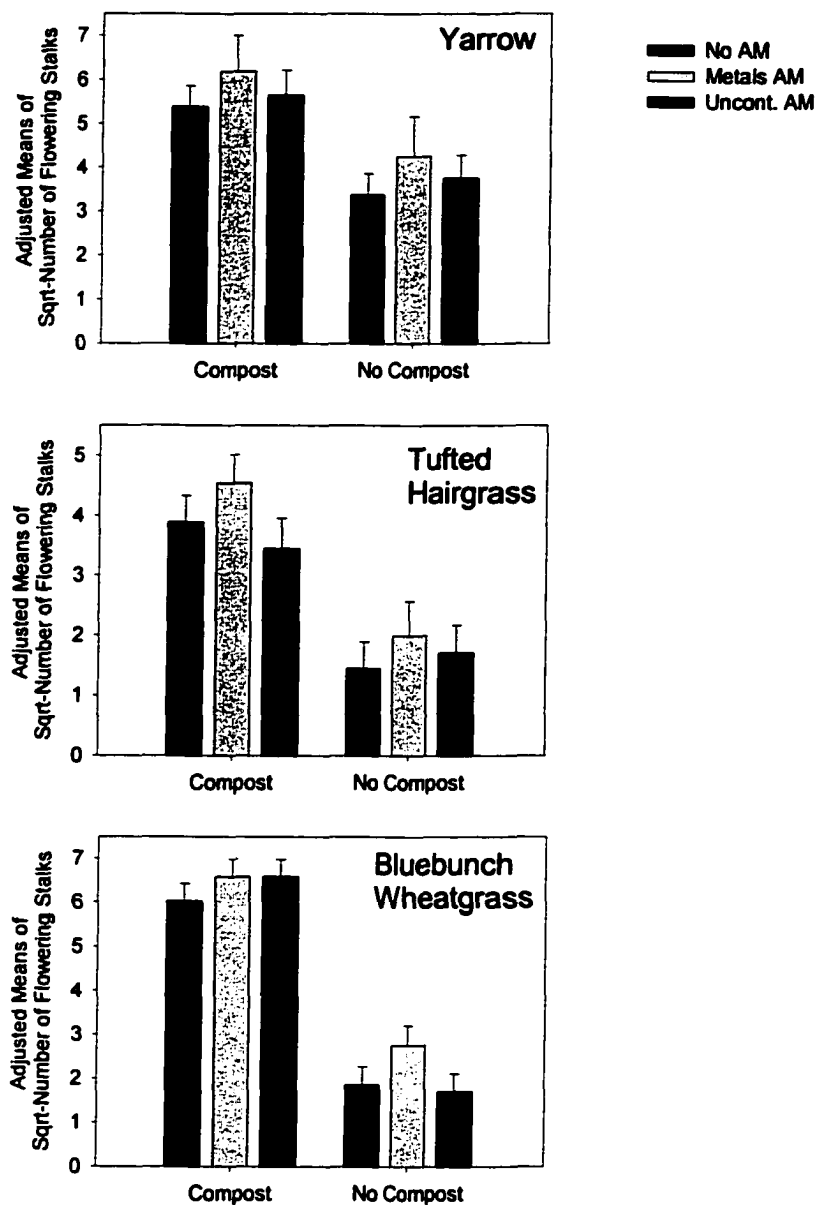


Figure 3. Number of flowering stalks, comparing AM effects within compost treatments for each plant species. Adjusted means reflect square root-transformed number of flowering stalks, adjusted for the covariate (initial plant vigor) and the unbalanced split-split-plot design. Error bars represent standard error of the mean. There were no significant differences between AM treatments within compost treatments.

