TECHNICAL BULLETIN NO. 01-15 IDAHO BUREAU OF LAND MANAGEMENT AUGUST 2001

RESTORING PALOUSE AND CANYON GRASSLANDS: PUTTING BACK THE MISSING PIECES

Compiled and Edited
by
Bertie J. Weddell

Restoring Palouse and Canyon Grasslands: Putting Back the Missing Pieces

A. Restoration of Palouse and Canyon Grasslands: A Review. B.J. Weddell and J. Lichthardt

B. Soil Biological fingerprints from Meadow Steppe and Steppe Communities with Native and Non-native Vegetation. B.J. Weddell, P. Frohne, and A.C. Kennedy

C. Experimental Test of Microbial Biocontrol of Cheatgrass. B.J. Weddell, A. Kennedy, P. Frohne, and S. Higgins

D. Experimental Test of the Effects of Erosion Control Blankets on the Survival of Bluebunch Wheatgrass Plugs. B.J. Weddell

Complied and edited by
Bertie J. Weddell
draba Consulting
1415 NW State Street
Pullman, WA 99163

March 2000

for the
Bureau of Land Management
Cottonwood Field Office
Route 3, Box 181
Cottonwood, ID 83522

Table of Contents

Contributors ---------- iii

Acknowledgments ---------- iv

Overview ---------- v

1. Restoration of Palouse and Canyon Grasslands: A Review, B.J. Weddell and J. Lichthardt ---------- 1

1.1 Introduction ---------- 1

1.2. Native steppe and meadow steppe ecosystems ---------- 2

1.3. Techniques used in restoration ---------- 3

1.3.1. Manipulation of soil biota ---------- 3

3.1.2. Weed control ---------- 4

1.3.3. Propagation of native plants ---------- 5

1.3.4. Fire management ---------- 6

1.4. Literature cited ---------- 6

2. Research Reports ---------- 12

2.1. Soil Biological Fingerprints from Meadow Steppe and Steppe Communities with Native and Non-native Vegetation, B.J. Weddell, P. Frohne, and A.C. Kennedy ---------- 12

2.1.1. Introduction ---------- 12

2.1.2. Methods ---------- 12

2.1.3. Results ---------- 15

2.1.4. Discussion ---------- 18

2.1.5. Literature cited ---------- 21

2.2. Experimental Test of Microbial Biocontrol of Cheatgrass, B.J. Weddell, A. Kennedy, P. Frohne, S. Higgins ---------- 22

2.2.1. Introduction ---------- 22

2.2.2. Methods ---------- 22

2.2.3. Results ---------- 23

2.2.4. Discussion ---------- 24

2.2.5. Literature cited ---------- 25

2.3. Experimental Test of the Effects of Erosion Control Blankets on the Survival of Bluebunch Wheatgrass Plugs, B.J. Weddell ---------- 27

2.3.1. Introduction ---------- 27

2.3.2. Description of the study area ---------- 28

2.3.3. Methods ---------- 28

2.3.4. Results and discussion ---------- 29

2.3.5. Literature cited ---------- 31

Contributors

Pamela Frohne
U.S. Department of Agriculture
Agricultural Research Service
221 Johnson Hall
Washington State University
Pullman, WA 99164-6421

Stewart Higgins
Department of Crop and Soil Sciences
Washington State University
Pullman, WA 99164-6420

Ann C. Kennedy
U.S. Department of Agriculture
Agricultural Research Service
221 Johnson Hall
Washington State University
Pullman, WA 99164-6421

Juanita Lichthardt
Idaho Conservation Data Center
P.O. Box 25
Boise, ID 83707

Bertie J. Weddell
Draba Consulting
1415 NW State Street
Pullman, WA 99163

Acknowledgments

This project was funded through challenge cost-share agreements between the Palouse Land Trust of Moscow, Idaho, and the Bureau of Land Management (BLM), Cottonwood Resource Area. In addition, many people contributed to this project. Archie and Mary George, Jacie and Wayne Jensen, Washington State University, and the Washington Department of Transportation allowed their property to be used as study sites. Karen Gray, Ed Haagen, Janice Hill, Mark Stannard, and Sarah Walker provided helpful information. For the studies at the Waha site, Vern McMaster and Archie George assisted with collecting field data, and Mary Fauci and Juanita Lichthardt helped with setting up field trials. Karen Gray assisted with identification of mosses and lichens, Stewart Higgins provided statistical guidance, and Margaret Dibble grew the bluebunch wheatgrass plugs. All of these contributions are gratefully acknowledged. The support and assistance provided by Craig Johnson of the BLM are especially appreciated.

Overview

Prior to the middle of the nineteenth century, the natural vegetation of the Palouse region of southeastern Washington, northeastern Oregon, and adjacent parts of northern Idaho was dominated by steppe and meadow steppe plant associations. Beginning in the 1830s, Euroamerican settlement of the region led to dramatic changes. Deep soils were cultivated, and shallower soils were grazed by livestock. The extent and quality of native grasslands declined as a result of these changes in land use. For this reason, there is now considerable interest in restoring native vegetation in the Palouse region. This volume looks at the prospects for restoration of native steppe and meadow steppe communities, with an emphasis on methods that do not create areas of bare soil. Section 1 reviews work that has been done on the restoration of native steppe and meadow steppe. Section 2 reports on the results of pilot projects undertaken to (A) compare microbial soil communities in native and non-native vegetation at two meadow steppe and two steppe sites, (B) test the efficacy of bacterial control of a severe cheatgrass infestation, and (C) investigate the effectiveness of artificial ground covers on survival of transplanted native grass plugs.

1. Restoration of Palouse and Canyon Grasslands: A Review

B.J. Weddell and J. Lichthardt

1.1. Introduction

The Bureau of Land Management (BLM) manages extensive areas of bunchgrass steppe in all types of condition. Restoration of certain areas, if feasible, could be used to safeguard priority habitats and species and to improve conditions for wildlife. The purpose of this report is to summarize research and field trials relevant to steppe restoration in the Palouse region of southeastern Washington and adjacent Idaho.

Almost all Palouse steppe vegetation has been cultivated or grazed by domestic livestock. Remaining examples of uncultivated native steppe or meadow steppe vegetation are primarily found in the breaks of the Snake and Columbia Rivers. Most of these sites have been degraded by domestic livestock grazing and alien weed invasion. The conversion of vast areas of native steppe vegetation to alien-dominated communities has been the primary impetus for a growing interest in grassland restoration in the Palouse region.

The National Academy of Sciences attempted to standardize the terminology used in discussing land restoration following surface coal mining, and their definitions were reiterated by Allen (1988). "Restoration" is defined as a return to the natural processes that sustain a native ecosystem; "reclamation" as the return of similar organisms and ecological functioning; and "rehabilitation" as the return of the land to a useful condition, but probably to a use different than the original. These definitions have been widely adopted.

In practice, restoration of both the biota and processes of degraded grasslands is usually not possible due to the ubiquitous presence of opportunistic alien species which have altered natural succession. For this reason, grassland restoration projects may have to limit their scope to a specific goal, for example, the establishment of native vegetation with a composition and physiognomy needed to support native wildlife or particular species of conservation concern. Ideally, both reclamation and restoration should use material from locally adapted native populations collected in close proximity to the target site, because genetic material from non-local sources can genetically undermine locally adapted genotypes.[1]

This review focuses primarily on the semiarid bunchgrass steppe and meadow steppe of western North America (Daubenmire 1970; Franklin and Dyrness 1988). It considers re-establishment of native steppe vegetation on degraded sites, including sites converted to agricultural use (old fields), but it does not extend to the reclamation of non-native soils such as mine spoil.

The past was not static. Because natural disturbances, human activities, and arrivals of novel species cause changes on short-term time scales, and geological and climatic changes cause long-term changes, we cannot identify a single snapshot in time as "natural" (Botkin 1990; Sprugel 1991). The initial step in any ecological restoration project, therefore, is to answer the

[1] It has been suggested that this is what happened with reed canarygrass (*Phalaris arundinacea*). This species is native to the Pacific Northwest but has become highly invasive, perhaps because of hybridization between agronomic cultivars and native material (Merigliano and Lesica 1998).

question: what is the target condition to which we seek to return? Information about past conditions can be used to help make this decision. The vegetation of southeastern Washington and adjacent Idaho prior to 1917 was described by Weaver (1917). Areas that have received relatively light impacts can also serve as reference sites. Fortunately, extensive areas of steppe vegetation that remain in the region's major river canyons probably have a flora very similar to that of the former Palouse grasslands (Lichthardt and Moseley 1997; Weddell and Lichthardt 1998).

1.2. Native steppe and meadow steppe ecosystems

Native steppe and meadow steppe ecosystems were historically dominated by caespitose perennial grasses, especially Idaho fescue (*Festuca idahoensis*), bluebunch wheatgrass (*Pseudoroegneria spicata* ssp. *spicata* [=*Agropyron spicatum*]), Sandberg's bluegrass (*Poa secunda*), and junegrass (*Koeleria macrantha* [=*cristata*]). In the relatively mesic meadow steppe associations, these were accompanied by diverse native forbs and low shrubs. Though less obvious, organisms in and on the soil, including cyanobacteria, bacteria, algae, microfungi, lichens, bryophytes, protozoa, and nematodes, also performed important functions.

A well developed microbiotic (or cryptobiotic) crust is characteristic of many arid and semiarid ecosystems of the Inland Northwest and throughout the world. In North America, cryptobiotic crusts are prevalent in the Great Basin, the Columbia Basin, and the Colorado Plateau. Because these crusts affect surface stability, soil fertility and structure, water infiltration, seedling establishment, and plant growth (Fletcher and Martin 1948; Harper and Marble 1988; St. Clair et al. 1984; 1993; West 1990; Belnap and Gardner 1993; Harper and Pendleton 1993; Belnap 1994; Kaltenecker and Wicklow-Howard 1994; Williams 1994; Leonard et al. 1995; Quigley and Arbelbide 1997), they are potentially important in restoring steppe, shrub steppe, and desert vegetation. The soil crusts of grasslands in the Palouse region were characterized by Cooke (1955).

Crust composition varies with climate and successional stage. The cryptobiotic crust may be removed or damaged by burning, trampling, cultivation, or burial (for instance, by volcanic ash) (Harris et al. 1987). Disturbance of the crust can result in changes in rates of ecosystem processes such as nitrogen fixation (Evans and Belnap 1999). The nitrogen-fixing properties of surface cyanobacteria may be of limited importance for grassland restoration, however, because native grasses thrive under conditions of low nitrogen availability, and higher nitrogen levels may selectively benefit annuals (Morgan 1994). Algae and cyanobacteria are the primary microbial photosynthetic agents of the below-ground ecosystem. They also contribute to nitrogen fixation and soil particle aggregation.

Mycorrhizae (fungi associated with roots) play a pivotal role in structuring steppe and shrub steppe communities through their influences on nutrient uptake, growth, and reproduction in associated vascular plants (Dhillion and Friese 1992; Harnett and Wilson 1999). Endomycorrhizae, also known as arbuscular mycorrhizae, form important associations with the roots of many native grasses and shrubs in steppe and shrub steppe communities. Sagebrush (*Artemisia* spp.), rabbitbrush (*Chrysothamnus* spp.), and native bunchgrasses are highly dependent upon arbuscular mycorrhizae. By contrast, many alien annual grasses such as cheatgrass and medusahead (*Taeniatherum caput-medusae*), are nonmycorrhizal or facultatively

mycorrhizal (Goodwin 1992; Wicklow-Howard 1994; 1998).

