THESIS

FACTORS CONTROLLING LONG-TERM COMMUNITY DEVELOPMENT OF A SAGEBRUSH STEPPE ECOSYSTEM

Submitted by

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY TIMOTHY B. HOELZLE ENTITLED FACTORS CONTROLLING LONG-TERM COMMUNITY DEVELOPMENT OF A SAGEBRUSH STEPPE ECOSYSTEM BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

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ABSTRACT OF THESIS

FACTORS CONTROLLING LONG-TERM COMMUNITY DEVELOPMENT OF A SAGEBRUSH STEPPE ECOSYSTEM

A study was established in 1984 in the Piceance Basin of northwest Colorado to examine how nutrient availability, soil organisms, and seed availability affect plant and microbial community development following disturbance. Initial results showed that increased nitrogen (N) availability and removal of soil organism limited plant community recovery, while decreased N availability and seeding with late seral species accelerated community development. Nutrient addition and immobilization treatments continued through 1999. Here, I examined how these treatments affected plant and microbial community composition 25 years after the initial disturbance.

Supporting earlier findings, repeated N addition limited plant community succession, while phosphorus (P) addition had little effect. However, addition of N and P together worked synergistically to further retard successional recovery through the promotion of the invasive winter annual, *Bromus tectorum* L.. Although nutrient additions resulted in differences in the rate of ecosystem development, few differences were observed in microbial biomass and composition, indicating that these treatments did not strongly affect these communities.

Initial results showed that the rate of plant community development was accelerated by N immobilization through the addition of sucrose; however, I found that plant community composition was similar between these communities and those receiving N, indicating convergence in successional trajectories ten years after the cessation of treatments. Soil fungi, which often increase with community development, were higher in plots receiving the sucrose amendment. This suggests that, even though differences in successional development of the plant community were not found, succession in the belowground system was accelerated through sucrose additions.

Although removal of soil organisms by fumigation initially slowed plant ecosystem recovery, these differences were no longer apparent 25 years later, illustrating that plant and microbial communities can recover from this type of disturbance. However, differences in successional trajectories were observed as a result of seed mix. Seeding with early seral species resulted in a community with significantly more exotic species and mid seral shrubs, while seeding with late seral species resulted in a community dominated by perennial grasses. This suggests that seed mix can alter successional trajectory, providing long-term evidence for the role of priority effects in community development.

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PART ONE

LONG-TERM COMMUNITY DEVELOPMENT IN RESPONSE TO REPEATED NITROGEN AND PHOSPHORUS ADDITION

I. INTRODUCTION

It is well accepted that terrestrial ecosystem productivity is generally limited by nitrogen (N) and phosphorus (P) (Walker & Syers, 1976; Vitousek & Howarth, 1991; Vitousek & Reiners, 1991; Elser et al., 2007). Numerous investigations have been conducted attempting to elucidate the impact of nutrient levels on plant communities (DiTommaso & Aarssen, 1989), and specifically their role in ecosystem development following severe disturbance (Wali, 1999). Where nutrients are limited, their addition results in increased plant productivity and decreased species richness (Inouye & Tilman, 1995; Foster & Gross, 1998), often dominated by species especially adapted to high nutrient conditions (Bakelaar & Odum, 1978; Maly & Barrett, 1984; Carson & Barrett, 1988) and lacking a native woody component (Tilman, 1987). Nutrient addition to systems that evolved with low nutrient inputs often results in significant increases in non-native species production at the expense of native species (Holmes, 2001; Brooks, 2003; Rickey & Anderson, 2004; Leishman & Thomson, 2005), and in the western US, effects of elevated N (Lowe et al., 2003; Saetre & Stark, 2005) and P levels (Miller et al., 2006; Belnap & Sherrod, 2009) on *Bromus tectorum* L. productivity are of particular concern. This persistent winter annual of Eurasia has infested an estimated 40,000,000 hectares of the western US (Rosentreter, 1994). Establishment of significant *B. tectorum* populations has been shown to impede ecosystem succession through increased fire frequency (Epanchin-Niell et al., 2009), shifts in water dynamics

(Kulmatiski et al., 2006), and decreased N availability to native species (Sperry et al., 2006).

Most research on community development has focused on competition between plants (Thompson et al., 2001), but more recently studies have begun to focus on the role soil microorganisms play in controlling vegetation composition and succession (van der Heijden et al., 1998; Reynolds et al., 2003). Soil microbial activity can influence plant community diversity and succession through suppression of dominant plant species (De Deyn et al., 2003) or promotion of rare species (van der Heijden et al., 1998). Vegetation succession has been linked to an increase in the ratio of fungal: bacterial biomass in the soil (van der Wal et al., 2006), however it is unclear which component drives this relationship (Harris, 2009). Soil nutrient levels can also affect the interactions between these organisms. Presence of soil biota in the absence of fertilizer has been shown to increase plant species diversity and promote later successional plant species, however an increase in soil nutrient levels resulted in a decline in plant species diversity and a shift in community composition toward early successional species (De Deyn et al., 2004). Increased soil nitrogen availability results in greater plant productivity (Inouye & Tilman, 1995), which can lead to increased soil microbial biomass through increased availability of soil carbon and nitrogen (Wardle, 1992). Diversity of arbuscular mycorrhizal (AM) fungi, which play an important role in plant nutrient and water acquisition (Smith and Read, 1997), has been linked to increased plant species diversity and productivity, as well as ecosystem stability (van der Heijden et al., 1998). Addition of P has been shown to decrease AM fungal diversity (Raznikiewicz et al., 1994)

and colonization (Duke et al., 1994), indicating a mechanism for the role of P in microbial community development.

A majority of studies assessing community succession in response to elevated nutrient levels have been conducted for short time periods and/or under controlled, greenhouse conditions, limiting our understanding of long-term development of plant and soil communities. Inouye and Tilman (1995) highlight the importance of long-term studies of vegetation dynamics in response to fertilization, suggesting that short-term studies offer only a snapshot of community development and do not provide information on the effects of nutrient addition on long-lived species.

This study examines the response of plant and microbial populations to repeated N and P addition in an *Artemisia tridentata* Nutt. ecosystem 24 and 25 years after disturbance. Initial investigations (Carpenter et al., 1990; McLendon & Redente, 1991) of the plant community suggested that N addition impeded ecosystem recovery, while P addition did not significantly affect plant community development. Application of N retarded secondary succession by increasing the productivity of annual plant species, resulting in decreased presence of perennial plant species. After five years of N addition, plots continued to be dominated by annuals, while perennial grasses and shrubs dominated in the unfertilized, control plots (McLendon and Redente, 1991).

By revisiting these experiments 24 and 25 years after the initial disturbance, the aim was to examine differences in plant community composition and production in plots receiving repeated input of N and P in relation to relevant attributes of the soil microbial community. This information will be useful in a variety of applications, including

improving our understanding of ecosystem development and successional processes, determining effective methods to restore arid lands impacted by disturbance, and assisting in the development of regulatory guidelines for reclamation of disturbed lands. The specific objective of this study was to understand how repeated addition of N and P affects plant and microbial community composition and soil nutrient levels 25 years after a physical disturbance. I hypothesized that (1) N addition would result in a plant community composition dominated by early-seral (annual) species with no differences between N applied alone and N and P applied together, (2) P addition alone would have no effect on plant community composition, and (3) relative to an unfertilized control and undisturbed native system, addition of N would result in greater microbial biomass and a lower fungal: bacterial biomass ratio, while P addition would result in decreased AM fungi activity and biomass.

II. METHODS

Study Description

The study site is located 65 km northwest of Rifle, Colorado, USA in the Piceance Basin (UTM 12 S 722198 4420302) at an elevation of 2,030 m. The climate is semiarid, with a mean annual precipitation of 297 mm, approximately half occurring as snowfall (HPRCC, 2010). The main soil type is Yamac loams (fine-loamy mixed, Borollic Camborthids), supporting a big sagebrush steppe community (USDA, 1982). In August 1984, research plots were established, fenced to exclude cattle grazing, and a disturbance, similar to that of proposed resource extraction activity, was conducted within the *A. tridentata* community. All vegetation and the top 5 cm of soil were removed from the site, and the next 25 cm of soil were thoroughly mixed. The disturbance resulted in a reduction of >90% of the soil seed bank (Carpenter et al., 1990). Following the disturbance, four blocks of treatment plots were established in a factorial design. Each block consisted of one 500-m^2 plot of each treatment (unfertilized control, nitrogen only, phosphorus only, and nitrogen + phosphorus) and an undisturbed reference plot of equal size. Nutrient treatments were added three times yearly from 1984 through 1999. N was applied as ammonium nitrate at a rate of 100 kg N ha^{-1} yr^{-1} and P was applied as triple super phosphate at a rate of 100 kg P ha^{-1} yr^{-1}. Refer to McLendon and Redente (1991) for detailed treatment descriptions.

Vegetation Estimation

Vegetation sampling occurred during summer of 2008 and 2009. Sampling was conducted twice during the growing season (early- and late-summer) in an effort to capture the peak biomass of both the cool-season and warm-season plant species (Lauenroth et al., 1986). During each sampling period, eight-0.5 m^2 quadrats were randomly placed within each plot and aboveground biomass from the current year was harvested to ground level, separated by species, and collected. For shrubs, only the biomass produced during that year was collected. Vegetation was then dried to constant mass at 55˚C and weighed. Total annual aboveground production for each species was estimated by using the greater of the two mass values within each year. This method of biomass estimation may result in an underestimation of shrub productivity (Kirmse & Norton, 1985); therefore, shrub density data were also collected. Density was estimated in November 2009 within each plot by establishing 1-m belt transects (60 m in length) and counting the number of each shrub species encountered.