CHAPTER 6

Incorporating Ecological Concepts into Mine Restoration Practices

INTRODUCTION

The practice of planning and implementing restoration of mine sites in Montana is necessarily interdisciplinary. A great variety of considerations – ecological, social, economic, political, and legal – influence each stage of the process. Scientists are charged with providing rigorous, defensible scientific information, and restoration practitioners are charged with selecting an appropriate course of action based on that scientific evidence, while constrained by many non-ecological considerations. While non-ecological considerations constrain the decision-space of practitioners, a project's success or failure ultimately rests on whether ecological goals are met. Successful restoration, therefore, depends heavily on effective information exchange between researchers and practitioners.

My Training WEB internship was designed to explore the status of and potential for improving current information exchange between research and management, while emphasizing the importance of disseminating scientific information to restoration practitioners and land managers. This chapter presents my internship experiences in the context of ecological restoration of mine wastes in Montana. I describe the problem of mine waste remediation and current perspectives on information exchange. I then present the rationale for and the design of the internship, provide an assessment of components of the internship, and conclude by offering suggestions for improving information exchange between researchers and practitioners.

ECOLOGICAL RESTORATION OF MINE WASTES

Mine Waste Reclamation

Over a century of mining in Montana has resulted in large quantities of mine wastes affecting public and private lands, including thousands of abandoned mine sites and one of the largest Superfund complexes in the nation (Moore and Luoma 1990). Agencies responsible for regulating and protecting public and private lands are faced with the mandate to manage these contaminated areas. Because of the recalcitrance of the contaminants, the remoteness and magnitude of some sites, and the sheer number of contaminated areas, mitigation of these mine wastes will be difficult and expensive. Successful reclamation of these sites depends on the amelioration of physical, geochemical and biological factors. Agencies base reclamation decisions on current scientific knowledge, which is rapidly evolving and interdisciplinary in nature.

A major component of restoration is revegetation, which may reduce surface runoff, increase soil stability, and provide complex organic molecules to immobilize metals (Munshower 1994). Infertile, acidic, metal-contaminated soils make plant colonization and development difficult, and many revegetation efforts have had limited success. In general, the great majority of restoration resources are often committed to physical and chemical treatment of wastes, leaving relatively little resources for revegetation and subsequent monitoring (Wiener et al. 1980). With increasing time to evaluate project performance since early reclamation projects, the need for multi-disciplinary perspectives to define the

best reclamation methods is apparent.

Ecological Restoration of Plant-Soil Systems

Significant advances in plant ecology have improved our understanding of plant community structure and function. Restorationists can use principles of plant ecology to develop more effective and efficient methods for reconstructing stable plant communities. The relationship between plant communities and the soil environment may be especially important for mine revegetation, because mine disturbance is often associated with abiotically harsh and biologically inert soils. My dissertation research (Chapters 2-5) adds to the growing evidence that plant growth in mine-disturbed soils can be significantly affected by soil microorganisms. While mycorrhizae in mine wastes are the focus of my research, they are just one component of complex and dynamic soil ecosystems that determine plant community structure and function. Restoration practitioners need a basic understanding of plant community function and interaction with soil environments to develop more successful and reliable revegetation strategies.

INFORMATION EXCHANGE: BASIC SCIENCE AND MANAGEMENT

While basic scientific research is generating crucial information regarding plant community development and function, this information is not efficiently transferred to restoration management. Communication between academic scientists and managers can be limited by many factors relating to both academic and management approaches (Huenneke 1995, Meffe 1998,

Robertson 2000). Conservation biology has struggled with these issues in recent years, as seen in several commentaries recommending changes in academic training and communication between scientists and managers. A primary cause of poor information exchange between research science and natural resource management may be differences in training of many conservation managers and traditional academic scientists (Noss 1997, Meffe 1998). Natural resource managers must be trained across multiple disciplines so they can assimilate a variety of scientific and social data that are needed to guide decisions (Noss 1997, Meffe 1998). Academic scientists, in contrast, are trained to specialize in a narrow scientific discipline, and do not necessarily have the background to recognize management implications of their research. Several other issues make communication between basic research science and conservation management difficult, including differences in motivation, goals, finances, staffing, and time frames (Huenneke 1995).