Because arbuscular mycorrhizae enhance resource acquisition in some species and not others, they can affect competitive interactions between species. The colonization of rangeland by nonmycorrhizal species is associated with declines in arbuscular mycorrhizal fungi (Goodwin 1992). When arbuscular mycorrhizae are absent, nonmycorrhizal species capture soil resources more effectively than mycorrhizal species such as caespitose grasses (Goodwin 1992). In some contexts, however, mycorrhizal fungi indirectly enhance non-native species. In greenhouse experiments, arbuscular mycorrhizae increased the effect of spotted knapweed (*Centaurea bieberstenii* [=*maculosa*]) on Idaho fescue (Marler et al. 1999).

One of the most important influences of soil crusts may be their effects on seedling establishment, but these effects are not well understood. (See Harper and Marble 1988 for review.) In some studies, cryptobiotic crusts enhanced the establishment of seedlings (Belnap 1994; Belliveau 1998); but in other studies cryptogams were associated with negative effects on seedling establishment (St. Clair et al. 1984). Negative effects might result because of inhibitory substances produced by crusts (Pyatt 1967) or because crusts present a physical barrier to germinating seeds (Schlatterer and Tisdale 1969).

1.3. Techniques used in restoration

1.3.1. Manipulation of soil biota

Because organisms in the soil and in soil crusts are such an important part of steppe and meadow steppe ecosystems, restoration efforts should take them into consideration. First of all, care should be taken to insure that weed control measures undertaken for the sake of restoration do not do more harm than good by harming microbiotic crusts. This can be a problem with fire and herbicides. (See sections 3.2. and 3.4.)

Second, restoration efforts should include restoration of soil microbiota. Attention should be paid to enhancing the survival and colonization of residual propagules such as spores and mycorrhizal fungi and to creating environments that promote the establishment and growth of desirable microorganisms (Wicklow-Howard 1994; Johnston and Belnap 1997). Belnap (1993) reported some success in hastening the recovery of cyanobacterial-lichen crusts by inoculation with crusts from undisturbed sites, although recovery rates were low. Soil algae and cyanobacteria can be used to enhance the fertility of agricultural fields (Zimmerman 1993), and could be used in ecosystem restoration. Green algae that produce extracellular polysaccarides which stabilize soil aggregates and improve soil tilth are used commercially for agricultural purposed in the Pacific Northwest. (See Zimmerman 1993 for review.)

Mycorrhizal inoculation can promote the revegetation of severely disturbed soils where few native plants remain. Thorne et al. (1998) demonstrated that inoculation with native arbuscular mycorrhizal fungi enhanced establishment and growth of the cultivar Secar bluebunch wheatgrass on mine spoils. Mycorrhizal hyphae in root fragments are probably the most important source of inoculum (Dhillion and Friese 1992), which can be added to the soil of containerized planting stock.

3.1.2. Weed control

The degree of degradation of terrestrial ecosystems is often diagnosed by the presence and extent of alien plant species (e.g., Andreas and Lichvar 1995); frequently their presence is related to soil disturbance and overgrazing. Increasingly, however, aggressive aliens are becoming established even in ostensibly undisturbed bunchgrass vegetation, wherever their seed can reach. The most notorious alien species in the Palouse region are upland species that can dominate and exclude perennial grasses over a wide range of elevations and substrate types.

Alien annual plants are now the primary impediment to restoration of grassland sites. Weed control is therefore central to reclamation and restoration of grasslands in the Palouse region. Usually, this step must precede and/or accompany efforts to establish native vegetation. Even where communities of exotics can successfully be replaced with native species, an ongoing program of weed control is generally necessary to protect the site. Several aggressive alien perennials have also become widespread.

Much of the research on grassland restoration focuses on techniques for enhancing perennial grass growth by suppressing the growth of aggressive alien annuals (Youtie 1997; Youtie et al. 1998, 1999). Intensive methods for reducing weeds are often impractical, however, due to steep terrain, extent of the infestation, and low land values. Weed reduction involves site- and weed-specific methods of control. The selection of appropriate methods also depends on whether a treatment area contains native species and their phenology. Herbicides should be applied when they will do maximum damage to exotics and minimal damage to native species.

The Nature Conservancy has experimented with hand-pulling, mowing, chemical treatments, and fire in attempts to decrease weed infestations and reestablish native bunchgrasses in Palouse grassland (Youtie 1997, Youtie et al. 1998, 1999; Weddell 1996, 1997). In trials at Rose Creek Preserve in eastern Washington, weeding and fall application of herbicide resulted in reduced coverage of medusahead (Weddell 1996, 1997). At the Lawrence Memorial Grassland Preserve in north-central Oregon, Youtie and her coworkers tested the effectiveness of spring application of herbicides, prescribed summer burning, and repeated mowing to control cheatgrass and medusahead in a mounded prairie dominated by Idaho fescue and bluebunch wheatgrass. Although each treatment was initially effective, the effects were only temporary, and the alien annual grasses returned to pretreatment levels within two years after the treatments were applied (Youtie et al. 1998). Research on controlling weeds in canyon grasslands by hand-pulling or herbicide application and the effects of these treatments on rare plants is in progress at Craig Mountain in Idaho (Janice Hill, The Nature Conservancy, Idaho Chapter, personal communication).

Biological control with soil microorganisms is another approach to weed problems. Some success has been obtained in using *Pseudomonas* bacteria to suppress growth of cheatgrass (*Bromus tectorum*) in wheat fields in a steppe zone (Kennedy et al. 1991); however, preliminary attempts to control a heavy cheatgrass infestation in a meadow steppe restoration project were unsuccessful. (See Weddell et al., this volume.)

1.3.3. Propagation of native plants

Establishment of grass cover is indispensable for grassland reclamation. Not only do grasses dominate in native communities, but they are essential to controlling weeds, and are the easiest elements to establish. Seed should generally be collected from the local vicinity and then planted and increased off-site, a time-consuming process.

The steep terrain of canyon grasslands severely limits the options available for seeding and site preparation. Most sites must be chemically treated to control competing weeds. This is usually followed by broadcast seeding. Unfortunately, broadcast seeding is very undependable and requires twice the application rate of drill seeding. In a project in Asotin Canyon, in southeastern Washington, more than 90% of the seed broadcast on a plowed site was lost to rodents or birds (Nelson et al. 1970). Losses of only 23% occurred on unplowed, chemically prepared sites, however.

Reclamation of old fields on Palouse uplands is less problematic than on rugged canyon sites because machinery can be used to prepare a seedbed and drill seed. Drilling places seed in an optimum environment for germination and seedling development and requires less seed than broadcast seeding (Nelson et al. 1970). Native grass seed must be mixed with rice hulls if it is to feed evenly through the drill. Native forbs and shrubs should not be added to the grass seeding, so that chemical weed treatments will be an option during the initial years of grass establishment.

Although most grassland reclamation projects in the Inland Northwest are in only their early stages, grass seeding for erosion control and soil improvement under the Conservation Reserve Program has been a common practice for decades. Improved varieties of alien grasses are generally used in these plantings, but the same methods for seedbed preparation and planting are applicable to planting natives, at least on rolling uplands of the Palouse (USDA-NRCS 1998).

Even with drill seeding, it may take two or more years to develop grass cover that can out-compete annual weeds. In the interim, chemicals can be used to control broadleaf weeds and mowing to keep annuals from reseeding. Slender wheatgrass (*Elymus* [=*Agropyron*] *trachycaulum*) or mountain brome (*Bromus marginatus*) are sometimes used as nurse crops because they establish more quickly than bluebunch wheatgrass or Idaho fescue but are not long-lived. When a nurse crop is used, it must be mowed to keep it from reseeding, which can delay the development of cover by target species (Mark Stannard, U.S. Department of Agriculture, Plant Materials Center, Pullman, WA, personal communication).

Transplanting young plants is another approach. Weddell (1997) reported that transplanting plugs of bluebunch wheatgrass was moderately successful in the short term at Rose Creek Preserve, and Youtie et al. (1998) found that the survival of transplanted bunchgrasses (Idaho fescue, bluebunch wheatgrass, and squirreltail, *Elymus elymoides*) at the Lawrence Memorial Grassland ranged from 43-87% per mound after one year. Although this method can produce good survival, it requires large inputs of labor and time. Other disadvantages of this approach are that mortality may be substantial in hot, dry weather (Wright and Bunting 1986) and it selects for genotypes adapted to germinate and emerge under greenhouse conditions rather than under field conditions.

1.3.4. Fire management

Some publications (e.g. Adams 1989; Fedrizzi 1998; Johnson 1998) recommend using prescribed fire to restore or improve native steppe vegetation. Fire is considered beneficial in terms of its effects on site productivity, species composition, and litter removal. Although there is considerable interest in restoring "natural" fire regimes, however, the effects of fire on Palouse and canyon grasslands are not well understood. In the Midwest, fire is an important tool in maintaining prairie remnants. The historical role of fire in the steppe and meadow steppe vegetation of the Palouse region is less clear (Weddell 2001). Daubenmire (1970) dismissed it as relatively unimportant, whereas others conclude that fires were probably more prevalent in the recent past than at present (Morgan et al. 1996). The lack of information about the pre-settlement fire frequency of steppe and meadow steppe ecosystems makes it difficult to emulate the natural fire regime in restored communities.

Fire also has some disadvantages. It destroys or reduces diversity in the soil crust (Antos et al. 1983; Youtie et al. 1999). While the algal component may reestablish relatively rapidly, estimated recovery times for lichens and mosses are on the order of decades or even centuries (Johansen et al. 1984; Belnap 1993). In addition, burning and crust destruction favor the establishment of disturbance-adapted species that are present in the seed bank or nearby. These are usually non-natives. For this reason, the benefits of fire must be weighed against its effects on weed invasion in situations where exotics are ubiquitous (Weddell 2001).

1.4. Literature cited

Adams, G.R. 1989. Why burn rangelands? Pp. 101-102 *in* Prescribed fire in the intermountain region: Forest site preparation and range improvement, symposium proceedings. D.M. Baumgartner, D.W. Breuer, B.A. Zamora, L.R. Neuenschwander, and R.H. Wakimoto, comps. and eds. Cooperative Extension, Washington State University, Pullman.

Allen, E.B. 1988. The reconstruction of disturbed arid lands: An ecological approach. Westview Press. Boulder, CO.

Andreas, B.K. and R.W. Lichvar. 1995. Floristic index for establishing assessment standards: A case study for northern Ohio. U.S. Army Corps of Engineers. Wetlands Research Program Technical Report WRP-DE-8.

Antos, J.A., B. McCune, and C. Bara. 1983. The effect of fire on an ungrazed western Montana grassland. American Midland Naturalist 110:354-364.

Belliveau, W.M. 1998. Cryptobiotic soil crusts in the sagebrush steppe grasslands of western Montana. Kelseya (Journal of the Montana Native Plant Society) (Summer):1,4.

Belnap, J. 1993. Recovery rates of cryptobiotic crusts: Inoculant use and assessment methods. Great Basin Naturalist 53:89-95.