Soil Nutrients

Soil samples were collected during late May 2009 to a depth of 10 cm for use in determining relevant soil abiotic and biotic variables. Thirty-six samples were taken from each plot in a systematic pattern and composited. Soils were kept on ice until sieved at 2 mm and stored at 4°C (-20°C for fatty acid, see below). Soil nutrient levels were analyzed by AgSource Harris Laboratories at the University of Nebraska for percent organic matter (percent loss on ignition), nitrate-N and ammonium-N (2.0M KCl

extraction followed by colorimetric analysis), total N (Kjeldahl), and extractable P (Bray-1). Potential net N mineralization rates were determined using 3 g of freshly collected soil held at field capacity and at 20°C over a 21-day laboratory incubation (Drury & Beauchamp, 1991). Extracts (2.0M KCl) were stored at -20°C until colorimetric analysis (OI Analytical Flow Solution IV) to determine the increase in nitrate and ammonium concentration.

Microbial Community Estimation

The soil microbial community was analyzed for microbial biomass C (MBC) and N (MBN), ester-linked fatty acid methyl ester (EL-FAME), and mycorrhizal inoculum potential (MIP). Microbial biomass analysis was estimated using the chloroform fumigation extraction method without subtraction of a fumigated control (Franzluebbers et al., 1999) on 8 g of freshly collected soil. Extracts (0.5M K_2SO_4) were stored at -20°C until analysis of total organic carbon and total nitrogen using Shimadzu TOC-V and TNM-1, respectively. EL-FAME analysis was determined using the method outlined by Schutter and Dick (2000) on 3 g of soil archived at -20°C. Briefly, hexane extracts were evaporated under N_2, stored at -20°C, and analyzed by gas chromatograph (Agilent model 6890 with flame ionization detector) by the University of Delaware Plant and Soil Sciences Laboratory. Peaks were named using the Sherlock Eukary program supplied by MIDI Microbial ID (Newark, DE). The FAMEs 10Me-C17:0, 10Me-C18:0, C16:1ω7, cy-C17:0, cy-C19:0, a-C15:0, i-C15:0, i-C16:0, a-C17:0, and i-C17:0 represented bacterial biomass (Frostegard & Baath, 1996; Zelles, 1997; Drenovsky et

al., 2004). FAME C16:1ω5 was used for AM fungi (Olsson, 1999), while C18:1ω9 and C18:2ω6 represented saprophytic fungi (Frostegard & Baath, 1996; Stahl & Klug, 1996; Zelles, 1997). The FAME C16:0 is found in membranes among all soil organisms (Denef et al., 2009) and was used only in calculations of total microbial biomass derived from EL-FAME anlaysis. The MIP assay (Moorman & Reeves, 1979) used maize (*Zea mays*) as the host plant grown for 28 days in the Colorado State University Greenhouse (16: 8 hours day: night at 23 ± 8˚C). Plants were grown in Cone-tainers™ (3.8 x 21 cm) in each field soil (1:1 soil:sand). Roots were harvested, fixed, stained, and mounted according to the methods outlined by Koske and Gemma (1989) before being viewed for percent infection using the magnified intersections method (McGonigle et al., 1990) at 400x. One hundred fields of view were observed per plant to estimate percent colonization using hyphae, arbuscules, and vesicles as indicators of mycorrhizae presence.

Statistical Analysis

Relative values for plant production (percent composition) were used to reduce heterogeneity due to variation in abiotic variables, such as precipitation totals and timing (Doerr et al., 1984). Percent composition was achieved by dividing species production values by the total plant production for that year. Shrub density values were converted to number of individuals per square meter by dividing the total number of shrubs encountered within the belt transect by the total transect length. When necessary, values were transformed to satisfy the assumptions of normality and heterogeneity of variance using log, square, or square root transformations. Vegetation

composition, soil nutrient, and microbial attributes were analyzed with the MIXED procedure of SAS statistical software, version 9.2 (SAS Institute, Cary, NC, USA) using block as a random variable. Tukey's Studentized Range Test (HSD) was used to explore differences between treatment means.

III. RESULTS

Vegetation

In 2008, the undisturbed reference area was dominated by native, perennial vegetation with high species richness and low total production (Table 1.1). Major species (greater than 5% of total production) were the perennial forb *Phlox hoodii* Richardson, the perennial grasses *Hesperostipa comata* (Trin. & Rupr.) Barkworth, *Pascopyrum smithii* (Rydb.) A Löve, and *Poa secunda* J. Presl, and the late seral shrub *A. tridentata* (Table 1.2). The disturbed control had high species richness and was dominated by perennial grasses and mid seral shrubs. Major species were the annual grass *B. tectorum*, the perennial grasses *Agropyron cristatum* (L.) Gaertn., *H. comata*, and *P. smithii*, the mid seral shrub *Ericameria nauseosa* (Pall. ex Pursh) G.L. Nesom & Baird, and the late seral shrub *A. tridentata* (Table 1.2).

Species richness was lower in the N, N + P, and P addition treatments relative to the control, while the N + P addition treatment contained a lower number of species compared to the reference as well (Table 1.1). In contrast to the control and reference, the N + P addition treatment had significantly greater production of the annual forb *Sisymbrium altissimum* L. and the annual grass *B. tectorum* (Table 1.2). Annual species were also a significant component of the N and P addition treatments, where annual forb production in the N addition treatment was greater than the control and reference, but, similar to the P addition treatment, annual grass production was greater than the

reference but not the control (Table 1.1). Native species production was greater in the reference than all three nutrient addition treatments (Table 1.1). A majority of the differences in annual grass and non-native production was attributed to *B. tectorum* (Table 1.2). The N + P addition treatment had greater *B. tectorum* production than the control and reference, while the N and P addition treatments had greater *B. tectorum* production than the reference only. Other major species found in all three of the nutrient addition treatments were the perennial grass *P. smithii*, the mid seral shrub *E. nauseosa*, and the late seral shrub *A. tridentata*.

In 2009, the reference was again dominated by native, perennial species with high species richness and low total production (Table 1.3). Major species were the perennial forb *P. hoodii*, the perennial grasses *A. cristatum* and *Poa fendleriana* (Steud.) Vasey, and the late seral shrub *A. tridentata* (Table 1.4). The control had high species richness and was dominated by perennial grasses, perennial forbs, and mid seral shrubs (Table 1.3). Major species encountered were the perennial forb *Sphaeralcea coccinea* (Nutt.) Rydb., the perennial grasses *A. cristatum*, *Elymus repens* (L.) Gould, *H. comata*, and *P. smithii*, and the mid seral shrub *E. nauseosa* (Table 1.4).

The P addition treatment was dominated by perennial grasses and late seral shrubs with a significant proportion of annual grasses and perennial forbs (Table 1.3). Major species were the perennial forb *S. coccinea*, the annual grass *B. tectorum*, the perennial grasses *A. cristatum* and *P. smithii*, the mid seral shrub *E. nauseosa*, and the late seral shrub *A. tridentata* (Table 1.4). There were significantly fewer species encountered in the N addition treatment relative to the reference, while the N + P

addition treatment had lower species richness compared to both the control and reference (Table 1.3). The N addition treatment had greater production of the annual forb *Alyssum alyssoides* (L.) L. compared to all other treatments, with the annual grass *B. tectorum*, the perennial grasses *H. comata*, and *P. smithii*, the mid seral shrub *E. nauseosa*, and the late seral shrub *A. tridentata* also present as major species (Table 1.4). The N + P addition treatment was dominated by exotic and annual grass production, where this treatment exhibited greater exotic productivity relative to the reference and greater annual grass productivity compared to all other treatments (Table 1.3). Again, *B. tectorum* played a major role in community composition as a result of this treatment, where *B. tectorum* production was greater here than all other treatments (Table 1.4).

Figure 1.1 illustrates differences in total shrub density broken out by late-seral and mid-seral shrub components. Total shrub density was lower in the N + P addition treatment compared to the reference. The reference had greater late-seral shrub density than all disturbed treatments, and no differences were observed in late seral shrub densities among nutrient addition treatments. The only late-seral shrub encountered was *A. tridentata*. Mid-seral shrub density was lower in both the N + P addition treatment and the reference compared to the control. Mid-seral shrubs encountered were *Atriplex canascens* (Pursh) Nutt., *Chrysothamnus viscidiflorus* (Hook.) Nutt., *E. nauseosa*, *Gutierrezia sarothrae* (Pursh) Britton & Rusby, and *Sarcobatus vermiculatus* (Hook.) Torr.

Soil Nutrients

No differences were observed in soil organic matter or pH between treatments (Table 1.5). Additionally, no differences were observed in total N, however differences were seen between extractable inorganic N and mineralizable N. Nitrate-N was significantly higher in the N + P addition treatment than the P addition treatment, control, and reference, and net N mineralization potential was greater in the N and N + P addition treatments than all others (Table 1.3). Extractable P was greater in the N + P and P addition treatment than all others.

Microbial

Table 1.6 illustrates differences in soil biotic parameters, where MBC and MBN were lower in the N addition treatment than the control, however no differences were observed among any of the other treatments. Results from the EL-FAME assay showed the N and N + P treatments had a lower fungal: bacterial biomass ratio than the P addition treatment and control, while the N + P addition treatment was lower than the reference as well (Table 1.4). However, no differences were observed in total bacterial, total fungal, or AM fungal biomass. In terms of potential AM fungal colonization, no differences were observed in hyphal or arbuscular structures, however more vesicles were observed in the N addition treatment than the control and N + P addition treatment (Table 1.4).