Ecological restoration is hindered by similar impediments to those highlighted by conservation biologists. Many people actually implementing restoration (such as mine personnel and contractors) are faced with immediate pressures of meeting regulations and specifications within budget and time constraints. Many regulators, responsible for developing specifications and regulations, are too time-constrained to independently seek out primary scientific literature and innovative approaches for restoration. In addition to the lack of accessible channels for communication, there is little published research examining restoration scenarios, and without training in specific scientific

disciplines, primary literature may be incomprehensible to many restoration managers. There is a need for scientific research done in realistic contexts and at appropriate scales for restoration, as well as better forums for communication and translation of those research findings to managers.

THE INTERNSHIP

Lack of information transfer between basic soil science and management, as well as a desire to understand the role of science in disturbed land management, motivated me to design and conduct an internship focused on this issue of natural resource management. The internship was designed to facilitate communication between restoration practitioners and basic scientific researchers, with an overarching goal of promoting the incorporation of ecological and soil biology principles in restoration strategies. The internship was sponsored by the Training Within Environmental Biology (Training WEB) program at the University of Montana and the Environmental Protection Agency (E.P.A.), and took place February 1, 2000 through December 31, 2000. The primary objectives of the internship were: 1) to develop an information transfer program with agency personnel to promote the incorporation of ecological concepts into restoration designs and 2) to participate in various stages of government agency restoration project planning to develop an understanding of the role of science in restoration management and provide assistance in applying ecological concepts to restoration. I pursued these objectives through two activities: workshops and participation in technical advising. Workshops were

conducted to improve communication with restoration practitioners about basic soil ecology research and its relevance to restoration, and then assess its efficacy based on feedback from workshop participants. Technical advising was included in the internship to allow more direct input to restoration projects, and to gain perspective on how effectively rigorous scientific information is applied to on-going, large-scale remediation planning processes.

Workshops

I conducted half- and full-day workshops to provide current information about relevant soil biology and ecology concepts to restoration practitioners. These workshops, titled "Ecological Restoration of Plant-Soil Systems," were held at the Billings Reclamation Symposium, the Mine Design Operations and Closures Conference, and for the Glacier National Park Restoration Group. Participants were primarily restoration managers from government agencies, including E.P.A., Montana Department of Environmental Quality, U.S.D.A. Forest Service, National Parks Service, and Office of Surface Mining, private consultants, academic scientists, and mining company reclamation managers.

Workshops began with an overview of restoration ecology, emphasizing the benefits of using principles from natural systems to guide reconstruction of disturbed lands. The first section of the workshop provided a brief overview of ecological concepts that are particularly relevant to restoration, including succession, plant-microbe interactions, and the relative importance of competition and facilitation. Once a context of ecological function was

established, I presented basic concepts in soil biology, including a description of the soil biota, common biological interactions, and principles of nutrient cycling. The final section of the workshop was an interactive discussion of factors limiting mine revegetation, the relevance of soil ecology to those limitations, and strategies for ameliorating restoration limitations. I provided each participant with a forty-page booklet summarizing discussion topics and listing literature and Internet references for locating additional information.

Technical Advising

A primary limitation in the interaction between basic scientists and agency restoration planners is a lack of understanding regarding the role of scientific knowledge in restoration management. To gain insight into this issue, I participated as a technical advisor on three mine waste reclamation projects at various stages of planning.

Clark Fork River Feasibility Study. The Clark Fork River is a high-priority Superfund site in western Montana, where historic mining activities near Butte, MT resulted in vast quantities of mine wastes being deposited downstream (Moore and Luoma 1990). After years of monitoring and assessment, including both human health and ecological risk assessments, E.P.A. conducted a feasibility study in 2000 as a formal evaluation of plausible alternatives for remediation of the river and floodplain. This process involved extensive document review, discussions, and negotiations, as E.P.A. and other involved parties identified the most appropriate actions to attain effective and feasible

remedies for the Clark Fork River Operable Unit. Participating in this process gave me insight into the complexities of working toward large-scale environmental protection in the context of mixed scientific, political, social, economic, and legal considerations, and the necessity of having rigorous and defensible science guide the formulation of alternatives as well as the decision-making process.