Belnap, J. 1994. Potential role of cryptobiotic soil crusts in semiarid rangelands. Pp. 179-185 *in* S. B. Monsen and S. G. Kitchen, eds. Proceedings—Ecology and management of arid rangelands. USDA Forest Service, General Technical Report INT-GTR-313.

Belnap, J. and J.S. Gardner. 1993. Soil microstructure in soils of the Colorado Plateau: The role of the cyanobacterium *Microcoleus vaginatus*. Great Basin Naturalist 53:40-47.

Botkin, D.B. 1990. Discordant harmonies: A new ecology for the twenty-first century. Oxford University Press, New York.

Cooke, W.B. 1955. Fungi, lichens, and mosses in relation to vascular plant communities in eastern Washington and adjacent Idaho. Ecological Monographs 25:119-180.

Daubenmire, R.F. 1970. Steppe vegetation of Washington. Washington Agricultural Experiment Station, Washington State University, Technical Bulletin 62.

Dhillion, S.S. and C.F. Friese. 1992. The occurrence of mycorrhizas in prairies: Application to ecological restoration. Pp. 103-114 *in* R.G. Wickett, P.D. Lewis, A. Woodliffe, and P. Pratt, eds., Proceedings of the Thirteenth North American Prairie Conference: Spirit of the Land, Our Prairie Legacy. Department of Parks and Recreation, Windsor, Ontario.

Evans, R.D. and J. Belnap. 1999. Long-term consequences of disturbance on nitrogen dynamics in an arid ecosystem. Ecology 80:150-160.

Fedrizzi, J. 1998. Craig Mountain Cooperative Management Area fire management plan. M.S. thesis, University of Idaho, Moscow.

Fletcher, J.E. and W.P. Martin. 1948. Some effects of algae and molds in the rain-crust of desert soils. Ecology 29:95-100.

Franklin, J.F. and C.T. Dyrness. 1988. Natural vegetation of Oregon and Washington. Oregon State University Press, [Corvallis].

Goodwin, J. 1992. The role of mycorrhizal fungi in competitive interactions among native bunchgrasses and alien weeds: A review and synthesis. Northwest Science 66:251-260.

Harnett, D.C. and G.W.T. Wilson. 1999. Mycorrhizae influence plant community structure and diversity in tallgrass prairie. Ecology 80:1187-1195.

Harper, K.T. and J.R. Marble. 1988. A role for nonvascular plants in management of arid and semiarid rangelands. Pp. 135-169 *in* P.T. Tueller, ed., Vegetation science applications for rangeland analysis and management. Kluwer Academic Publishers, Dordrecht.

_____. and R.L. Pendleton. 1993. Cyanobacteria and cyanolichens: Can they enhance availability of essential minerals for higher plants? Great Basin Naturalist 53:59-72.

Harris, E., R.N. Mack, and M.S.B. Ku. 1987. Death of steppe cryptogams under the ash from Mount St. Helens. American Journal of Botany 74:1249-1253.

Johansen, J.R., L.L. St. Clair, B.L. Webb, G.T. Nebeker. 1984. Recovery patterns of cryptogamic soil crusts in desert rangelands following fire disturbance. Bryologist 87:238-242.

Johnson, C.G., Jr. 1998. Vegetation response after wildfires in national forests of northeastern Oregon. U.S. Department of Agriculture Forest Service. R6-NR-ECOL-TP-06-98.

Johnston, R.L. and J. Belnap. 1997. Potential for influencing native bunchgrass restoration by manipulating soil biota. Pp. 99-104 *in* T.N. Kaye, A. Liston, R.M. Love, D.L. Luoma, R.J. Meinke and M.B. Wilson, eds., Conservation and Management of Native Plants and Fungi. Native Plant Society of Oregon, Corvallis.

Kaltenecker, J. and M. Wicklow-Howard. 1994. Microbiotic soil crusts in sagebrush habitats of southern Idaho. Report to USDA Forest Service and USDI Bureau of Land Management, on file with the Interior Columbia Basin Ecosystem Management Project, 112 E. Poplar, Walla Walla, WA 99362.

Kennedy A.C., L.F. Elliott, F.L. Young, and C.L. Douglas. 1991. Rhizobacteria suppressive to the weed downy brome. Soil Science Society of America Journal 55:722.

Leonard, S.G., R. Rosentreter, and M.G. Karl. 1995. Microbiotic crusts: Ecological roles and implications for rangeland management. Report to USDA Forest Service and USDI Bureau of Land Management, on file with the Interior Columbia Basin Ecosystem Management Project, 112 E. Poplar, Walla Walla, WA 99362.

Lichthardt, J. and R.K. Moseley. 1997. Status and conservation of the Palouse Grassland in Idaho. Report to the U.S. Fish and Wildlife Service, on file at Idaho Department of Fish and Game Conservation Data Center, P.O. Box 25, Boise, ID 83707.

Marler, M.J., C.A. Zabinsky, and R.M. Callaway. 1999. Mycorrhizae indirectly enhance competitive effects of an invasive forb on a native bunchgrass. Ecology 80:1180-1186.

Merigliano, M.F. and P. Lesica. 1998. The native status of reed canarygrass (*Phalaris arundinacea* L.) in the Inland Northwest, USA. Natural Areas Journal 18:223-230.

Morgan, J.P. 1994. Soil impoverishment. Restoration and Management Notes 12:55-56.

Morgan, P., S.C. Bunting, A.E. Black, T. Merrill, and S. Barrett. 1996. Fire regimes in the Interior Columbia River Basin: Past and present. Final Report, RJVA-INT-94913. Intermountain Fire Sciences Laboratory, USDA Forest Service, Intermountain Research Station, Missoula, MT.

Nelson, J.R., A.M. Wilson, and C.J. Goebel. 1970. Factors influencing broadcast seeding in bunchgrass range. Journal of Range Management 23:163-170.

Pyatt, F.B. 1967. The inhibitory effects of *Peltigera canina* on the germination of graminaceous seeds and the subsequent growth of the seedlings. Bryologist 70:326-329.

Quigley, T.M. and S.J. Arbelbide, tech. eds. 1997. An assessment of ecosystem components in the Interior Columbia Basin and portions of the Klamath and Great Basins. USDA Forest Service, General Technical Report PNW-GTR-405.

Schlatterer, E.F. and E.W. Tisdale. 1969. Effects of litter of *Artemisia*, *Chrysothamnus*, and *Tortula* on germination and growth of three perennial grasses. Ecology 50:869-873.

Sprugel, D. 1991. Disturbance, equilibrium, and environmental variability: What is 'natural' vegetation in a changing environment? Biological Conservation 58:1-18.

St. Clair, L.L. and J.R. Johansen. 1993. Introduction to the symposium on soil crust communities. Great Basin Naturalist 53:1-4.

_____., B.C. Webb, J.R. Johansen, and G.T. Nebeker. 1984. Cryptogamic soil crusts: Enhancement of seedling establishment in disturbed and undisturbed areas. Reclamation and Revegetation Research 3:129-136.

_____., J.R. Johansen, and S.R. Rushforth. 1993. Lichens of soil crust communities in the intermountain area of the western United States. Great Basin Naturalist 53:5-12.

Thorne, M.E., B.A. Zamora, and A.C. Kennedy. 1998. Sewage sludge and micorrhizal effects on Secar bluebunch wheatgrass in mine spoil. Journal of Environmental Quality 27:1228-1233.

USDA-NRCS. 1998. Conservation reserve program planting considerations. USDA Natural Resources Conservation Service, Pullman Plant Materials Center, Pullman, WA.

Weaver, J.E. 1917. A study of the vegetation of southeastern Washington and adjacent Idaho. University Studies 17(1). Lincoln, NB.

Weddell, B. 1996. Final site specific report: Rose Creek Preserve monitoring and restoration, National Park Service challenge cost share grant. Unpublished report prepared for The Nature Conservancy, Washington Chapter.

_____. 1997. Final progress report: Rose Creek Preserve monitoring and restoration. Unpublished report prepared for The Nature Conservancy, Washington Chapter.

Weddell, B.J. 2001. Fire in steppe vegetation of the northern intermountain region. Idaho Bureau of Land Management, Technical Bulletin No. 01-14.

Weddell, B.J. and J. Lichthardt. 1998. Identification of conservation priorities for and threats to Palouse Grassland and Canyon Grassland remnants in Idaho, Washington, and Oregon. Idaho Bureau of Land Management, Technical Bulletin No. 98-13.

West, N.E. 1990. Structure and function of microphytic soil crusts in wildland ecosystems of arid and semi-arid regions. Pp. 179-223 *in* M. Begon, A.H. Fitter, and A. McFadyen, eds. Advances in Ecological Research Vol. 20. Academic Press, London.

Wicklow-Howard, M. 1994. Mycorrhizal ecology of shrub-steppe habitat. Pp. 207-210 *in* S.B. Monsen and S.G. Kitchen, eds. Proceedings—Ecology and management of arid rangelands. USDA Forest Service, General Technical Report INT-GTR-313.

_____. 1998. The role of mycorrhizal fungi in rangelands. Pp. 23-24 *in* R. Rosentreter and A. DeBolt, eds., The Ellen Trueblood symposium: Highlighting Idaho's rare fungi and lichens. Idaho Bureau of Land Management, Technical Bulletin No. 98-1.

Williams, J.D. 1994. Microbiotic crusts: A review. Report to USDA Forest Service and USDI Bureau of Land Management, on file with the Interior Columbia Basin Ecosystem Management Project, 112 E. Poplar, Walla Walla, WA 99362.

Wright, G. and S. Bunting. 1986. Palouse prairie restoration at Nez Perce National Historic Site (Idaho). Restoration and Management Notes 4:23-24.

Youtie, B. 1997. Weed control as the first step in protecting and restoring native plant communities on northeast Oregon natural areas. Pp. 78-82 *in* T.N. Kaye, A. Liston, R.M. Love, D.L. Luoma, R.J. Meinke and M.B. Wilson, eds., Conservation and Management of Native Plants and Fungi. Native Plant Society of Oregon, Corvallis.

Youtie, B., J. Ponzetti, D. Salzer, and J. Soll. 1998. Controlling annual exotic grasses on relict grasslands (Oregon). Restoration and Management Notes 16:2.

_____., _____, and _____. 1999. Fire and herbicides for exotic annual grass control: Effects on native plants and microbiotic soil organisms. Pp. 590-591 *in* VIth International Rangeland Congress Proceedings, Vol. 2.

Zimmerman, W.J. 1993. Microalgal biotechnology and applications in agriculture. Pp. 457-478 *in* F.B. Metting, Jr., ed., Soil microbial ecology: Applications in agricultural and environmental management. Marcel Dekker, Inc., New York.

2. Research Reports

2.1. Soil Biological Fingerprints from Meadow Steppe and Steppe Communities with Native and Non-Native Vegetation

B.J. Weddell, P. Frohne, and A.C. Kennedy

2.1.1. Introduction

All cells contain fatty acids that can be extracted and esterified with methanol to form fatty acid methyl esters (FAMEs) (Klug and Tiedje 1993; Kennedy 1994). When the FAMEs extracted from a soil sample are analyzed using gas chromatography, the resulting profile of fatty acids constitutes a "fingerprint" of the organisms in the sample. Soil microbial communities can be compared by extracting FAMEs from soil and using multivariate statistical techniques on the resulting FAME profiles to analyze any differences.