IV. DISCUSSION

Twenty-five years after the initial disturbance and ten years after nutrient additions ceased, clear differences persisted in areas receiving additional N compared to the disturbed control and undisturbed reference areas. Areas receiving N exhibited higher production of annual and exotic vegetation with lower species richness compared to the control or reference (Tables 1.1, 1.3). However, differences were also found between the two treatments receiving N, where succession in the N + P addition treatment was further impeded through increased productivity of the invasive, annual *B. tectorum* (Tables 1.2, 1.4) and lacked a significant mid seral shrub component (Tables 1.1, 1.3; Figure 1.1).

Although initial findings (McLendon & Redente, 1991) attributed changes in community composition solely to elevated N levels, the changes observed between the N and N + P addition treatments indicate that P may play an important role in determining the persistence of an exotic, annual grass dominated system. Soil N levels have been widely shown to control *B. tectorum* dominance in western landscapes (Link et al., 1995; Lowe et al., 2003; Adair et al., 2008; Rowe et al., 2009). While disturbance with N addition could have been the catalyst for dominance by annual plants, the coupled addition of P may have promoted *B. tectorum* to remain as a significant vegetation component. Table 1.5 shows that the N + P addition treatment exhibited significantly higher levels of extractable P, nitrate-N, and N-mineralization potential

compared to the control, while the N treatment exhibited elevated N mineralization potential only. A meta-analysis of primary producer response to N and P addition conducted by Elser et al. (2007) produced a synergistic relationship between N and P addition, especially in grassland systems, and concluded that N addition alone could result in a system limited by P. Differences in plant community composition between the N, N + P, and P treatments (Tables 1.1-4) suggests that N was the limiting soil nutrient in this ecosystem, and its addition resulted in limitation by P. The coupled addition of N and P further retarded the rate of ecosystem development, illustrating Liebig's Law of the Minimum (Hooker, 1917).

Total shrub density was significantly lower in the N + P treatment than the reference area (Figure 1.1). However, there was no difference in mid seral shrub density between the N + P treatment and reference, while the control had significantly higher mid seral shrub density than both. Mid and late seral shrubs were largely absent from the N + P treatment, but were present in the treatments receiving N or P alone. Although not statistically significant, these results were mirrored in vegetation composition, where the N + P treatment exhibited less mid seral shrubs than the other disturbed treatments (Tables 1.1, 1.3). This indicates that the addition of N and P together may work synergistically to prevent shrub establishment and growth, and may be a result of *B. tectorum* establishment and persistence.

Observations of the untreated control and reference plots over the 25 years of community development of this study, as well as observations from a similar study at this research site (McLendon & Redente, 1990) show the general progression of

vegetation as annual forb > annual grass > perennial grass > mid seral shrub > late seral shrub. Repeated additions of N in this study caused the vegetation community to remain dominated by the annual forbs *A. alyssoides* and *S. altissimum* and the annual grass *B. tectorum* for a longer period of time than the disturbed control (McLendon & Redente, 1991; Tables 1.2, 1.4). P addition resulted in a vegetation community more similar to that of the control, but still had a minor annual vegetation component (Tables 1.1, 1.3). The control was dominated by perennial grasses and mid seral shrubs with a minor contribution by the late seral shrub *A. tridentata* (Tables 1.1-4). The relative abundance of annual and perennial vegetation (Tables 1.1, 1.3) and major species (Tables 1.2, 1.4), as well as shrub density (Figure 1.1) among treatments suggests a gradient from early to late successional status of treatments to be N + P > N > P > control > reference. A physical disturbance, such as the one examined in this study, is one way to alter community structure, while repeated fertilization represents another type of disturbance. Repeated N and P additions resulted in soil nutrient levels outside of the normal range of *A. tridentata* ecosystems (Table 1.5), slowing the rate of plant community development.

The ratio of fungal: bacterial biomass (Table 1.6) was higher in treatments linked to later successional vegetation communities (P addition, control, and reference) than earlier successional communities (N + P and N addition), supporting findings linking the relative abundance of soil fungi to vegetation succession (van der Wal et al., 2006; Harris, 2009). In addition to differences in fungal: bacterial biomass, differences were also found in total microbial biomass and infection potential by AM fungi (Table 1.6).

MBC and MBN were lower in the N addition treatment than the control, but no differences were observed in individual microbial components. This indicates that repeated addition of N decreased the growth of microbial communities at this site. There were no differences between hyphal or arbuscule root infection, but greater root infection by vesicles were observed in the N addition treatment than control or N + P addition treatment. Vesicles are AM fungal structures used to store carbohydrates exchanged in the symbiotic relationship with plants (Sylvia, 2005), but are not found in all arbuscular mycorrhizal fungi (Morton & Benny, 1990). Increased vesicle colonization may indicate that there is a greater exchange between plant and AM fungi in areas of high N or that addition of N may have resulted in a shift in the structure of AM fungi toward vesicular AM fungi of the suborder Glomineae (Morton & Benny, 1990).

These results partially support hypothesis 1 in that N addition resulted in a community dominated by annual species, but there was a marked difference between the N and N + P treatments. Hypothesis 2 was accepted, as there were few differences in community composition between the P and control treatments. Hypothesis 3 was partially accepted, as a lower fungal: bacterial biomass ratio was found in N and N + P addition treatments, but lower total microbial biomass was found in the N addition treatment relative to the control and no differences were found in terms of AM fungi due to P addition.

V. CONCLUSION

Initial investigations (Carpenter et al., 1990; McLendon & Redente, 1991) indicated that N addition impeded successional processes, resulting in a plant community dominated by annual forbs and grasses, while addition of P had no effect. These results show that after 25 years of community development with repeated nutrient input, addition of N continued to retard successional development, while P alone had little effect relative to the disturbed control. This is a contrary result to previous findings where N and P addition has been shown to accelerate community succession in more productive systems (DiTommaso & Aarssen, 1989). Coupled addition of N and P resulted in a community dominated by *B. tectorum* with minimal shrub development, while addition of N alone had significantly less *B. tectorum* productivity and shrub development similar to the unfertilized control. These results point to *B. tectorum* establishment and persistence as a main determinant of the rate of community development in fertilized, ungrazed environments.

VI. TABLES AND FIGURES

Table 1.1 Mean and standard error of 2008 plant community composition (% of total biomass) and relevant community parameters by treatment. Variables include composition by lifeform and nativity, as well as total plant biomass and species richness. Treatments were addition of nitrogen only, nitrogen and phosphorus together, phosphorus only, an unfertilized control, and an undisturbed reference. Means followed by different letters within rows indicate significant differences ($p<0.05$) using Tukey's HSD.

		Nitrogen		Nitrogen + Phosphorus		Phosphorus		Control		Reference	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Annual Forb	%	19.6ab	4.6	45.7a	15.5	12.6ab	6.6	4.3bc	1.7	1.3c	0.5
Annual Grass	%	16.5ab	4.5	28.1a	7.9	13.3ab	5.6	8.5bc	4.9	1.2c	0.8
Biennial Forb	%	0	0	0.2	0.2	0	0	1.6	0.6	0.1	0.1
Perennial Forb	%	6.3ab	1.6	2.7b	0.8	10.8ab	3.1	9.3ab	4.4	14.3a	6.9
Perennial Grass	%	26.5	5.0	15.0	14.78	37.3	11.6	50.6	7.5	55.6	7.0
Mid Seral Shrub	%	19.4	12.4	3.6	3.3	15.9	5.7	19.3	3.3	0.4	0.1
Late Seral Shrub	%	11.6	6.9	4.8	4.7	9.9	4.0	6.4	0.7	27.1	8.7
Exotic	%	38.9a	7.1	69.9a	16.4	39.8a	12.2	28.4ab	4.7	6.5b	2.0
Native	%	61.0b	7.0	30.1b	16.4	60.1b	12.29	71.6ab	4.7	93.5a	2.0
Total Plant Biomass	$g\ m^{-2}$	170a	10	134ab	14	103b	15	129ab	12	60c	4
Species Richness	#	14.8cd	0.9	11.5d	1.3	18bc	2.0	23a	0.4	21.8ab	0.3

Table 1.2 Mean and standard error of 2008 major species composition (% of total biomass). Major species were determined as any species greater than 5% of community composition among treatments or years. Treatments were addition of nitrogen only, nitrogen and phosphorus together, phosphorus only, an unfertilized control, and an undisturbed reference. Means followed by different letters within rows indicate significant differences ($p<0.05$) using Tukey's HSD.

		Nitrogen		Nitrogen + Phosphorus		Phosphorus		Control		Reference	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Agropyron cristatum	%	0.1	0.1	1.1	1.1	12.0	12.0	10.7	6.7	2.3	2.2
Alyssum alyssoides	%	16.7a	3.7	1.0b	0.6	5.5b	1.3	4.2b	1.6	1.3b	0.5
Artemisia tridentata	%	11.6ab	6.9	4.8b	4.7	9.9ab	4.0	6.2ab	0.8	26.8a	8.8
Bromus tectorum	%	16.5ab	4.5	28.1a	7.9	13.3ab	5.6	8.5bc	4.9	1.2c	0.8
Descurainia sophia	%	1.3	1.3	6.9	4.3	0	0	0	0	0	0
Elymus lanceolatus	%	0	0	0	0	2.7	2.7	0.9	0.9	1.8	1.7
Elymus repens	%	0.8ab	0.8	0b	0	0b	0	3.4a	1.6	0b	0
Ericameria nauseosa	%	19.4a	12.4	3.6ab	3.3	14.9a	5.2	15.7a	4.9	0b	0
Hesperostipa comata	%	5.2a	2.0	0b	0	2.7a	1.5	14.9a	3.5	6.4a	1.9
Pascopyrum smithii	%	16.3	7.6	11.9	11.9	14.6	8.4	10.2	2.6	11.5	1.6
Phlox hoodii	%	0b	0	0b	0	0b	0	0b	0	7.2a	5.4
Poa fendleriana	%	0	0	0	0	0	0	0	0	2.6	2.6
Poa secunda	%	0.3b	0.2	0b	0	0.8ab	0.7	2.1ab	1.0	13.5a	3.5
Sisymbrium altissimum	%	1.5b	0.9	37.5a	19.4	7.0b	7.0	0b	0	0b	0
Sphaeralcea coccinea	%	3.5	0.9	1.8	1.0	5.3	1.2	2.9	0.4	0.5	0.4

Table 1.3 Mean and standard error of 2009 plant community composition (% of total biomass) and relevant community parameters by treatment. Variables include composition by lifeform and nativity, as well as total plant biomass and species richness. Treatments were addition of nitrogen only, nitrogen and phosphorus together, phosphorus only, an unfertilized control, and an undisturbed reference. Means followed by different letters within rows indicate significant differences ($p<0.05$) using Tukey's HSD.