Butte Commercial AM Inoculum Revegetation Project. As part of efforts to improve reclamation of mined lands in Butte, managers with the Permitting and Enforcement Division of the Montana Department of Environmental Quality were interested in potential benefits of AM inoculation as part of their remediation design. While several companies actively market AM inoculum products in Montana, very little information is available to guide restoration managers as they evaluate and select a product. We designed and implemented an experiment to directly compare four commercial inoculum products to each other and to non-inoculated control plots. When completed, this project will provide important and useful information to restoration practitioners faced with deciding between commercial AM inoculum products.

U.S.D.A. Forest Service Technical Revegetation Workshop. Several U.S.D.A. Forest Service offices in Montana organized a panel discussion and workshop to discuss reclamation and revegetation issues for abandoned mine lands under their management. This workshop involved revegetation experts from academia, Forest Service researchers, and private consultants, as well as Forest Service restoration managers, to discuss philosophies and technologies

for reclamation and revegetation. As a workshop participant, I was able to clarify many misconceptions about microbial inoculation and re-emphasize the role of soil microorganisms as an important and natural part of plant-soil systems.

WORKSHOP ASSESSMENT

Discussions and post-workshop evaluation forms from two of the workshops (Appendix B) allowed participants to comment on the workshop experience and present views on interactions between academicians and restoration practitioners. Participants responded very positively to the workshop content and format, and 90% of evaluation respondents (N=31) stated that they would use information from the workshop in future reclamation efforts. The paragraphs below summarize recurring and pertinent comments from workshop participants.

Concept of Dynamic Living Soil. Many workshop participants appreciated the presentation of soils as dynamic living systems that include plants, rather than just engineered growth substrate. Further, they stated that understanding interactions between plants and other soil biota, and knowing environmental factors that promote healthy soil communities will help in reclamation projects such as those requiring construction of soil from overburden.

Biology and Ecology of Mycorrhizae. Many workshop participants found the information about mycorrhizal fungi to be the most useful part of the workshop. I emphasized the natural functions of mycorrhizae and provided information to help restoration practitioners evaluate their needs for inoculum and

compare commercially available inoculum sources. I included a list of questions to ask about commercial products, and qualities to use in comparing them. Many participants expressed satisfaction with having a demystified appreciation for mycorrhizae as important natural symbioses rather than a magic bullet for restoration.

For example, the Glacier National Park Restoration Group was interested in using AM to enhance seedling transplant success on various revegetation projects. They have established strict restoration guidelines that require preservation of the purity of plant genetic material within different areas of the park. Yet, after being convinced by an inoculum vendor that AM inocula are the same worldwide, they were about to introduce a commercial inoculum product containing fungi from a completely unknown origin. After the workshop, they realized that it was more appropriate and efficient to collect native topsoil inoculum for most of their work, which is often on the scale of campsite revegetation. By understanding AM biology, they were able to evaluate the commercial product and develop a more appropriate solution to meet their specific restoration needs.

Discussion of Concept Application to Restoration. Many participants welcomed the interactive discussion on applications of soil ecological concepts to restoration as a very useful part of the workshop. This process was especially helpful because it allowed for immediate application of the new information, with the instructor present as a resource for biological information and other participants providing insights from their experiences.

Workbook and References. The workbook accompanying the workshop was greatly appreciated as a source of information to return to as issues arise in future reclamation projects. Several people commented that it would be helpful to have access to this type of information on the Internet. Some sections of the workshop materials will soon be included on the Ecosystem Restoration Website that is currently being created by the Reclamation Research Unit at Montana State University.