Different soil microorganisms are associated with the roots of different plant species (Westover et al. 1997). The soil biological fingerprints associated with non-native plants may differ from those associated with natives, either because alien species colonize sites with particular soil microbiota, or because the microbial community changes as a result of colonization by exotics. In this study, we compared the biological fingerprints of surface soils from meadow steppe and steppe communities in Palouse grasslands dominated by non-native and native vascular plants.

2.1.2. Methods

Study sites

We obtained data on vegetation, cryptogams, and soil biota at two meadow steppe sites, Kramer and Paradise Ridge, and two steppe sites, Waha and Colfax (Figure 1). Kramer and Paradise Ridge support Idaho fescue/common snowberry (*Festuca idahoensis/Symphoricarpos albus*) associations, while Waha and Colfax support bluebunch wheatgrass/Sandberg's bluegrass (*Pseudoroegneria spicata* spp. *spicata* [=*Agropyron spicatum*]-*Poa secunda*) associations (Weddell and Lichthardt 1998). At meadow steppe sites, native perennial grasses and low to medium shrubs are accompanied by a well developed forb component, whereas at steppe, perennial bunchgrasses are the sole dominants (Daubenmire 1970). The major non-native plants present were bulbous bluegrass (*Poa bulbosa*), hairy vetch (*Vicia villosa*), and alien annual bromes (*Bromus* spp.) at Colfax; yellow star-thistle (*Centaurea solstitialis*), Kentucky bluegrass (*Poa pratensis*), piedmont bedstraw (*Cruciata pedemontana*), and annual grasses including medusahead (*Taeniatherum caput-medusae*), ventenata (*Ventenata dubia*), and annual

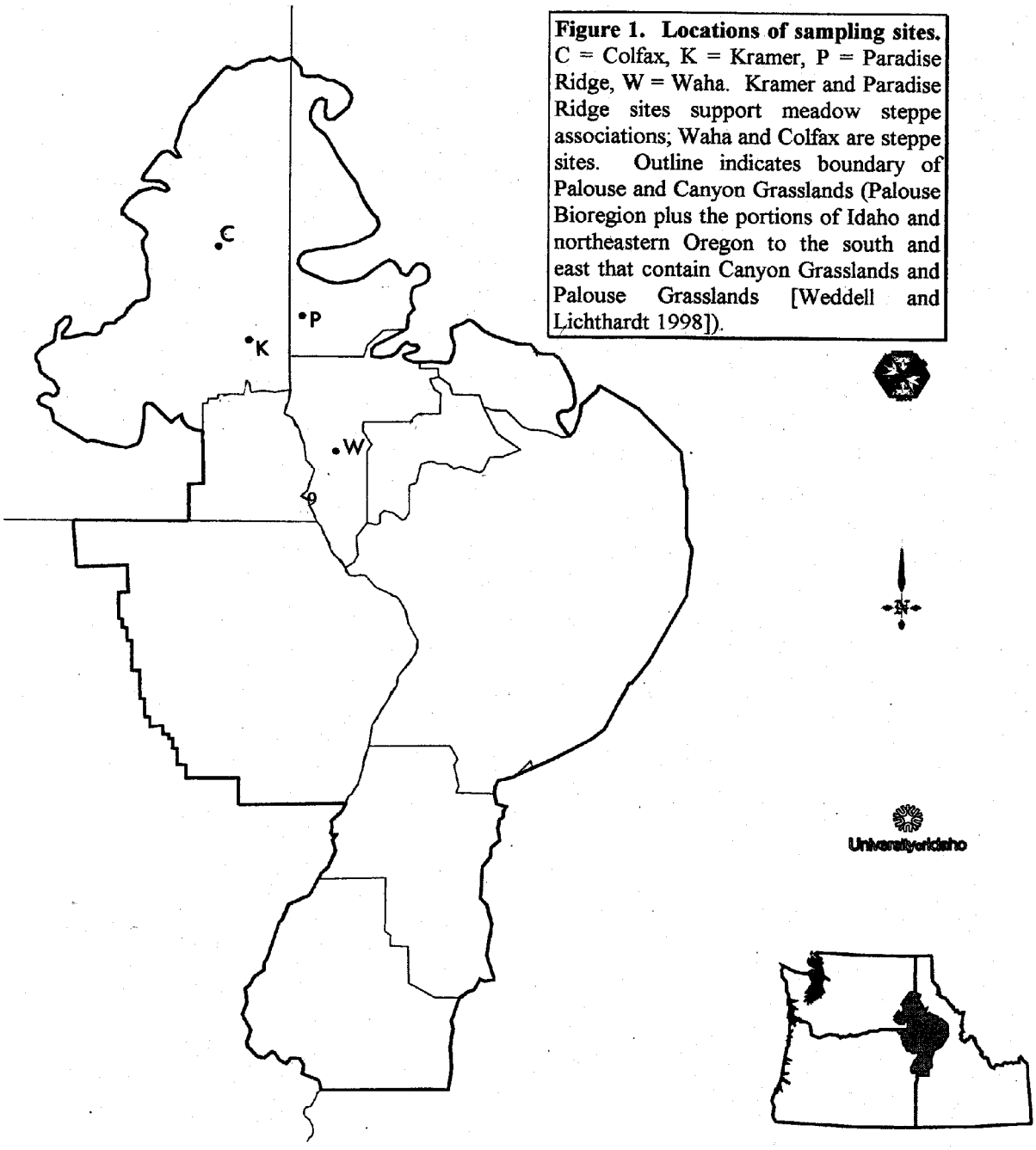


Figure 1. Locations of sampling sites. C = Colfax, K = Kramer, P = Paradise Ridge, W = Waha. Kramer and Paradise Ridge sites support meadow steppe associations; Waha and Colfax are steppe sites. Outline indicates boundary of Palouse and Canyon Grasslands (Palouse Bioregion plus the portions of Idaho and northeastern Oregon to the south and east that contain Canyon Grasslands and Palouse Grasslands [Weddell and Lichthardt 1998]).

bromes at Waha; cheatgrass (*Bromus tectorum*) at Paradise Ridge; and Kentucky bluegrass at Kramer.

Collection of field data and samples

Soil samples and data on vegetation at the four study sites were collected between May 22 and June 4, 1999. At each study site, one 20-m transect was set up in vegetation dominated primarily by native plants, and a similar transect was established nearby in vegetation dominated primarily by non-native species. At Kramer, Waha, and Colfax, the transects were the same as those used in 1998 to monitor threats to native vegetation (Weddell and Lichthardt 1998; transect 73 at Kramer). Vegetation was sampled using a 20-x-50-cm plot frame placed at 0.5-m intervals along each transect, and the canopy coverages of all species of vascular plants except annual grasses were recorded using six coverage classes (0-5%, 5-25%, 25-50%, 50-75%, 75-95%, and 95-100%). Mean canopy coverage values for all species of vascular plants, except annual grasses, was computed using the midpoints of the coverage classes, and coverage values were summed by life form for exotic and native species (i.e., native perennial graminoids, alien perennial graminoids, native perennial forbs, etc.). The canopy coverage of native annual grasses as a group was also estimated; as was the coverage of alien annual grasses. The coverages of rocks, litter, and bare ground were estimated in the same way. Soil temperature at a depth of 7 cm was measured at 5, 10, and 15 m along the transects. Soil stoniness was estimated using a sharpened piece of rebar 9 mm in diameter with a cross-bar at the top. The rod was thrust into the soil at right angles to the surface at 0, 5, 10, 15, and 20 m along the transects, and the depth of penetration until a rock fragment or bedrock stopped the bar was measured. Soil strength was measured with a penetrometer at 1-m intervals along each transect. Soil samples were collected 5, 10, and 15 m along the transects and kept on ice until they were transported to the laboratory.

Fatty acid extraction

FAMEs were extracted from two 1-g subsamples from each soil sample using the procedure described by Kennedy and Busacca (1995). We used the Sherlock Microbial Identification System protocol (MIDI Labs, Inc., Newark, DE), with the addition of an internal standard (nonadecanoic acid methyl ester). Samples were hydrolyzed with sodium hydroxide in aqueous methanol. To hydrolyze the samples, 1 mL of a solution formed from 45 g NaOH in 150 mL methanol and 150 mL deionized water was added, and the samples were heated for 30 min in a 100°C water bath. After the samples were cooled, the fatty acids were methylated by adding 2 mL 6.0 N HCl in aqueous methanol (325 mL 6.0 N HCl in 275 mL methanol). FAMEs were extracted from the aqueous phase to an organic phase with 1 mL hexane:methyl-tert-butyl-ether (1:1 volume:volume). One hundred L of nonadecanoic acid methyl ester (161 M) in hexane:methyl-tert-butyl-ether (1:1 volume:volume) were added, and the samples were placed on an end-over-end mixer for 10 min. Samples were centrifuged at 3,000 rpm for 2 min, and the organic phase was transferred to an acid-washed tube. The extraction process was then repeated without the internal standard. The combined organic phases were base-washed by the addition 3

mL of 1.2% NaOH in deionized water with 5 min of end-over-end rotation. Samples were allowed to evaporate over night, after which they were resuspended in 150 L of hexane:methyl-tert-butyl-ether.

Gas chromatography of fatty acids

Samples were analyzed by a 5890A Hewlett Packard gas chromatograph with a flame ionization detector, HP-IB communications, and HP 3365 ChemStation software, using the Eukary method of the Sherlock Microbial Identification System (Sasser 1990). ChemStation operated the sampling, analysis, and integration of the samples under Eukary method parameters. The temperature was ramped at 5 ºC/min from 170ºC-300ºC, where it remained until the end of the 38-min run. A Hewlett Packard Ultra-2 nonpolar fused silica capillary column (25 m by 0.20 mm by 0.33 m) was used. Hydrogen was the carrier gas, nitrogen was the make up gas, and air was used to support the flame at a flow rate of 30, 30, and 400 mL/min.

Statistical analysis

We used a pattern recognition program to identify differences and similarities between the fatty acid fingerprints of the different subsamples (Sasser 1990). The data for FAMEs with up to 20 carbons were analyzed using principal component analysis (PCA) in SAS (SAS Institute 1988). We limited our analysis to FAMEs with less than 21 carbons, since these are the dominant fatty acids in bacteria (Haack et al. 1994). PCA is a type of multivariate analysis that expresses the similarities and differences of data sets in terms of a small number of principal components. The variance-covariance structure of a data set is explained through a few linear combinations of the original variances, with coefficients equal to the eigenvectors of the correlation matrix (Jolliffe 1986). The statistical component that gave the greatest separation of the groups was designated PRIN1, the component that gave the next greatest separation was designated PRIN2, and so on (Kennedy and Busacca 1995).

2.1.3. Results

Characteristics of the vegetation and soils at the four study areas are summarized in Table 1, Figure 2 and Figure 3. The steppe sites (Waha and Colfax) tended to have higher coverage of perennial graminoids than the meadow steppe sites (Kramer and Paradise Ridge) (Figure 2). Cryptogam cover tended to be higher at the steppe sites than at the meadow steppe sites, and higher in the native vegetation at those sites than in the non-native vegetation (Figure 3). At the steppe sites, the non-native plant transects were generally less rocky (as evidenced by lower coverage of surface rock and deeper penetration of the test bar) than the native plant transects (Table 1).