		Nitrogen		Nitrogen + Phosphorus		Phosphorus		Control		Reference	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Annual Forb	%	13.9a	3.8	5.0ab	1.7	3.5ab	0.9	2.3b	0.6	0.2c	0.1
Annual Grass	%	10.9b	4.2	50.3a	12.17	12.3b	11.0	2.9b	1.6	0.2b	0.1
Biennial Forb	%	0.1	0.1	4.5	3.5	0.7	0.5	0.9	0.7	0.3	0.2
Perennial Forb	%	6.6b	3.3	5.9ab	0.7	15.1a	2.6	15.9a	4.6	12.0ab	3.3
Perennial Grass	%	47.5	8.6	16.4	13.3	39.3	9.7	57.0	7.8	42.1	5.6
Mid Seral Shrub	%	11.5	5.9	7.6	5.4	8.0	4.5	16.4	4.4	1.5	0.9
Late Seral Shrub	%	9.6b	5.1	10.4b	4.3	21.2ab	9.4	4.7b	2.8	43.7a	5.5
Exotic	%	25.1ab	3.8	60.7a	8.5	27.5ab	9.5	24.4ab	9.7	7.5b	4.2
Native	%	74.9ab	3.8	39.3b	8.5	72.5ab	9.5	75.6ab	9.7	92.5a	4.2
Total Biomass	$g\ m^{-2}$	89ab	9	112a	24	64ab	5	74ab	3	61b	3
Species Richness	#	16.3bc	2.0	13.0c	2.2	19.3abc	2.8	21.8ab	1.4	25.8a	1.7

Table 1.4 Mean and standard error of 2009 major species composition (% of total biomass). Major species were determined as any species greater than 5% of community composition among treatments or years. Treatments were addition of nitrogen only, nitrogen and phosphorus together, phosphorus only, an unfertilized control, and an undisturbed reference. Means followed by different letters within rows indicate significant differences ($p<0.05$) using Tukey's HSD.

		Nitrogen		Nitrogen + Phosphorus		Phosphorus		Control		Reference	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Agropyron cristatum	%	1.3	1.1	1.6	1.1	10.9	7.5	8.6	5.5	6.8	4.4
Alyssum alyssoides	%	11.8a	4.1	1.5b	1.4	3.5b	0.9	2.3b	0.6	0.2b	0.1
Artemisia tridentata	%	9.6ab	5.1	10.4ab	4.3	21.2ab	9.4	4.7b	2.8	40.6a	7.2
Bromus tectorum	%	10.8b	4.2	50.3a	12.2	12.3b	11.0	2.9b	1.6	0.2b	0.1
Descurainia sophia	%	0	0	0	0	0	0	0	0	0	0
Elymus lanceolatus	%	1.6	1.6	7.4	7.4	1.1	0.8	0.5	0.3	4.2	1.3
Elymus repens	%	0b	0	0b	0	0b	0	9.7a	5.4	0.2ab	0.2
Ericameria nauseosa	%	11.5	5.9	3.8	3.0	6.0	3.7	11.9	4.5	1.0	0.7
Hesperostipa comata	%	7.6ab	5.3	0b	0	2.5ab	1.8	11.4a	4.6	4.5a	2.0
Pascopyrum smithii	%	31.3	14.1	7.0	5.2	20.4	11.5	7.7	2.5	2.5	0.3
Phlox hoodii	%	0b	0	0b	0	0.2b	0.2	0.6b	0.6	5.7a	3.0
Poa fendleriana	%	0	0	0	0	0.2	0.1	2.0	0.7	10.0	4.3
Poa secunda	%	0b	0	0b	0	0.7a	0.6	0.1ab	0.1	0.7a	0.5
Sisymbrium altissimum	%	0.3	0.3	3.2	1.3	0	0	0	0	0	0
Sphaeralcea coccinea	%	4.9	3.0	4.6	0.6	7.2	3.0	7.1	2.6	1.0	0.4

Table 1.5 Mean and standard error of relevant soil properties in the 0-10 cm soil layer by treatment for samples collected in May 2009. Variables include organic matter, total nitrogen, nitrate and ammonium expressed as nitrogen, net nitrogen mineralization potential, extractable phosphorus, and soil pH. Treatments were addition of nitrogen only, nitrogen and phosphorus together, phosphorus only, an unfertilized control, and an undisturbed reference. Means followed by different letters within rows indicate significant differences ($p<0.05$) using Tukey's HSD.

		Nitrogen		Nitrogen + Phosphorus		Phosphorus		Control		Reference	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Organic Matter	g kg^{-1}	27	1	25	1	24	3	20	1	22	1
Total Nitrogen	g kg^{-1}	1.2	0.1	1.2	0.1	1.0	0.1	1.0	0.1	1.3	0.2
Nitrate-N	mg kg^{-1}	4.0ab	0.4	4.5a	0.5	2.5b	0.5	2.5b	0.3	2.5b	0.3
Ammonium-N	mg kg^{-1}	2.6ab	0.5	3.6a	1.0	1.0b	0.0	1.7ab	0.4	2.2ab	0.5
Potential Nitrogen Mineralization	mg kg^{-1}	1.1a	0.2	1.1a	0.1	0.5b	0.1	0.5b	0.1	0.5b	0.0
Phosphorus	mg kg^{-1}	6.3b	1.0	111.8a	47.2	35.0a	10.4	5.0b	0.7	6.5b	0.9
pH		7.9	0.1	7.7	0.2	8.0	0.1	8.0	0.1	8.0	0.1

Table 1.6 Mean and standard error of relevant soil microbial attributes in the 0-10 cm soil layer by treatment for samples collected in May 2009. Variables microbial biomass carbon (MBC) and nitrogen (MBN) determined by chloroform fumigtion extraction, fungal: bacterial biomass ratio, bacterial biomass, fungal biomass, and arbuscular mycorrhizal (AM) fungal biomass determined through fatty acid methyl ester, and mycorrhizal infection potential of AM fungal structures (vesicles, arbuscules, and hyphae). Treatments were addition of nitrogen only, nitrogen and phosphorus together, phosphorus only, an unfertilized control, and an undisturbed reference. Means followed by different letters within rows indicate significant differences ($p<0.05$) using Tukey's HSD.

		Nitrogen		Nitrogen + Phosphorus		Phosphorus		Control		Reference	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
MBC	$\mu g\ g^{-1}$	224b	11	300ab	69	341ab	41	667a	101	369ab	38
MBN	$\mu g\ g^{-1}$	37b	2	53ab	11	62ab	7	77a	7	57ab	5
Fungi: Bacteria	$nmol\ g^{-1}$	1.0bc	0.1	0.8c	0.1	1.5a	0.2	1.5a	0.2	1.3ab	0.1
Bacteria	$nmol\ g^{-1}$	93	31	104	36	49	17	50	12	42	6
Fungi	$nmol\ g^{-1}$	88	28	81	27	71	27	79	23	54	6
AM Fungi	$nmol\ g^{-1}$	18	7	12	4	18	7	22	6	16	3
Vesicle	%	0.27a	0.09	0.02b	0.06	0.08ab	0.03	0.02b	0.01	0.08ab	0.01
Arbuscule	%	0.27	0.18	0.03	0.02	0.04	0.04	0.02	0.01	0.10	0.10
Hyphae	%	3.74	0.70	2.41	0.63	2.72	0.39	1.96	0.23	1.77	0.33