Interactions Between Basic Scientists and Restoration Practitioners

Inadequate Interactions. Of 18 workshop participants asked specifically about interaction levels, 100% reported that they believed there was not adequate interaction between academic scientists and restoration practitioners, and cited a variety of potential reasons. Only 44% of workshop participants completing evaluations (N=31) reported ever interacting with research scientists during reclamation planning and design. Some participants said they value academic input and would enjoy more interaction, but most stated that time and financial constraints were the primary reason for lack of communication. One participant cited fundamental differences in goals and work schedules, writing "Scientists don't publish anything until a concrete solution is discovered, which could take years...I just don't have time with my budget to wait on research projects." Other participants noted that they wouldn't know whom to contact for help, and some felt that academicians tend to isolate themselves or won't participate unless grant money is involved. Many people noted that they believe

that basic scientists are too concerned with fine details and unable to offer practical advice about real-world reclamation. Another plausible (and non-exclusive) explanation is that practitioners do not keep abreast of current research in their fields, whatever the reason may be.

Inadequate Communication. Other comments focused on problems in communication between academic scientists and practitioners. One workshop participant noted that in their experience most scientists were unable to communicate research in layman's terms, while another wrote, "...usually I get lots of information and none of it is useful." Discussions and evaluation form responses both indicated that participants feel that there are not enough forums for interaction.

Ideas for Improvement. Many workshop participants volunteered ideas for improving interaction between basic scientists and restoration practitioners, although it was clear that time and money are primary limiting factors. A common suggestion was to simply develop more forums for interaction and develop better communication skills. Many workshop participants praised the workshop format which allowed me to provide basic biological and ecological background information, coupled with a presentation of basic research examining the role of soil microbes in restoration, and directly followed by an active discussion of how these concepts could be applied to different restoration scenarios. Another participant suggested developing some sort of academic apprenticeship for restoration workers, so that people who did not want to return to school for several years of study could have a brief academic experience to

develop knowledge in certain scientific areas.

CONCLUSIONS AND RECOMMENDATIONS

The interdisciplinary nature of this internship allowed me to identify and evaluate impediments to interactions between basic science and restoration management. The development of workshops and participation in technical advising in on-going restoration projects allowed me to actively address those impediments. The success of the workshops was demonstrated by very positive evaluations, by the statements by most participants that they intend to use workshop concepts in future restoration efforts, and by the efforts by several agencies to conduct research and projects that include an ecologically-based strategies discussed in the workshop.

Many of the participants' suggestions for improvement of information exchange between researchers and practitioners necessarily require time and funding commitments. A primary reason for the effective execution of the workshops I conducted was that my time was funded and sanctioned by the Training WEB program and the E.P.A. Further, my Ph.D. research and personal interests in ecological restoration were fully integrated into the workshop design.

Workshop feedback and my own experiences in presenting the workshops and participating in technical advising point to three primary recommendations for improvement of information exchange between researchers and practitioners. They are: 1) improving current channels of communication, 2) creating new channels of communication, and 3) integrating research and restoration practice.

Current forums for communication could be improved by increasing attendance of practitioners at academic conferences and academicians at management-oriented conferences. Conference organizers should be encouraged to increase this cross-disciplinary participation by recruiting experts from relevant fields, and by organizing symposia and panel discussions that address information exchange and application of the most current research to management.

New channels of communication might include joint agency and university funding of workshops like those I presented. Sharing the time and funding requirements of such workshops would reduce individual burdens while providing forums for dissemination of basic research, maintaining open communication between research interests and management needs, and establishing contacts for potential funding sources for management-oriented research. Further, increased encouragement of graduate students to participate in structured internships can more readily provide current scientific information to managers, while also preparing students to effectively work in natural resource management after completing academic training. Some workshop participants suggested a similar program for managers to participate in an “academic internship,” to allow for intense exposure to current developments in research, without having to commit to a full-time graduate program.