	Kramer		Paradise Ridge		Waha		Colfax	
	Native	*Non-native*	Native	*Non-native*	Native	*Non-native*	Native	*Non-native*
Plant community type	Meadow steppe		Meadow steppe		Steppe		Steppe	
Vegetation								
Shrubs	13.4	*18.6*	30.3	*10.5*	0.0	*0.0*	0.0	*0.0*
Perennials								
Graminoids								
Native perennial graminoids	21.0	*17.4*	15.4	*18.6*	54.4	*5.9*	29.8	*7.9*
Alien perennial graminoids	**19.8**	***3.3***	**0.0**	***0.0***	**0.0**	***6.9***	**17.9**	***9.4***
Forbs								
Native perennial forbs	87.4	*72.8*	23.5	*24.1*	37.1	*31.8*	9.3	*0.4*
Alien perennial forbs	**0.3**	***0.0***	**0.0**	***0.0***	**0.0**	***0.0***	**0.0**	***0.0***
Coverage native perennial forbs and graminoids	108.4	*90.1*	38.9	*42.8*	91.5	*37.6*	39.0	*8.3*
Percent native perennial graminoids	12.7	*10.2*	15.2	*21.5*	46.6	*6.5*	44.8	*7.9*
Annuals/biennials								
Graminoids								
Native annual grasses	0.0	*0.0*	0.0	*0.0*	0.0	*0.0*	0.6	*0.0*
Alien annual grasses	**1.8**	***13.7***	**6.9**	***7.8***	**16.9**	***25.6***	**2.0**	***33.5***
Forbs								
Native annual/biennial forbs	14.5	*24.1*	21.1	*17.1*	3.6	*2.3*	0.4	*13.0*
Alien annual/biennial forbs	**7.0**	***21.0***	**4.3**	***8.5***	**4.8**	***17.5***	**6.5**	***35.4***
Total vascular canopy coverage	165.0	*170.8*	101.4	*86.6*	116.8	*89.9*	66.4	*99.5*
Total coverage exotics	28.8	*37.9*	11.1	*16.3*	21.6	*50.0*	26.4	*78.3*
Percent exotic coverage	16.8	*22.2*	11.0	*18.8*	18.5	*55.6*	*39.7*	*78.6*
Total coverage annuals	23.3	*58.8*	32.3	*33.4*	25.3	*45.4*	9.5	*81.9*
Percent annual coverage	14.1	*34.4*	31.8	*38.5*	21.6	*50.5*	14.3	*82.3*
Total exotic annuals	8.8	*34.7*	11.1	*16.3*	21.6	*43.1*	8.5	*68.9*
Percent exotic annuals	5.3	*20.3*	11.0	*18.8*	18.5	*48.0*	12.8	*69.2*
Mosses	14.5	*1.0*	1.1	*7.1*	25.9	*14.0*	12.9	*0.0*
Lichens	0.1	*0.0*	0.5	*3.3*	1.4	*0.0*	6.4	*0.0*
Total cryptogam coverage	14.6	*1.0*	1.6	*10.4*	27.3	*14.0*	19.3	*0.0*
Bare ground	5.6	*11.0*	17.5	*22.0*	2.4	*6.0*	16.4	*11.3*
Rock	0.0	*0.0*	3.6	*5.5*	15.0	*0.5*	18.9	*5.9*
Litter	76.9	*52.1*	54.8	*56.5*	52.3	*91.3*	34.5	*68.4*
Soils								
Soil strength (lbs per sq. inch)	0.49	*0.19*	0.10	*0.16*	0.21	*0.17*	1.58	*1.75*
Soil temperature (degrees C)	17.7	*18.3*	13.7	*12.3*	19.7	*15.7*	36.7	*31.0*
Soil stoniness (cm to rock)	54.0	*45.8*	23.4	*23.6*	13.6	*36.4*	5.8	*24.6*
Slope (%)	11	*13*	25	*23*	9	*11*	22	*24*
Aspect	NW	*WSW*	ENE	*ENE*	SW	*SW*	E	*E*
Elevation (m)	850	*850*	1,070	*1,070*	1,100	*1,100*	670	*670*
Soil type	Palouse-Thatuna silt loam		Schumacher variant loam		Mallory-Jacket complex		Gwin-Linville complex	

Table 1. Characteristics of vegetation and soils at two meadow steppe sites (Kramer and Paradise Ridge) and two steppe sites Waha and Colfax).

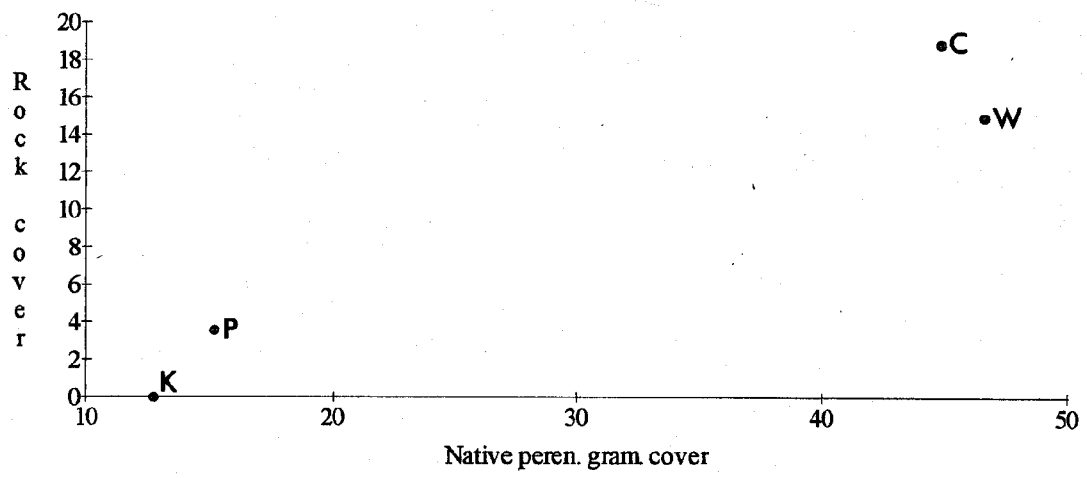


Figure 2. Rock cover and native perennial graminoid cover of sampling sites. Only data for transects dominated by native vegetation are shown. C = Colfax, K = Kramer, P = Paradise Ridge, W = Waha.

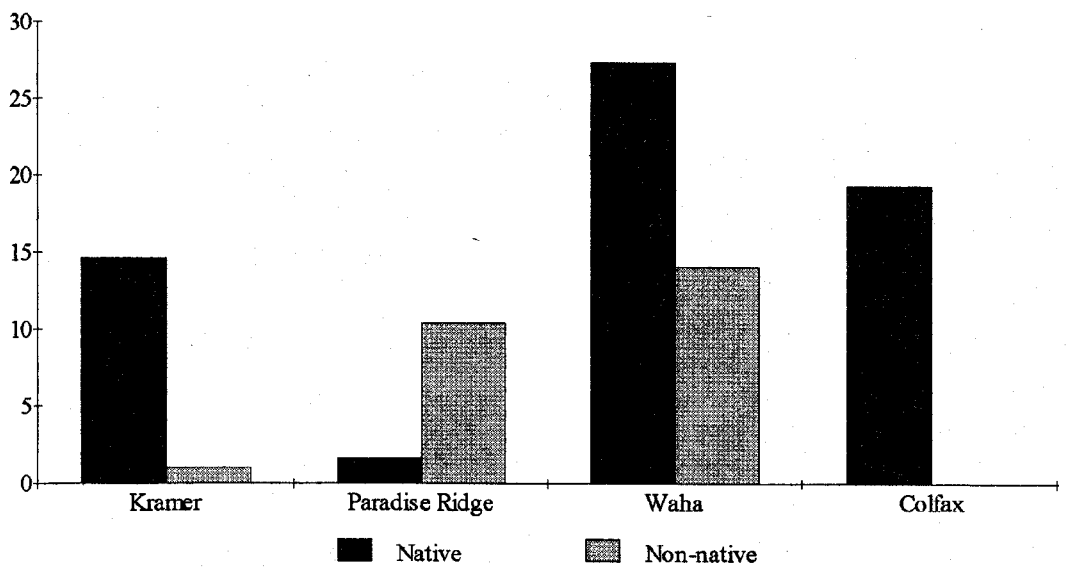


Figure 3. Comparison of coverage of mosses and lichens along transects dominated by native and non-native vegetation at four study sites. Waha and Colfax are steppe sites; Kramer and Paradise Ridge are in meadow steppe.

We analyzed 12 subsamples (2 subsamples/sample, 3 samples/transect, 2 transects/site) from each site except Colfax. We encountered technical difficulties with two of the Colfax subsamples, so at that site we had only 10 subsamples. Figure 4 shows an example of a gas chromatogram fatty acid fingerprint for one of the subsamples.

The distribution of the subsamples according to the first two principal components is plotted in Figure 5. The first principal component (PRIN1) explained 15.6% of the variance, and the second (PRIN2) accounted for 11.8% of the variance in the subsamples. Plant association seemed to have more effect on soil microbiota than the degree of coverage by non-native species. The profiles from bluebunch wheatgrass-Sandberg's bluegrass associations at Waha and Colfax (shown as circles on Figure 5)—tended to occupy high positions along the PRIN1 axis and low positions along the PRIN2 axis. The reverse was true for the profiles from meadow steppe vegetation—Idaho fescue/common snowberry associations at Paradise Ridge and Kramer—(shown as triangles in Figure 5); they scored relatively high on PRIN2 and low on PRIN1.

The separation among FAME profiles from transects dominated by native and non-native vegetation (solid and open symbols respectively) was less dramatic, although some differences were apparent. Samples taken in native vegetation (solid symbols) tended to have a narrower distribution along PRIN2 than samples from non-native vegetation (open symbols).

2.1.4. Discussion

Using profiles of fatty acid methyl esters extracted from surface soil samples, we found differences between the microbial communities associated with Idaho fescue/common snowberry associations and bluebunch wheatgrass-Sandberg's bluegrass associations. We did not find pronounced differences between the microbiota of stands dominated by non-native and native plants, however, although some differences were apparent. This may be because non-native species—especially annual bromes, such as cheatgrass and ventenata, and, at Kramer, the perennial Kentucky bluegrass—are present even in communities dominated by native grasses and forbs. We did not look at the microbial associations of particular species. Such an analysis might reveal differences in soil microorganisms that were not revealed in this study.