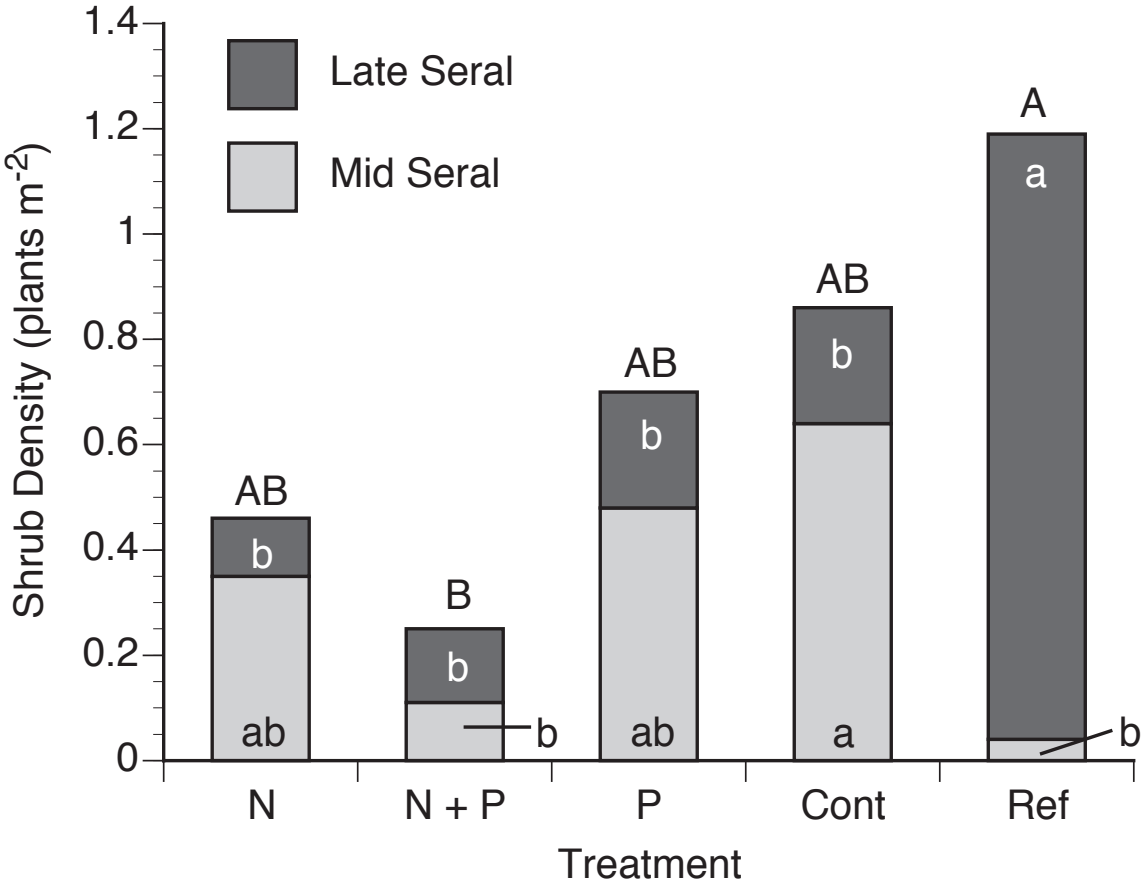

Figure 1.1 Mean of total, mid seral, and late seral shrub density (plants m^{-2}) by treatment for 2009. Treatments were addition of nitrogen only, nitrogen and phosphorus together, phosphorus only, an unfertilized control, and an undisturbed reference. Different upper-case letters indicate significant differences in total shrub density, different white lower-case letters indicate significant differences between late seral shrub density, and different black lower-case letters indicate significant differences between mid seral shrub density using Tukey's HSD ($p<0.05$).

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PART TWO

PLANT AND MICROBIAL COMMUNITY DEVELOPMENT IN RESPONSE TO REPEATED NITROGEN AND CARBON ADDITION

I. INTRODUCTION

Ecosystem succession can be defined as a progressive change in community composition and dynamics over time (Putman, 1994). During secondary succession, soil nitrogen (N) generally decreases with time since disturbance (Vitousek et al., 1989). Plants typical of early successional communities have rapid growth rates, high biomass and seed production, and short life cycles (Odum, 1969; Grime, 1977). These characteristics require high N use and when this resource is in adequate supply, these types of species can outcompete slower growing, longer living competitors (Grime, 1977; Chapin, 1980). Invasive plant species tend to share characteristics of early successional species described above (Ehrenfeld, 2003; Leishman et al., 2007); therefore, soil N can also be a major determinant of invasion success and persistence (McLendon & Redente, 1992; Blumenthal et al., 2003; Brooks, 2003; Prober & Lunt, 2009). Reduction in soil N allows late successional species to gain a competitive advantage over the high N-use species typical of early successional communities (Odum, 1969; Grime, 1977; McLendon & Redente, 1992; Paschke et al., 2000). Late successional communities provide many important ecological services over that of early successional communities, including reduced invasibility, (Blumenthal et al., 2005; Prober & Lunt, 2009), greater ecosystem stability through increased species diversity and functional redundancy (Tilman, 1996; van Ruijven & Berendse, 2007), decreased

erosion potential (Jiao et al., 2007), and improved wildlife habitat of native species (Watters et al., 2002; Walker et al., 2007).

In addition to changes in vegetation composition, microbial community size and structure change as ecosystems develop. Total microbial biomass generally increases during ecosystem succession (Insam & Domsch, 1988; Holtkamp et al., 2008). While bacterial biomass remains relatively constant, fungal biomass often increases, which may cause the soil microbial community to become fungal-dominated (Klein et al., 1995; Chabrerie et al., 2003; de Vries et al., 2006; van der Wal et al., 2006). Therefore, the ratio of fungal: bacterial biomass can be a useful indicator for the successional state of soil microbial communities (Wardle et al., 1995; de Vries et al., 2006). Increased soil N levels have been shown to result in decreased total microbial biomass (DeForest et al., 2004; Chapter 1) and a shift in structure toward more bacterial-dominated communities (Grayston et al., 2001; de Vries et al., 2007).

Over successional time, plant-available soil N often decreases where processes regulating N outputs (leaching, erosion, denitrification and volatilization, plant uptake, and microbial immobilization) become greater than those regulating N inputs (N_2 fixation and atmospheric deposition) (Vitousek & Howarth, 1991). Recent research has focused on manipulating soil N through vegetation removal, burning, and grazing, topsoil removal, establishment of low N-use plant species, and N immobilization (Perry et al., 2010). Microbial-mediated N immobilization is limited by labile carbon (C) sources that regulate microbial growth and reproduction (Ruess & Seagle, 1994; Zak et al., 1994). By supplementing soils with additional C sources, such as sucrose, sawdust,

or woodchips, this process can be manipulated to decrease soil N (Morgan, 1994; Zink & Allen, 1998).

Managing for low soil N can allow ecosystems to develop faster, providing for suppression of annual species, promotion of native species, increased perennial species production, and increased shrub establishment, as well as decreasing potential for invasive plant species establishment and persistence (Zink & Allen, 1998; Paschke et al., 2000; Perry et al., 2004; Blumenthal et al., 2005; Szabó et al., 2008; Rowe et al., 2009). McLendon and Redente (1992) established an experimental N gradient through N addition and immobilization by sucrose addition. After three years (1988-1990), they found sucrose addition promoted rapid species replacement with greater cover of mid successional species and higher species richness compared to N addition. Nitrogen and sucrose addition, which began in 1987, continued through 1999. The aim of the current study was to revisit the McLendon and Redente (1992) research site to examine the plant and soil microbial communities for differences in community development. The specific hypotheses were 1) sucrose addition would result in vegetation characteristic of a later successional community, with greater representation of perennial grasses, forbs and shrubs, greater species richness, and less total production, compared to areas amended with nitrogen, and 2) sucrose addition would result in a soil microbial community with a greater contribution of soil fungi and greater total microbial biomass.

II. METHODS

Study Description

The study site is located 65 km northwest of Rifle, Colorado, USA in the Piceance Basin (UTM 12 S 722198 4420302) at an elevation of 2,030 m. The climate is semiarid, with a mean annual precipitation of 297 mm, approximately half occurring as snowfall (HPRCC, 2010). The main soil type is Yamac loams (fine-loamy mixed, Borollic Camborthids), supporting a big sagebrush steppe community (USDA, 1982). In August 1984, research plots were established, fenced to exclude cattle grazing, and a disturbance, similar to that of proposed resource extraction activity, was conducted within the *A. tridentata* community. All vegetation and the top 5 cm of soil were removed from the site, and the next 25 cm of soil were thoroughly mixed. The disturbance resulted in a reduction of >90% of the soil seed bank (Carpenter et al., 1990). Following the disturbance, the areas were weeded by hand to further reduce the seedbank. In the fall of 1987, four blocks of treatment plots were established in a factorial design. Each block consisted of one 160-m^2 plot of each treatment. Treatments were addition of nitrogen (100 kg ha^{-1} yr^{-1}), addition of sucrose (1600 kg ha^{-1} yr^{-1}), and an untreated control. All plots were seeded with a mixture of early and late seral species common to the site in November of 1987. Nitrogen and sucrose were applied from 1987 through 1999. Refer to McLendon and Redente (1992) for detailed treatment descriptions.

Vegetation Estimation

Vegetation sampling occurred during summer of 2008 and 2009. Sampling was conducted twice during the growing season (early- and late-summer) in an effort to capture the peak biomass of both the cool-season and warm-season plant species (Lauenroth et al., 1986). During each sampling period, eight-0.5 m^2 quadrats were randomly placed within each plot and aboveground biomass from the current year was harvested to ground level, separated by species, and collected. For shrubs, only the biomass produced during that year was collected. Vegetation was then dried to constant mass at 55˚C and weighed. Total annual aboveground production for each species was estimated by using the greater of the two mass values within each year.

Soil Nutrients

Soil samples were collected during late May 2009 to a depth of 10 cm for use in determining relevant soil abiotic and biotic variables. Thirty-six samples were taken from each plot in a systematic pattern and composited. Soils were kept on ice until sieved at 2 mm and stored at 4°C (-20°C for fatty acid, see below). Soil nutrient levels were analyzed by AgSource Harris Laboratories at the University of Nebraska for percent organic matter (percent loss on ignition), nitrate-N and ammonium-N (2.0M KCl extraction followed by colorimetric analysis), total N (Kjeldahl), and extractable P (Bray-1). Potential net N mineralization rates were determined using 3 g of freshly collected soil held at field capacity and at 20°C over a 21-day laboratory incubation (Drury & Beauchamp, 1991). Extracts (2.0M KCl) were stored at -20°C until colorimetric analysis

(OI Analytical Flow Solution IV) to determine the increase in nitrate and ammonium concentration.