Perhaps the most direct way to ensure the application of rigorous science in practical restoration ecology is to better integrate research and management from the outset. Collaboration on restoration projects would allow researchers to

develop and test methods and theories for restoration, while meeting the overriding need of the manager to design and implement a feasible project quickly. Researchers can take advantage of the fact that restoration projects will be funded and implemented with or without their involvement. Practitioners, on the other hand, would do well to embrace concepts of adaptive management, and consider management as experimentation. Restoration managers will be better able to utilize current research findings if the scale and context (i.e., field versus greenhouse) of research are compatible with those of management.

At the most fundamental level, these changes require that the science-management interaction becomes a priority to both natural resource managers and restoration ecologists. The Training WEB fellowship allowed me to view both perspectives, and shape my academic program such that I could integrate contributions to science and management. By recognizing the objectives and needs of various parties involved, it is possible to increase understanding of natural systems through reconstruction of disturbed lands and develop more reliable and stable restoration strategies.

REFERENCES

- Huenneke, L.F. 1995. Involving academic scientists in conservation research: perspectives of a plant ecologist. *Ecological Applications* 5:209-214.
- Meffe, G.K. 1998. Softening the Boundaries. *Conservation Biology* 12:259-260.
- Moore, J.N. and S.N. Luoma. 1990. Hazardous wastes from large-scale metal extraction. *Environmental Science and Technology* 24:1278-1285
- Munshower, F.F. 1994. *Practical Handbook of Disturbed Land Revegetation*, Lewis Publishers, Inc. Boca Raton, Florida. 140pp.
- Noss, R.F. 1997. The failure of universities to produce conservation biologists. *Conservation Biology* 11:1267-1269.
- Robertson, A.I. 2000. Communicating about research - we still have a long way to go. *Ecological Management & Restoration* 1:161-162.
- Wiener, D.P., J. DiStefano, and R. Lanoue. 1980. *Reclaiming the West: The Coal Industry and Surface-Mined Lands*, INFORM, Inc. New York. 450pp.

APPENDIX A

Quality Assurance / Quality Control for ICP-AES of Soil Water Extracts and Soil and Plant Digest

**QA/QC for ICP-AES analysis of water-extracts
October 6, 2000**

		Practical Quantifiable Limit (PQL)	Lab and Equipment Blanks	Instrument Performance Checks	Analytical Duplicates	Extraction Duplicates	Fortified Blank Spike Recovery	Fortified Sample Spike recovery
<i>Criteria for Exceedance</i>			above PQL	> +/- 10%	> +/- 10%	> +/- 10%	> +/- 30%	> +/- 30%
<i>Number Conducted</i>			5	5	4	3	1	4
Element	Units	PQL	# Exceeding	# Exceeding	# Exceeding	# Exceeding	# Exceeding	# Exceeding
Al	mg/kg	0.04	0	0	2*	0	0	0
As	mg/kg	0.05	0	0	0	0	0	0
B	mg/kg	0.01	0	0	0	0	0	0
Ba	mg/kg	0.003	0	0	0	1	0	0
Be	mg/kg	0.001	0	0	0	0	0	0
Ca	mg/kg	0.2	0	0	0	0	0	0
Cd	mg/kg	0.005	0	0	0	0	0	0
Co	mg/kg	0.005	0	0	0	0	0	0
Cr	mg/kg	0.05	0	0	0	0	0	0
Cu	mg/kg	0.03	0	0	0	0	0	0
Fe	mg/kg	0.08	0	0	0	0	0	0
Hg	mg/kg	0.05	0	0	0	0	0	0
K	mg/kg	0.4	0	0	0	0	0	0
Li	mg/kg	0.005	0	0	0	0	0	0
Mg	mg/kg	0.4	0	0	0	0	0	0
Mn	mg/kg	0.04	0	0	0	2*	0	0
Mo	mg/kg	0.01	0	0	0	0	0	0
Na	mg/kg	0.1	0	0	0	0	0	0
Ni	mg/kg	0.01	0	0	0	0	0	0
P	mg/kg	0.05	0	0	0	0	0	0
Pb	mg/kg	0.04	0	0	0	0	0	0
Sb	mg/kg	0.08	0	0	0	0	0	0
Se	mg/kg	0.08	0	0	0	0	0	0
Si	mg/kg	0.02	0	5	0	1	0	0
Sn	mg/kg	0.03	0	0	0	0	0	0
Sr	mg/kg	0.01	0	0	0	1	0	0
Ti	mg/kg	0.1	0	0	0	0	0	0
Tl	mg/kg	0.08	0	0	0	0	0	0
V	mg/kg	0.02	0	0	0	0	0	0
Zn	mg/kg	0.008	0	0	0	0	0	0