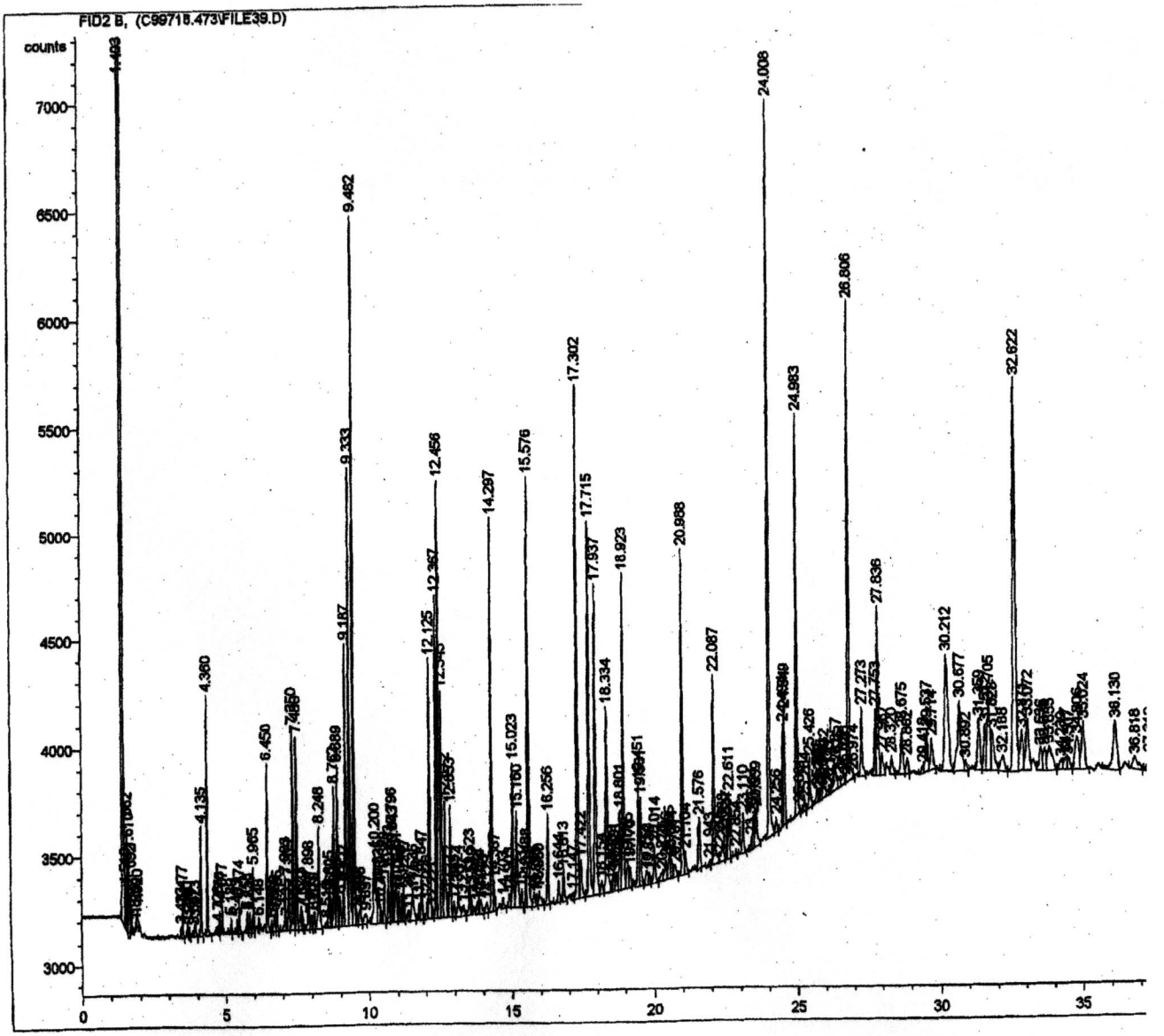
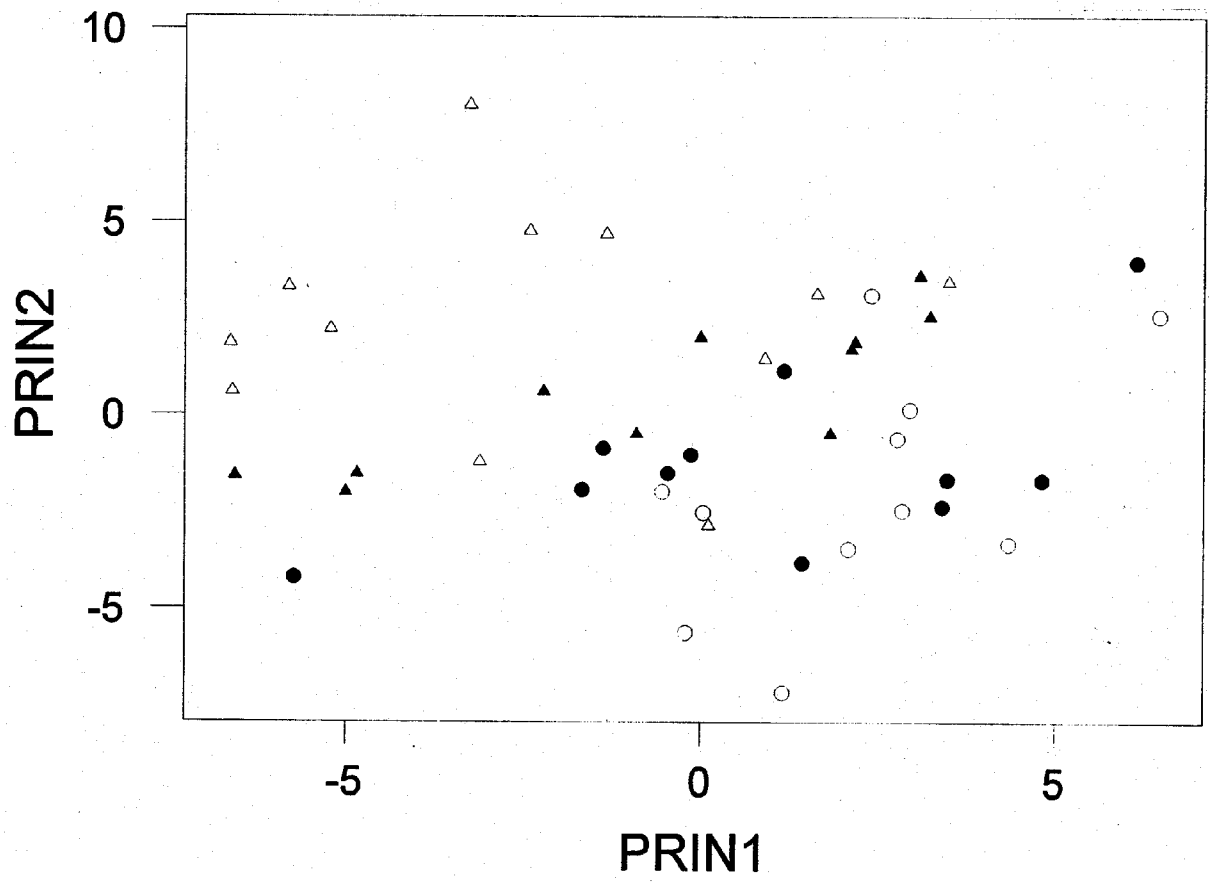


Figure 4. Fatty acid fingerprint of a soil subsample collected along the native transect at the Kramer site.

Figure 5. Two-dimensional plot of the first two principal components derived from profiles of FAMEs extracted from soils dominated by native and non-native vegetation at two meadow steppe and two steppe sites. Open circles: non-native steppe (Waha and Colfax); open triangles: non-native meadow steppe (Kramer and Paradise Ridge). Filled circles: native steppe (Waha and Colfax); filled triangles: native meadow steppe (Kramer and Paradise Ridge). Note that the separation of meadow steppe and steppe samples (circles and triangles) is greater than the separation of native and non-native samples (open and filled symbols).



2.1.5. Literature cited

Daubenmire, R.F. 1970. Steppe vegetation of Washington. Washington Agricultural Experiment Station, Washington State University, Technical Bulletin 62.

Haack, S.K., H. Garchow, D.L. Odelson, L.J. Forney, and M.J. Klug. 1994. Accuracy, reproducibility, and interpretation of fatty acid methyl ester profiles of model bacterial communities. Applied and Environmental Microbiology 60:2483-2493.

Jolliffe, I.T. 1986. Principal component analysis. Springer-Verlag, New York.

Kennedy, A.C. 1994. Carbon utilization and fatty acid profiles for characterization of bacteria. Pp. 543-556 *in* R. Weaver, J.S. Angle, P.S. Bottomley, and D. Bezdiecek, eds., Methods of Soil Analysis, Part 2: Microbiological and chemical properties. American Society of Agronomy, Madison. WI.

Kennedy, A.C. and Busacca, A. 1995. Microbial analysis to identify source of PM-10 material. Pp. 670-675 *in* Proceedings of the Air and Water Waste Management Association Specialty Conference on Particulate Matter: Health and Regulatory Issues, Pittsburgh, PA.

Klug, M.J. and J.M. Tiedje. 1993. Response of microbial communities to changing environmental conditions: Chemical and physiological approaches. Pp. 371-374 *in* R. Guerrero, and C. Pedros-Alio, eds., Trends in microbial ecology. Spanish Society for Microbiology, Barcelona, Spain.

SAS Institute. 1988. User's guide. Statistics Version 6. Statistical Analytical Institute, Cary, NC.

Sasser, M. 1990. Identification of bacteria by gas chromatography of cellular fatty acids. MIDI Technical Note #101. MIDI Labs, Inc., Newark, DE.

Weddell, B.J. and J. Lichthardt. 1998. Identification of conservation priorities for and threats to Palouse Grassland and Canyon Grassland remnants in Idaho, Washington, and Oregon. Idaho Bureau of Land Management, Technical Bulletin No. 98-13.

Westover, K.M., A.C. Kennedy, and S.E. Kelley. 1997. Patterns of rhizosphere microbial community structure with co-occurring plant species. Journal of Ecology 85:863-873.

2.2. Experimental Test of Microbial Biocontrol of Cheatgrass

B.J. Weddell, A. Kennedy, P. Frohne, S. Higgins

2.2.1. Introduction

The establishment of alien annual grasses in disturbed Palouse and canyon grasslands presents a major challenge for parties seeking to restore native bunchgrass vegetation (Youtie 1997; Youtie et al. 1998, 1999; **Section 3, this volume**). One promising area of research involves manipulating the microbiota of the rhizosphere (the portion of the soil dominated by plant roots) to control weeds. (See Metting 1993 for review.)

Rhizobacteria (bacteria that actively colonize roots) with deleterious effects on weedy species have the potential to control weeds without the undesirable effects associated with the application of herbicides (Cherrington and Elliott 1987; Kremer 1987, Kremer et al. 1990; Kennedy et al. 1991; Kennedy and Kremer 1996). They are highly selective in their phytotoxic effects, and their use does not entail application of synthetic chemicals. Studies on agar, in growth chambers, and in the field have demonstrated that bacteria of the genus *Pseudomonas* have negative effects on the growth of cheatgrass (*Bromus tectorum*) (Cherrington and Elliott 1987; Kennedy et al. 1991).

Cheatgrass, a native of Eurasia, is ubiquitous in croplands and rangelands throughout the West (Mack 1981; Morrow and Stahlman 1984). Recruitment is usually concentrated in late summer and fall (Mack and Pyke 1983). Its ability to extract moisture from the upper layers of soil in winter and spring makes it an effective competitor for water (Hulbert 1955; Evans et al. 1970).

Methods of controlling cheatgrass are non-selective (Morrow and Stahlman 1984). Cheatgrass control is especially problematic in areas where maintenance or restoration of native grassland vegetation is the goal. There is no herbicide that is specific for cheatgrass, and burning can cause unacceptable mortality in native bunchgrasses and soil crust species (Youtie et al. 1999). In this study, we tested the effectiveness of *Pseudomonas fluorescens* as a biological agent to control cheatgrass on a formerly cultivated site where a program to restore native perennial grasses is in progress.