Microbial Community Estimation

The soil microbial community was analyzed for microbial biomass C (MBC) and N (MBN), ester-linked fatty acid methyl ester (EL-FAME), and mycorrhizal inoculum potential (MIP). Microbial biomass analysis was estimated using the chloroform fumigation extraction method without subtraction of a fumigated control (Franzluebbers et al., 1999) on 8 g of freshly collected soil. Extracts (0.5M K_2SO_4) were stored at -20°C until analysis of total organic carbon and total nitrogen using Shimadzu TOC-V and TNM-1, respectively. EL-FAME analysis was determined using the method outlined by Schutter and Dick (2000) on 3 g of soil archived at -20°C. Briefly, hexane extracts were evaporated under N_2, stored at -20°C, and analyzed by gas chromatograph (Agilent model 6890 with flame ionization detector) by the University of Delaware Plant and Soil Sciences Laboratory. Peaks were named using the Sherlock Eukary program supplied by MIDI Microbial ID (Newark, DE). The FAMEs 10Me-C17:0, 10Me-C18:0, C16:1ω7, cy-C17:0, cy-C19:0, a-C15:0, i-C15:0, i-C16:0, a-C17:0, and i-C17:0 represented bacterial biomass (Frostegard & Baath, 1996; Zelles, 1997; Drenovsky et al., 2004). FAME C16:1ω5 was used for AM fungi (Olsson, 1999), while C18:1ω9 and C18:2ω6 represented saprophytic fungi (Frostegard & Baath, 1996; Stahl & Klug, 1996; Zelles, 1997). The FAME C16:0 is found in membranes among all soil organisms (Denef et al., 2009) and was used only in calculations of total microbial biomass derived from

EL-FAME anlaysis. The MIP assay (Moorman & Reeves, 1979) used maize (*Zea mays*) as the host plant grown for 28 days in the Colorado State University Greenhouse (16:8 hours day:night at 23±8˚C). Plants were grown in Cone-tainers™ (3.8 x 21 cm) in each field soil (1:1 soil:sand). Roots were harvested, fixed, stained, and mounted according to the methods outlined by Koske and Gemma (1989) before being viewed for percent infection using the magnified intersections method (McGonigle et al., 1990) at 400x. One hundred fields of view were observed per plant to estimate percent colonization using hyphae, arbuscules, and vesicles as indicators of mycorrhizae presence.

Statistical Analysis

Relative values for plant production (percent composition) were used to reduce heterogeneity due to variation in abiotic variables, such as precipitation totals and timing (Doerr et al., 1984). Percent composition was achieved by dividing species production values by the total plant production for that year. When necessary, values were transformed to satisfy the assumptions of normality and heterogeneity of variance using log, square, or square root transformations. Vegetation composition, soil nutrient, and microbial attributes were analyzed with the MIXED procedure of SAS statistical software, version 9.2 (SAS Institute, Cary, NC, USA) using block as a random variable. Tukey's Studentized Range Test (HSD) was used to explore differences between treatment means.

III. RESULTS

Vegetation

No statistical differences between treatments were observed in vegetation by lifeform or species richness in 2008 or 2009 (Tables 2.1, 2.3). However, mean annual forb composition in the N addition treatment was at least twice that of the other treatments and perennial forb composition in the control was greater than twice that of the other treatments in 2008 (Table 2.1). Increased annual forbs in the N addition treatment can be attributed to the non-native *Sisymbrium altissimum* L., while increased perennial forbs in the control are due to *Castilleja linariifolia* Benth. and *Eriogonum umbellatum* Torr. (Table 2.2). In 2009, the annual grass composition in the N addition treatment was at least triple that of the other treatments and composition of late seral shrubs in the N addition treatment was nearly one-third of that found in the sucrose addition treatment and nearly one-fifth of that found in the control (Table 2.3). Differences in annual grass is due to the non-naitve *Bromus tectorum* L. and differences in late seral shrubs is due to *A. tridentata* (Table 2.4). Native plant species composition was significantly lower in the N addition treatment than the control in 2008 (Table 2.1), while in 2009 native plants were significantly lower in both the N and sucrose addition treatments than the control (Table 2.3). In 2009, total vegetation production was greater in the N addition treatment than the control or sucrose addition treatment (Table 2.3), but no differences were observed in 2008 (Tables 2.1).

Soil and Tissue Nutrients

Soil organic matter, total soil N, and potential N mineralization were greater in the N addition treatment compared to the control (Table 2.5). No differences were observed in soil nitrate-N between treatments, however ammonium-N was found to be greater in soils of the N addition treatment than either the control or sucrose addition treatment. Soil pH was lower in the N addition treatment than the control (Table 2.5).

Microbial

Analysis of microbial fatty acids showed no differences between treatments in bacterial biomass, but fungal biomass was significantly greater in the sucrose addition treatment than either the control or N addition treatment (Figure 2.1). Additionally, the ratio of fungal: bacterial biomass was greater in the sucrose addition treatment than the N addition treatment (Figure 2.1). Arbuscular mycorrhizal (AM) fungi hyphal infection was greater in the sucrose addition treatment than the control or N addition treatment (Figure 2.1). No differences were found in vesicular (Nitrogen: 0.05%; Control: 0.07; Sucrose: 0.11) or arbuscular (Nitrogen: 0.02%; Control: 0.01; Sucrose: 0.03) mean root infection. Additionally, no differences were observed between treatment means for MBC (Nitrogen: 1140 ppm; Control: 900; Sucrose: 910) or MBN (Nitrogen: 123 ppm; Control: 107; Sucrose: 110).

IV. DISCUSSION

Carbon addition has been widely shown to accelerate ecosystem development through the reduction of early successional and non-native plant species (Zink & Allen, 1998; Blumenthal et al., 2003; Perry et al., 2004), while promoting late successional species (Blumenthal et al., 2003; Prober et al., 2005; Rowe et al., 2009). Similarly, initial results (1988-1990) of this study found sucrose amendments to accelerate community succession, with a greater contribution of annual grasses and perennial forbs, higher species richness, and less annual forbs compared to the N addition treatment (McLendon & Redente, 1990; McLendon & Redente, 1992). Studies examining these mechanisms are generally short-term (Inouye & Tilman, 1995), and as a result an understanding of how the initial rate of succession can impact community composition over longer time periods is not well developed. By revisiting this study 22 years after treatments began and 10 years after treatments ceased, we can observe how initial changes in community composition and seral state affect long-term community development and use this information to increase our understanding of successional theory.

Nearly two decades after the initial investigation, where initial differences in the rate of plant succession were observed (McLendon & Redente, 1992), vegetation communities were not statistically different across treatments. Although some differences were observed in total production and native plant species composition,

plant species richness and composition across all vegetation lifeforms were similar (Tables 2.1, 2.3). Although statistically significant differences in total production were only observed in 2009 (Table 2.3), this same relationship was observed in 2008 (Table 2.1). Additionally, the untreated control had significantly more native vegetation compared to N addition in 2008 and 2009 or sucrose addition in 2009 (Tables 2.1, 2.3). Although repeated N addition has been shown to increase non-native vegetation (Carpenter et al., 1990; Holmes, 2001; Brooks, 2003; Rickey & Anderson, 2004), this is not an expected result of sucrose addition (Zink & Allen, 1998; Paschke et al., 2000; Blumenthal et al., 2003; Perry et al., 2004). Major exotic species found in both of these treatments were the annual forb *Alyssum alyssoides* (L.) L., the annual grass *B. tectorum*, and the perennial grass *Agropyron cristatum* (L.) Gaertn, while the annual forb *S. altissimum* was also a major exotic species found in the N addition treatment in 2008 (Table 2.4). Additionally, N fertilization resulted in elevated soil N levels ten years after treatments ceased, but impacts of sucrose addition on the immobilization of N were no longer apparent (Table 2.5), indicating a stronger legacy effect of N addition than sucrose addition on long-term soil N levels.

Comparing the vegetation communities observed in 1991 to those found in 2008 and 2009, long-term successional trajectory has converged, resulting in a rejection of my first hypothesis. Regardless of the initial rate of succession or community composition, a common successional pathway was observed. Experimental studies examining priority effects have shown that species that establish early in succession exhibit strong influence on the resulting composition of plant communities (Drury & Nisbet, 1973;

Facelli & Facelli, 1993; Korner et al., 2008; Urban & De Meester, 2009). However, in this study the vegetation community composition were similar after 22 years of development, indicating that differences early in successional development did not affect community composition later in succession. Connell and Slatyer (1977) introduced a model of succession that explains plants can facilitate, tolerate, and inhibit community development. If inhibition were a key mechanism in this system, one would expect to see differences in community development due to the release of inhibition pressures in areas with greater initial rates of succession. These results provide evidence for the concepts of tolerance, where longer-living, slower-growing species displace shorter-living, faster-growing species following senescence, or facilitation, where early successional species aid in the establishment of later successional species (Connell & Slatyer, 1977), and supports a more deterministic view of ecosystem development with a common endpoint (Clements, 1916).

In recent decades, community assembly theory has attempted to explain patterns of succession by examining interactions between organisms that determine successional trajectory (Weiher & Keddy, 1999; Temperton, 2004). An understanding of assembly rules can provide possible pathways from which plant communities are created (Grover, 1994). Community assembly theory is the study of the explicit constraints that limit how assemblages are selected from a larger species pool (Weiher & Keddy, 1999). Propagule availability and timing have been shown to have strong controls on the trajectory and rate of community development (Tilman, 1997; Turnbull et al., 2000; Seabloom & van der Valk, 2003). At the beginning of the study, all plots

were seeded with a mix of early and late seral species common to the site. Early in community development environment variables, specifically soil N, may have selected for assemblages from the pool of seeded and recruiter species. Nitrogen addition would have promoted development of large species with rapid growth rates, while sucrose addition would have released this selection pressure through reduction in soil N, allowing the plant community to quickly progress through this early successional state. As larger, fast-growing species senesced later in succession, soil N may not have had as strong of a selection pressure, allowing greater expression of the species seeded in this study and promoting a convergence in the rate and trajectory of community development.