*Values very close to instrument detection limits

**QA/QC for ICP-AES analysis of soil digests
September 28, 2000**

		Practical Quantifiable Limit (PQL)	Lab and Equipment Blanks	Instrument Performance Checks	External Soil Standard (NIST2710)	Analytical Duplicates	Digest Duplicates	Fortified Blank Spike Recovery	Fortified Sample Spike recovery
Criteria for Exceedance			above PQL	> +/- 10%	> +/- 20%	> +/- 10%	> +/- 10%	> +/- 30%	> +/- 30%
Number Conducted			5	6	1	4	4	1	6
Element	Units	PQL	Number of Exceedances						
Al	mg/kg	0.04	0	0	0	0	0	0	1
As	mg/kg	0.05	0	0	0	0	1*	0	0
B	mg/kg	0.01	0	0	0	2*	2*	0	0
Ba	mg/kg	0.003	0	0	0	0	0	0	0
Be	mg/kg	0.001	0	0	0	0	0	0	0
Ca	mg/kg	0.2	0	0	0	0	0	0	0
Cd	mg/kg	0.005	0	0	0	2*	0	0	0
Co	mg/kg	0.005	0	0	0	0	0	0	0
Cr	mg/kg	0.05	0	0	0	0	0	0	0
Cu	mg/kg	0.03	0	0	0	0	0	0	0
Fe	mg/kg	0.08	0	0	0	0	0	0	0
Hg	mg/kg	0.05	0	0	0	0	0	0	0
K	mg/kg	0.4	0	1	0	0	0	0	1
Li	mg/kg	0.005	0	3	0	0	0	0	0
Mg	mg/kg	0.4	0	0	0	0	0	0	0
Mn	mg/kg	0.04	0	0	0	0	0	0	0
Mo	mg/kg	0.01	0	0	0	0	0	0	0
Na	mg/kg	0.1	0	0	0	0	0	0	0
Ni	mg/kg	0.01	0	0	0	0	0	0	0
P	mg/kg	0.05	0	0	0	0	0	0	0
Pb	mg/kg	0.04	0	0	0	0	0	0	0
Sb	mg/kg	0.08	0	0	0	0	0	0	0
Se	mg/kg	0.08	0	0	0	0	0	0	0
Si	mg/kg	0.02	0	5	0	2	4	0	0
Sn	mg/kg	0.03	0	0	0	0	0	0	0
Sr	mg/kg	0.01	0	0	0	0	0	0	0
Ti	mg/kg	0.1	0	0	0	0	0	0	0
Tl	mg/kg	0.08	0	0	0	0	0	0	0
V	mg/kg	0.02	0	0	0	0	0	0	0
Zn	mg/kg	0.008	1	0	0	0	0	0	0