2.2.2. Methods

We tested the effects of *Pseudomonas* on germination and emergence of cheatgrass in a portion of a field in Pullman, Washington. The site was cultivated until 1995, when efforts to restore native grasses and forbs were initiated. The site was seeded with bluebunch wheatgrass (*Pseudoroegneria spicata* ssp. *spicata* [=*Agropyron spicatum*]) and Idaho fescue (*Festuca idahoensis*) in the fall of 1996 and again in the fall of 1997, but coverage of native perennial grasses remained low, in part because of a heavy infestation of cheatgrass.

After the onset of the fall rains in 1999, we inoculated the treatment areas with a culture of *Pseudomonas fluorescens* strain D7 at a concentration of 10^9 cells per plot. Because of the uniformly high density of cheatgrass on the study site prior to the start of the experiment, we assumed that the seed banks of all plots contained high densities of cheatgrass seeds. The experimental design was a 2-x-2 factorial design, with two levels of inoculum and two levels of herbicide, in a randomized complete block. Two 8-by-10-m blocks were divided into four rows, and the inoculation treatment was randomly assigned to two rows in each block. Approximately half of each row had been sprayed with Roundup® the previous spring (isopropylamine salt of glyphosate). Within each row, two 1-by-1-m plots were established, one in the area that had received *Roundup*® and one in the unsprayed area. The inoculum was applied on October 30 and November 24, 1999. Substantial amounts of cheatgrass had emerged prior to October 30; these were removed from the plots prior to the first inoculation.

The number of cheatgrass seedlings in a 10-cm-diameter circular sample from within each 1-m^2 plot was counted on February 15 or February 20, 2000 and used to calculate the density of cheatgrass seedlings per m^2. The location of the sample within each plot was randomly selected using a 10-by-10 grid. The density of seedlings in each plot in February was also ranked as high or low to evaluate whether seedling densities in the circular samples reflected densities throughout the plots.

The coverage of perennial caespitose grasses in the plots was recorded on October 30, 1999 and again on February 23, 2000 to determine whether the inoculum negatively affected established bunchgrasses. The diameter of each bunchgrass clump was assigned to one of the following classes: 0-1 cm, 1-5 cm, 5-10 cm, and 10-15 cm. The canopy coverage of each bunch was estimated as the area of a circle with a diameter equal to the midpoint of the appropriate coverage class. Mean values for perennial grass cover in October and February were compared using a 2-tailed paired *t* test.

2.2.3. Results

The number of seedlings per sample in plots that were ranked as low in density ranged from 9 to 25 (mean 16.6 ± 5.4), whereas the number of seedlings per sample in the high density plots ranged from 44 to 118 (73.4 ± 28.5). This correspondence between seedling density in the 1-by-1-m plots and in the 10-cm-diameter samples suggests that a single sample per plot was representative of seedling density throughout the plot.

The densities of cheatgrass seedlings per square meter ranged from 1,146 to 15,021. Seedling densities were not lower in plots treated with *Pseudomonas* than in untreated plots, but they were significantly lower ($P = 0.001$) in plots that had been sprayed with Roundup® the previous spring (Table 1). The interaction between the *Pseudomonas* treatment and application of Roundup® was not significant ($P = 0.172$).

The coverage of perennial grasses February did not differ significantly from the coverage of perennial grasses in October for either the control plots ($P = 0.405$) or the inoculated plots ($P = 0.351$).

Table 1. Effects of herbicide and fall application of *Pseudomonas* on density of cheatgrass seedlings. Seedling densities per square meter were calculated on the basis of a 10-cm-diameter circular sample.

Mean number of seedlings/m^2 ± SD (range)		P level
Pseudomonas 6,588 ± 5,736 (1,146-15,021)	No *Pseudomonas* 6,031 ± 3,526 (1,655-11,457)	0.712
Roundup® 2,800 ± 2,057 (1,146-7,638)	No Roundup® 9,818 ± 3,624 (5,601-15,021)	0.001
Block A 6,954 ± 4,658 (1,655-15,021)	Block B 5,665 ± 4,781 (1,146-14,894)	0.400

2.2.4. Discussion

Inoculation with *Pseudomonas* did not adversely affect the growth of the perennial grasses in this experiment. Thus, it appears that biocontrol with *Pseudomonas* is appropriate for restoration of sites where the maintenance of native perennial grasses is desired; however, the inocula were not effective in suppressing cheatgrass either. Although *Pseudomonas* inocula have decreased the population size and growth of cheatgrass in growth chamber and field trials (Kennedy et al. 1991), in this experiment we did not find that the treatment had any effect on the density of cheatgrass seedlings, even in those plots that had been treated with *Roundup*® the previous spring. The densities of cheatgrass in the test plots were still fairly high (greater than 1,000 per m^2 in all cases), even after the first flush of seedlings, which had germinated prior to October 30, had been removed. Kennedy et al. 1991 tested moderate weed infestations. Since this study tested a heavy infestation, it might take multiple years for any inhibitory effects to appear. The question of whether the *Pseudomonas* inocula would be effective at sites with smaller seed banks is worth investigating.

We did not determine whether the test inoculum reduced the vigor of cheatgrass seedlings. It is possible that even if the size of the cheatgrass population did not decline, a reduction in cheatgrass vigor might give native perennial grasses a competitive advantage and reduce the impact of cheatgrass infestations at sites with native perennial grasses.

Kennedy et al. (1991) found that the negative effects of *Pseudomonas* inocula were more pronounced at Washtucna, WA, which receives less precipitation than Pullman and which received below-normal precipitation in the year of the test (annual precipitation in 1987 was 250 mm at Washtucna and 400 mm at Pullman). This suggests that water stress might enhance the susceptibility of cheatgrass to deleterious rhizobacteria and that biocontrol with *Pseudomonas* might be more effective in drier zones.

2.2.5. Literature cited

Cherrington, C.A. and L.F. Elliott. 1987. Incidence of inhibitory pseudomonads in the Pacific Northwest. Plant and Soil 101:159-165.

Evans, R.A., H.R. Holbo, R.E. Eckert, Jr., and J.A. Young. 1970. Functional environment of downy brome communities in relation to weed control and revegetation. Weed Science 18:154-162.

Hulbert, L.C. 1955. Ecological studies of *Bromus tectorum* and other annual bromegrasses. Ecological Monographs 25:181-213.

Kennedy, A.C., L.F. Elliott, F.L. Young, and C.L. Douglas. 1991. Rhizobacteria suppressive to the weed downy brome. Soil Science Society of America Journal 55:722-727.

_____. and R.J. Kremer. 1996. Microorganisms in weed control strategies. Journal of Production Agriculture 9:480-484.

Kremer, R.J. 1987. Identity and properties of bacteria inhabiting seeds of selected broadleaf weed species. Microbial Ecology 14:29-37.

Kremer, R.J., M.F.T. Begonia, L. Stanley, and E.T. Lanham. 1990. Characterization of rhizobacteria associated with weed seedlings. Applied and Environmental Microbiology 56:1649-1655.

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

Mack, R.N. and D.A. Pyke. 1983. The demography of *Bromus tectorum*: Variation in time and space. Journal of Ecology 71:69-93.

Metting, F.B., Jr. 1993. Microbial ecology of the rhizosphere. Pp. 27-63 *in* F.B. Metting, Jr., ed., Soil microbial ecology: Applications in agricultural and environmental management. Marcel Dekker, Inc., New York.

Morrow, L.A. and P.W. Stahlman. 1984. The history and distribution of downy brome in North America. Weed Science Supplement 32:2-6.

Youtie, B. 1997. Weed control as the first step in protecting and restoring native plant communities on northeast Oregon natural areas. Pp. 78-82 *in* T.N. Kaye, A. Liston, R.M. Love, D.L. Luoma, R.J. Meinke, and M.V. Wilson, eds. Conservation and management of native plants and fungi. Native Plant Society of Oregon, Corvallis.

Youtie, B., J. Ponzetti, and D. Salzer. 1999. Fire and herbicides for exotic annual grass control: Effects on native plants and microbiotic soil organisms. Pp. 590-591 *in* VIth International Rangeland Congress Proceedings, Vol 2.

_____, _____, _____, and J. Soll. 1998. Controlling annual exotic grasses on relict grasslands (Oregon). Restoration and Management Notes 16:2.

2.3. Experimental Test of the Effects of Erosion Control Blankets on the Survival of Bluebunch Wheatgrass Plugs

B.J. Weddell

2.3.1. Introduction

Non-native annuals have invaded native steppe vegetation throughout the intermountain region. To date, no satisfactory method has been developed for restoring native perennial bunchgrass associations once they have been colonized by exotic species. The primary reason for this is that the removal of vegetation, even non-native vegetation, creates conditions that favor non-natives. This paradoxical situation occurs because the Eurasian annuals that have invaded possess adaptations which allow them to exploit areas of bare soil created by disturbances, whereas native species of the intermountain steppe were not exposed to frequent, large-scale disturbances during their evolution and lack adaptations to cope with such disturbances (Tisdale 1961; Mack and Thompson 1982).

This creates a frustrating predicament for land managers who wish to improve or restore native steppe that has been invaded by exotics. A variety of techniques, including herbicides, fire, and mechanical removal, have been used in attempts to minimize populations of exotic annual grasses and forbs and to promote the growth of native perennials, but so far, the long-term results of this work have been disappointing. Although it is possible to suppress the growth of exotics temporarily, generally after a few years their populations rebound (Youtie et al. 1998). One way to circumvent this problem would be to devise a restoration strategy that enhances the coverage of native species and reduces exotics without creating areas of bare soil for exotics to reinvade. Ideally, such a strategy would also maintain or restore the microbiotic crust of mosses, lichens, algae, cyanobacteria, and fungi that typically covers much of the ground between grasses and forbs in intermountain steppe communities. Inoculation with crust material enhances the rate of recovery of disturbed areas, but even with inoculation, lichens are expected to take decades to recover and mosses might not recover for centuries (Belnap 1993). During that time, exposed soil is vulnerable to colonization by exotics.

Biodegradable ground covers are often used in horticulture and restoration to control weeds and minimize erosion while transplants become established. This study examined the efficacy of biodegradable erosion control blankets in suppressing the growth of exotics around transplanted plugs of bluebunch wheatgrass at a location where native steppe vegetation has been displaced by non-native species. The establishment of seedlings in patches of moss placed on bare soil was also monitored.

2.3.2. Description of the study area

The study area is a privately-owned parcel of undeveloped land on a ridge 1.9 km northeast of Waha, in the southeast ¼ of Section 4, T. 33 N., R. 4 W. The location is surrounded by homesites. On drier locations where native vegetation persists, bluebunch wheatgrass (*Pseudoroegneria spicata* var. *spicata*) is dominant, accompanied by native grasses and forbs such as few-flowered wild oatgrass (*Danthonia unispicata*), Sandberg bluegrass (*Poa secunda*), grass-widows (*Olysinium douglasii* var. *inflatum*), cous biscuit-root (*Lomatium cous*), and narrow-leaved skullcap (*Scutellaria angustifolia*), as well as non-native annuals. On more mesic portions of the site, Idaho fescue (*Festuca idahoensis*) and common snowberry (*Symphoricarpos albus*) dominate. In some areas, however, native species have been almost completely replaced by non-native grasses and forbs. In 1998, the yellow star-thistle (*Centaurea solstitialis*) canopy intercepted over 65% of four 50-m transects in a weedy portion of the site (Weddell and Lichthardt 1998). Field morning glory (*Convolvulus arvensis*), medusahead (*Taeniatherum caput-medusae*), erect cinquefoil (*Potentilla recta*), teasel (*Dipsacus fullonum*), tall oatgrass (*Arrhenatherum elatius*), ventenata (*Ventenata dubia*), annual bromes, and piedmont bedstraw (*Cruciata pedemontana*) are prevalent.