Recent research has attempted to link our knowledge of plant succession to changes in microbial succession and understand the feedbacks within these two related systems (Harris, 2009). Although rates and trajectories of plant community development have converged, differences in microbial community succession were observed. Relative to bacteria, soil fungi increase with ecosystem development (van der Wal et al., 2006) and fungal: bacterial biomass ratios (de Vries et al., 2006) as well as AM fungi (van der Heijden et al., 1998; Caravaca et al., 2003) have been used as indicators of successional state. While no differences in total bacterial biomass were observed between treatments (Figure 2.1a), sucrose additions did promote a community with significantly greater fungal biomass (Figure 2.1b), fungal: bacterial biomass ratio (Figure 2.1c), and AM fungal hyphal infection (Figure 2.1d), partially supporting my second hypothesis. Harris (2009) discusses the positive correlation between ecosystem

succession and fungal dominance in soils, explaining how restoration methods can provide a shortcut to community development and the need to examine the role of microorganisms in expediting plant succession. These results indicate microbial community development is strongly affected by repeated carbon addition and is not correlated with plant community response, similar to findings by Chabrerie et al. (2003).

V. CONCLUSION

Observations of plant and microbial communities over time and in different ecological systems is important in developing a greater understanding of successional processes and further honing successional theory. This study sheds light on how early successional species composition can affect long-term development. Although differences in the rate of succession were observed early in succession as a result of experimental manipulations of soil N, these changes were no longer apparent two decades later. Seed availability plays a likely role in promoting the observed convergence in community composition.

Although differences in the vegetation community were not observed between treatments, repeated additions of sucrose did result in increased fungal dominance in the soil. Increases in soil fungi have been linked to ecosystem maturation (Wardle et al., 1995; van der Heijden et al., 1998; van der Wal et al., 2006), indicating that carbon addition may have a beneficial effect on community development of the belowground system. However, in this study changes in soil fungi were not linked with changes in plant community composition.

VI. TABLES AND FIGURES

Table 2.1 Mean and standard error of 2008 plant community composition (% of total biomass) and relevant community parameters by treatment. Variables include composition by lifeform and nativity, as well as total plant biomass and species richness. Treatments were addition of nitrogen, an unamended control, and addition of sucrose. Means followed by different letters within rows indicate significant differences ($p<0.05$) using Tukey's HSD.

		Nitrogen		Control		Sucrose	
		Mean	SE	Mean	SE	Mean	SE
Annual Forb	%	22.4	7.2	7.3	1.6	11.2	2.8
Annual Grass	%	21.0	7.5	12.4	4.9	13.9	3.7
Biennial Forb	%	0.8	0.6	2.0	1.7	1.1	1.0
Perennial Forb	%	10.0	6.8	31.5	7.3	14.7	1.4
Perennial Grass	%	27.1	5.4	23.5	7.8	32.0	14.0
Mid Seral Shrub	%	13.5	5.9	17.2	9.4	19.9	11.6
Late Seral Shrub	%	5.2	3.5	6.2	2.3	6.8	3.0
Exotic	%	58.9a	11.4	25.9b	4.1	38.2ab	7.4
Native	%	41.1b	11.4	74.1a	4.1	61.4ab	7.4
Total Plant Biomass	$g\ m^{-2}$	114	27	73	13	85	3
Species Richness	#	14.3	2.4	19.5	1.6	18.5	2.2

Table 2.2 Mean and standard error of 2008 major species composition (% of total biomass). Major species were determined as any species greater than 5% of community composition among treatments or years. Treatments were addition of nitrogen, an unamended control, and addition of sucrose. Means followed by different letters within rows indicate significant differences ($p<0.05$) using Tukey's HSD.

		Nitrogen		Control		Sucrose	
		Mean	SE	Mean	SE	Mean	SE
Achnatherum hymenoides	%	0.6	0.6	1.2	0.7	6.3	4.0
Agropyron cristatum	%	12.9	5.4	3.0	2.6	11.5	7.4
Alyssum alyssoides	%	11.9	1.3	7.2	1.6	10.9	2.7
Artemisia tridentata	%	5.2	3.5	6.2	2.3	6.8	3.0
Bromus tectorum	%	21.0	7.5	12.4	4.9	13.9	3.7
Castilleja angustifolia	%	0	0	0.8	0.8	0	0
Castilleja linariifolia	%	0	0	7.6	7.2	0.4	0.2
Ericameria nauseosa	%	13.1	6.0	15.4	8.7	16.8	11.3
Eriogonum umbellatum	%	0	0	5.3	5.3	0.2	0.2
Hesperostipa comata	%	1.3	1.3	7.7	4.7	0.6	0.5
Linum lewisii	%	5.8	5.1	7.5	2.8	7.1	2.6
Pascopyrum smithii	%	7.6	2.5	6.2	2.8	7.4	3.0
Sisymbrium altissimum	%	8.3	7.8	0.1	0.1	0.3	0.2
Sphaeralcea coccinea	%	2.8	0.8	6.8	1.5	4.7	2.0

Table 2.3 Mean and standard error of 2009 plant community composition (% of total biomass) and relevant community parameters by treatment. Variables include composition by lifeform and nativity, as well as total plant biomass and species richness. Treatments were addition of nitrogen, an unamended control, and addition of sucrose. Means followed by different letters within rows indicate significant differences ($p<0.05$) using Tukey's HSD.

		Nitrogen		Control		Sucrose	
		Mean	SE	Mean	SE	Mean	SE
Annual Forb	%	8.0	3.2	3.2	1.5	10.0	2.8
Annual Grass	%	17.5	10.2	4.4	3.4	5.3	2.9
Biennial Forb	%	1.5	0.6	0.9	0.9	2.0	1.9
Perennial Forb	%	19.1	3.3	28.3	7.5	21.8	4.7
Perennial Grass	%	36.0	8.7	28.9	8.3	37.3	13.3
Mid Seral Shrub	%	13.5	5.9	17.2	9.4	19.9	11.6
Late Seral Shrub	%	5.4	3.2	25.8	5.5	15.2	9.2
Exotic	%	36.6a	9.0	13.0b	4.3	33.8a	2.9
Native	%	63.4b	9.0	87.0a	4.3	66.2b	2.9
Total Plant Biomass	$g\ m^{-2}$	70a	11	45b	9.5	46b	8
Species Richness	#	17.5	1.4	20.3	2.6	21.0	1.9

Table 2.4 Mean and standard error of 2009 major species composition (% of total biomass). Major species were determined as any species greater than 5% of community composition among treatments or years. Treatments were addition of nitrogen, an unamended control, and addition of sucrose. Means followed by different letters within rows indicate significant differences ($p<0.05$) using Tukey's HSD.

		Nitrogen		Control		Sucrose	
		Mean	SE	Mean	SE	Mean	SE
Achnatherum hymenoides	%	0.4	0.3	3.5	1.3	3.2	2.2
Agropyron cristatum	%	9.2	4.9	1.3	1.3	14.8	7.7
Alyssum alyssoides	%	6.9	2.8	2.9	1.6	9.5	2.4
Artemisia tridentata	%	5.4	3.2	25.8	5.5	14.5	9.2
Bromus tectorum	%	17.5	10.2	4.4	3.4	5.3	2.9
Castilleja angustifolia	%	0.6	0.5	1.5	1.1	5.5	2.1
Castilleja linariifolia	%	0	0	0	0	0	0
Ericameria nauseosa	%	12.2	4.0	7.8	2.9	6.0	2.0
Eriogonum umbellatum	%	0	0	6.3	6.3	0.6	0.6
Hesperostipa comata	%	6.5	4.9	5.4	4.8	6.5	4.1
Linum lewisii	%	10.0	6.1	12.6	6.3	4.4	1.9
Pascopyrum smithii	%	18.7	10.8	7.1	3.9	4.2	1.4
Sisymbrium altissimum	%	0.1	0.1	0	0	0	0
Sphaeralcea coccinea	%	7.4	3.6	6.9	2.6	6.8	1.4

Table 2.5 Mean and standard error of relevant soil properties in the 0-10 cm soil layer by treatment for samples collected in May 2009. Variables include organic matter, total nitrogen, nitrate and ammonium expressed as nitrogen, net nitrogen mineralization potential, extractable phosphorus, and soil pH. Treatments were addition of nitrogen, an unamended control, and addition of sucrose. Means followed by different letters within rows indicate significant differences ($p<0.05$) using Tukey's HSD.