*Values very close to instrument detection limits

QA/QC for ICP-AES analysis of plant tissue digests
July 12, 2001

			Practical Quantifiable Limit (PQL)	Lab and Equipment Blanks	Instrument Performance Checks	External Soil Standard (NIST2710)	Analytical Duplicates	Digest Duplicates	Fortified Blank Spike Recovery	Fortified Sample Spike recovery
Criteria for Exceedance			above PQL	> +/- 15%	> +/- 10%	> +/- 10%	> +/- 10%	> +/- 10%	> +/- 30%	> +/- 30%
Number Conducted			11	12	2	7	7	1	9	
Element	Units	PQL	Number of Exceedances							
Al	mg/kg	0.04	0	0	2	1	5	0	0	
As	mg/kg	0.05	0	0	0	0	0	0	0	
B	mg/kg	0.01	0	0	0	0	4	0	0	
Ba	mg/kg	0.003	0	0	0	0	0	1	9	
Be	mg/kg	0.001	0	0	0	0	0	0	0	
Ca	mg/kg	0.2	0	0	0	0	0	0	0	
Cd	mg/kg	0.005	0	0	0	0	1	0	0	
Co	mg/kg	0.005	0	0	0	1	1	0	0	
Cr	mg/kg	0.05	0	0	0	0	0	0	0	
Cu	mg/kg	0.03	0	0	0	0	1	0	0	
Fe	mg/kg	0.08	0	0	2	0	0	0	0	
Hg	mg/kg	0.05	0	0	0	0	0	0	0	
K	mg/kg	0.4	0	0	0	0	0	0	0	
Li	mg/kg	0.005	0	0	0	0	0	0	0	
Mg	mg/kg	0.4	0	0	0	0	0	0	0	
Mn	mg/kg	0.04	0	0	0	1	0	0	0	
Mo	mg/kg	0.01	0	0	0	0	1	0	0	
Na	mg/kg	0.1	0	0	0	0	2	0	0	
Ni	mg/kg	0.01	0	0	0	0	1	0	0	
P	mg/kg	0.05	0	0	0	0	0	0	0	
Pb	mg/kg	0.04	0	0	0	0	0	0	0	
Sb	mg/kg	0.08	0	11	0	0	0	1	0	
Se	mg/kg	0.08	0	0	0	0	0	0	0	
Si	mg/kg	0.02	0	11	0	3	7	0	0	
Sn	mg/kg	0.03	0	0	0	0	0	0	0	
Sr	mg/kg	0.01	0	0	0	0	0	0	0	
Ti	mg/kg	0.1	0	0	0	0	0	0	0	
Tl	mg/kg	0.08	0	0	0	0	0	0	0	
V	mg/kg	0.02	0	0	0	0	1	0	0	
Zn	mg/kg	0.008	0	0	0	0	0	0	0	

APPENDIX B

Workshop Evaluation Form

***Workshop Evaluation
Ecological Restoration of Plant-Soil Systems
Whitefish, MT - April 8, 2001***

Please rate the workshop content: Excellent Good Fair Poor Bad
Please rate the workshop instructor: Excellent Good Fair Poor Bad
Please rate the workshop overall: Excellent Good Fair Poor Bad

- The goals of this workshop were to 1) introduce basic concepts in soil biology and restoration ecology and 2) begin to understand how we can use this knowledge to improve restoration practices.
- Do you believe goal #1 was achieved? (yes, no, somewhat) Feel free to elaborate.

- Do you believe goal #2 was achieved? (yes, no, somewhat) Feel free to elaborate.

- Do you think you will use information from this workshop in your future reclamation efforts? Feel free to elaborate.

- What was the most useful information presented in today's workshop?

- What was the least helpful, or most confusing, information presented in today's workshop?

In general, do you think there is adequate interaction between academic scientists and restoration practitioners? Why do you think that is?

➤ **Do you interact with academic research scientists during your reclamation planning and design process?**

YES _____ NO _____

If YES, was the experience fruitful or rewarding?

If NO, why not? Please be as specific as possible.

➤ **Please note any other thoughts and comments about this workshop, and/or about interactions between basic scientists and restoration practice and management.**