2.3.3. Methods

In the autumn of 1999, bluebunch wheatgrass seeds were collected onsite. These were started in a greenhouse on November 30. On April 5, 2000, 48 of the resulting plugs were planted in a weedy section of the study site, after being hardened out of doors for 48 hours. The experimental design was a randomized complete block, with two blocks. Each block contained six rows of four plugs, spaced at 2.5-m intervals. Rows were 1.5-m apart. The vegetation in the blocks was heavily infested with weeds, so that virtually no native vascular plants or cryptogams remained. Half the plugs were planted in the center of two-layer, 1-m^2 coconut fiber erosion control blankets bonded with biodegradable netting (*Bon Terra*, available from Arrow Construction, Spokane, WA). This product is permeable to gases and liquids and is designed to biodegrade within 36 months. Mats were placed around the plugs on April 8, 2000, and anchored with 15-x-2.5-x-15-cm staples. Each plug was watered with 250 ml water immediately after planting and again three days later.

In addition, all vascular plants, cryptogams, and litter were removed from two 50-cm-x-70-cm test blocks. A 10-cm wide buffer was left around the block perimeter. Each of the two 30-cm-x-50-cm test areas that remained was divided into three rows, each of which contained five 10-cm-x-10-cm experimental plots. One of these received a mat of mosses (*Tortula* sp.) and lichens (*Peltigera* sp. and *Cladonia* sp.). Seven of the remaining plots received a 10-cm-x-10-cm patch of mosses (primarily *Homalothecium* sp.) gathered from the adjacent Idaho fescue/common snowberry stand, and half the plots remained bare. Recruitment of yellow star-thistle and other species in plots with and without moss was monitored on May 23 and September 17.

Survival of the bluebunch wheatgrass plugs was recorded on May 23 and October 10, 2000. Two variables—basal diameter and number of reproductive culms—were

measured on

plugs that survived until October and compared for treatments and controls using analysis of variance. Values for the number of spikes were transformed using $\sqrt{Y} + 0.5$ because the data included many zeros.

2.3.4. Results and discussion

All the transplanted plugs survived until May 23. A considerable amount of pre-existing vegetation had managed to grow up through the mats as well (Figure 1). By October 10, nearly one third of the plugs had died. The proportion of bluebunch wheatgrass plugs that survived was only higher for plugs surrounded by erosion control blankets (75%) than for controls (63%). The unprotected plugs were significantly smaller ($P < 0.01$) and produced significantly fewer spikes ($P = 0.04$) than the treatment plugs (Figure 2), however.

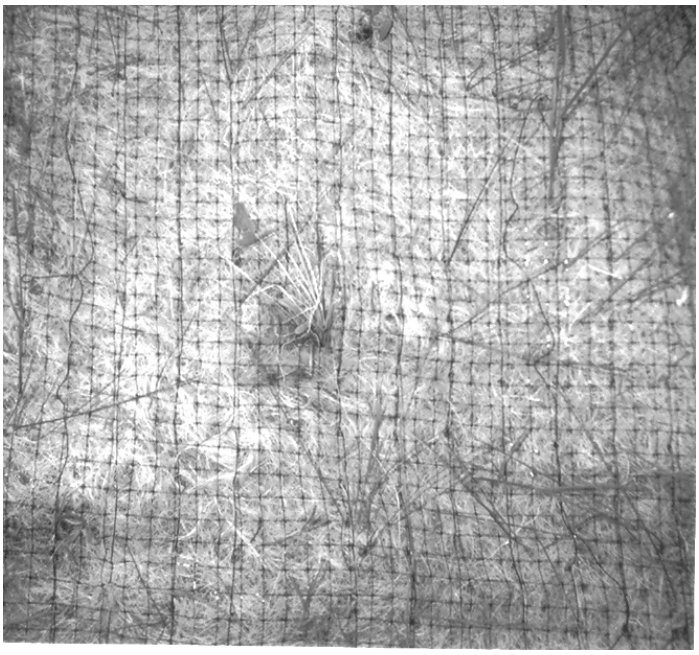


Figure 1. Biodegradable erosion control mat surrounding bluebunch wheatgrass plug 53 days after planting. Note how much of the pre-existing vegetation has penetrated the mats.

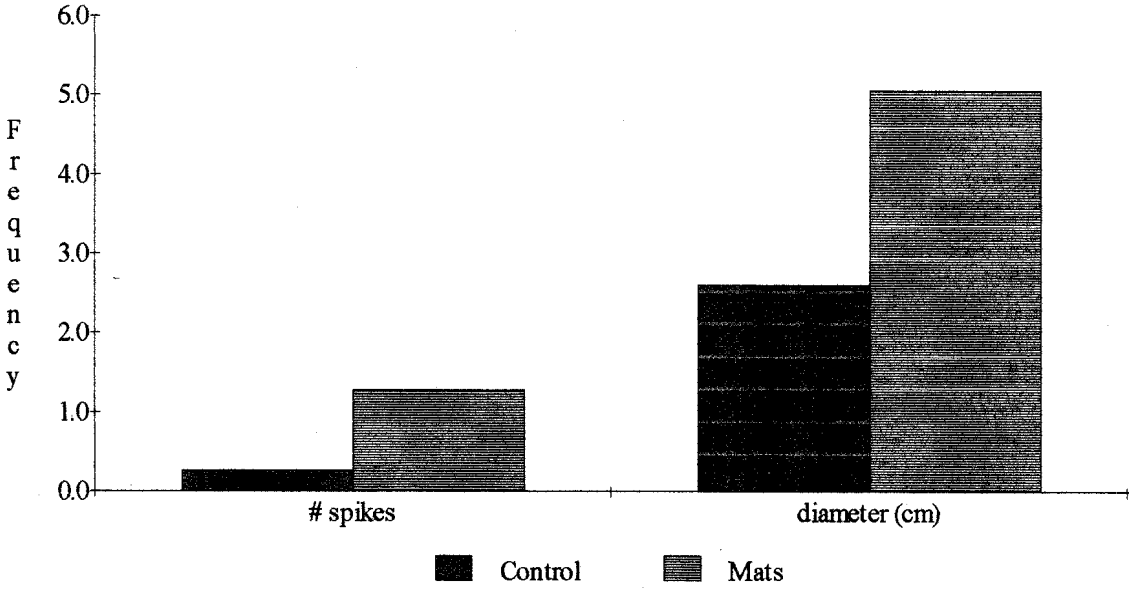


Figure 2. Comparison of number of spikes and crown diameters of bluebunch wheatgrass plugs surrounded by biodegradable mats and untreated plugs. Values are for plugs that survived until October 10.

The mosses in the crust patches were dry and appeared to be dead by August 4. Their appearance did not change after the onset of rains in September. Annuals or biennials that germinated both in the moss patches and on bare soil included yellow star-thistle, annual bromes, teasel, medusahead, ventenata, fiddleneck (*Amsinckia* sp.), thyme-leaf sandwort (*Arenaria serpyllifolia*), and Thale cress (*Arabidopsis thaliana*). All of these species except *Amsinckia* are exotics. The numbers of yellow star-thistle seedlings in the control and treatments plots were similar both in spring (15 individuals in plots with moss patches and 13 individuals in control plots) and fall (10 individuals in plots with moss patches and 11 individuals in control plots). Yellow star-thistle also germinated in plots with the moss/lichen patches.

These results suggest that biodegradable erosion control mats can enhance the establishment of native plants in areas with heavy infestations of non-native species. The fiber mats used in this experiment were not entirely successful in suppressing the vegetation they covered, however. Plastic mats would have been more effective in killing undesirable plants, but they would also have altered the soil environment appreciably. A permeable and biodegradable product was selected for this experiment to minimize such effects. Although the fiber matting probably has less dramatic effects on the soil and soil organisms than plastic, even the coconut fiber covering undoubtedly alters soil temperature. Furthermore, as it degrades, it will provide a source of carbon. The effects of these changes on soil microbiota are unknown. Other important

questions need to be answered as well. It is not known whether the mats will be a barrier to the establishment of non-native seedlings, and if so how long that effect will last. And we do not known whether cryptogams will become established on the mats as they degrade.

The microbiotic crust of steppe communities affects germination and seedling establishment directly by forming a mechanical barrier to root penetration (Schlatterer and Tisdale 1969) and through allelopathic effects, and indirectly through its effects on soil-water relations and nutrient status. (See Harper and Marble 1988 for review.) Thus, it is a critical element of steppe restoration programs. However, in this study, the moss patch trials did not demonstrate any effect of patches on the recruitment of annuals. This may be because the mosses did not survive. Although the soil surface was moist when the patches were put in place, and remained moist for several more weeks, the mosses became desiccated in summer and did not revive when moisture returned. For this reason, the effects of the moss patches may not have been very different from the effects of a non-living mat.

The collection of moss patches to transplant to disturbed sites creates bare areas in the donor area. In this study, this problem was minimized by collecting mosses from rocks and from beneath clumps of dense shrubs. In addition, the spatial scale of the trials was kept small (fractions of a meter) to minimize damage to intact crust. Larger-scale trials were avoided because it was felt that these would create unacceptably large patches of bare soil in the donor areas.

There is clearly a need for restoration techniques that enhance native species and remove non-natives without thereby creating further opportunities for colonization by non-native plants. In particular, a method of restoring damaged microbiotic crusts would be a boon to restoration efforts. In situations where native vegetation has been virtually eliminated, biodegradable erosion control blankets may be useful for protecting native species until they become established. This technique is labor intensive, however, so it is not practical for large areas. On the other hand, it avoids some of the disadvantages of other approaches to controlling exotic species, such as chemical and mechanical treatments and burning, which create bare areas vulnerable to exploitation by exotics.

2.3.5. Literature cited

Belnap, J. 1993. Recovery rates of cryptobiotic crusts: Inoculant use and assessment methods. Great Basin Naturalist 53:89-95.

Harper, K.T. and J.R. Marble. 1988. A role for nonvascular plants in management of arid and semiarid rangelands. Pp. 135-169 *in* P.T. Tueller, ed., Vegetation science applications for rangeland analysis and management. Kluwer Academic Publishers, Dordrecht.

Mack, R.N., and J.N. Thompson. 1982. Evolution in steppe with few large, hooved mammals. American Naturalist 119:757-773.

Schlatterer, E.F. and E.W. Tisdale. 1969. Effects of litter of *Artemisia*, *Chrysothamnus*,

and *Tortula* on germination and growth of three perennial grasses. Ecology 50:869-873.

Tisdale, E.W. 1961. Ecologic changes in the Palouse. Northwest Science 35:134-138.

Weddell, B.J. and J. Lichthardt. 1998. Identification of conservation priorities for and threats to Palouse Grassland and Canyon Grassland remnants in Idaho, Washington, cand Oregon. Idaho Bureau of Land Management, Technical Bulletin No. 98-13.

Youtie, B., J. Ponzetti, D. Salzer, and J. Soll. 1998. Controlling annual exotic grasses on relict grasslands (Oregon). Restoration and Management Notes 16:2.