		Nitrogen		Control		Sucrose	
		Mean	SE	Mean	SE	Mean	SE
Organic Matter	g kg^{-1}	25a	1	21b	1	23ab	2
Total Nitrogen	g kg^{-1}	1.2a	0.1	1.0b	0.1	1.1ab	0.1
Nitrate-N	mg kg^{-1}	3.0a	0.7	3.0a	0.7	2.8a	0.5
Ammonium-N	mg kg^{-1}	2.5a	0.5	1.5b	0.2	1.3b	0.1
Potential Nitrogen Mineralization	mg kg^{-1}	0.9a	0.2	0.3b	0.1	0.5ab	0.1
Phosphorus	mg kg^{-1}	5.0a	1.3	6.3a	0.4	6.0a	0.4
pH		7.9b	0.2	8.2a	0.1	7.9b	0.2

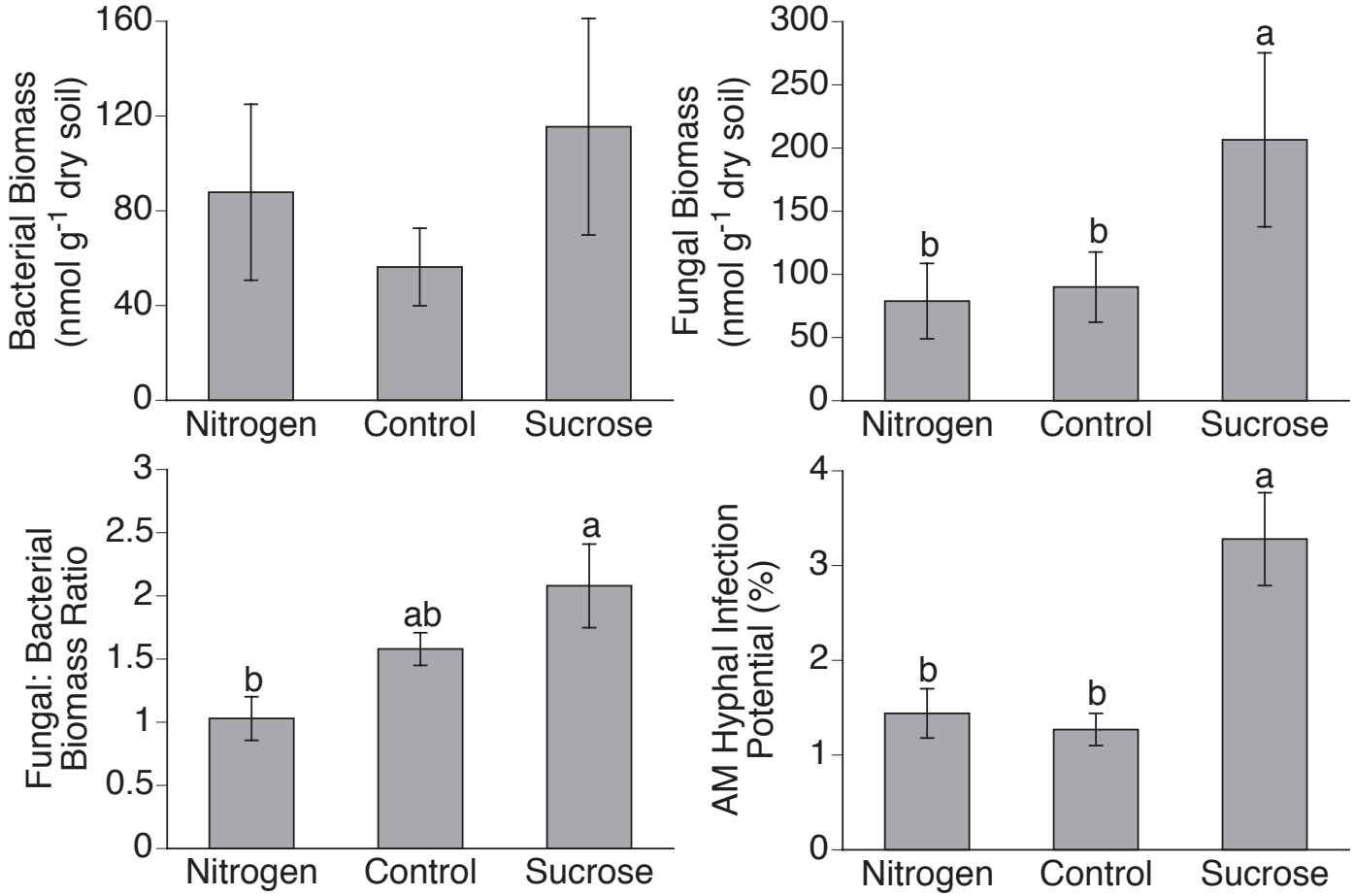

Figure 2.1 Mean and standard error of soil microbial attributes in the 0-10 cm soil layer by treatment for soil collected in May 2009. Microbial attributes include total bacterial biomass, total fungal biomass, fungal: bacterial biomass ratio, and arbuscular mycorrhizal (AM) hyphal infection potential. Biomass measures were determined by ester-linked fatty acid methyl ester assay and hyphal infection was determined by mycorrhizal inoculum potential assay. Treatments were addition of nitrogen, an unamended control, and addition of sucrose. Bars with different letters indicate statistically significant differences ($p<0.05$) using Tukey's HSD.

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PART THREE

EVIDENCE OF PRIORITY EFFECT IN LONG-TERM COMMUNITY DEVELOPMENT AS A RESULT OF SEED ADDITION

I. INTRODUCTION

Theories of succession and community assembly attempt to explain plant community development following disturbance (Young et al., 2001). Plants characteristic of early successional environments are gradually replaced by more competitive later successional species toward equilibrium (Egler, 1954), but the initial colonizers can influence future vegetation community composition (Belyea & Lancaster, 1999; Fukami et al., 2005). Early seral plants are generally short-lived and characterized by high growth rates with large seed production and dispersal capacity relative to late seral plants, which have a competitive advantage in high stress, low resource environments (Odum, 1969; Grime, 1977). Due to high growth rate and dispersal capacity, early seral species are able to colonize and outperform late seral species following disturbance. Early seral species persist until resources fall below thresholds required for their growth and reproduction or late seral species are able to establish and outcompete for these limiting resources (Grime, 1977; Tilman, 1988).

Recent research has explored successional processes in an effort to explain successional patterns in the context of community assembly theory (Weiher & Keddy, 1999; Temperton, 2004). Community assembly theory is the study of the explicit constraints that limit how assemblages are selected from a larger species pool (Weiher & Keddy, 1999). Supporting this theory is the concept of priority effects, which explain that the order and timing of seed arrival and establishment strongly influence future

vegetation composition (Belyea & Lancaster, 1999). Experimental studies found late seral species were present at low levels early in succession and removal of early seral plant species enhanced development of late seral species, indicating that early seral species can delay plant community development (Drury & Nisbet, 1973). This provided support to the theory that existing plant species can inhibit, rather than facilitate or tolerate, establishment of subsequent species (Connell & Slatyer, 1977) (first proposed by Egler [1954] as initial floristics). This theory of inhibition explained that initial colonizers slow successional development by preventing establishment of late seral species and suppressing growth of species already present (Connell & Slatyer, 1977), indicating that initial colonizers can delay the rate of community development.

The emerging field of restoration ecology is useful to test theories of ecosystem succession by examining community response to direct manipulations of ecosystem function and structure (Bradshaw, 1990). By manipulating ecosystem processes, such as propagule dispersal (Stevenson et al., 2000; Foster & Tilman, 2003), practitioners can alter the rate of ecosystem recovery while improving our understanding of the restrictions to ecosystem development (Young et al., 2005). On sites of low species richness or where seedbank and propagule sources are limited, addition of seed can be a useful method to accelerate ecosystem succession (Palmer et al., 1997). Studies examining succession of ex-arable lands found that seeding with both early and late seral species resulted in dominance by the former (Kleijn et al., 1997), but another study examining repeated seed addition over multiple growing seasons found early seral species could only successfully establish early in succession, while late seral species

were present throughout the study (Kleijn, 2003). Addition of late seral seeds to a disturbed *Artemisia tridentata* Nutt. ecosystem resulted in greater production of late seral grass and shrub species and decreased the early seral components (Stevenson et al., 2000). Foster and Tilman (2003) found addition of late seral species to an established tallgrass prairie resulted in increased species richness, a trait linked to ecosystem succession and stability (Tilman, 1996).

Relative to the length of typical ecologic studies, plant communities take long periods of time to develop (Inouye & Tilman, 1995), therefore most studies examining community development cannot fully assess the impacts of seed addition (Connell & Slatyer, 1977). In this study, I examine plant community development 25 years after seed addition of either early or late seral species and compare this response to an unseeded control and a reference *A. tridentata* community. My hypotheses were that the late seral seed treatment would result in greater composition of late seral species, greater species diversity, and greater late seral shrub establishment compared to the early seral or unseeded treatments.

II. METHODS

Study Description

The study site is located 65 km northwest of Rifle, Colorado, USA in the Piceance Basin (UTM 12 S 722198 4420302) at an elevation of 2,030 m. The climate is semiarid, with a mean annual precipitation of 297 mm, approximately half occurring as snowfall (HPRCC, 2010). The main soil type is Yamac loams (fine-loamy mixed, Borollic Camborthids), supporting a big sagebrush steppe community (USDA, 1982). In August 1984, research plots were established, fenced to exclude cattle grazing, and a disturbance, similar to that of proposed resource extraction activity, was conducted within the *A. tridentata* community. All vegetation and the top 5 cm of soil were removed from the site, and the next 25 cm of soil were thoroughly mixed. The disturbance resulted in a reduction of >90% of the soil seed bank (Carpenter et al., 1990). Following the disturbance, four blocks of treatment plots were established in a split-block design, where each block consisted of six 500-m^2 treatment plots, as well as an undisturbed reference of equal size. The first treatment level was fumigation (fumigated or not fumigated) and the second treatment level was seed mix (early seral, late seral, or unseeded control). A 24-hour application of methyl bromide was used as a soil fumigant in September 1984. In October-November 1984, early and late seral seed mixes (Table 3.1) were hand broadcast on specified plots, with the exception of *A.*

tridentata, which were transplanted at 6 months of age in addition to seeding. Refer to Stevenson et al. (2000) for detailed treatment descriptions.

Vegetation Estimation

Vegetation sampling occurred during summer of 2008 and 2009. Sampling was conducted twice during the growing season (early- and late-summer) in an effort to capture the peak biomass of both the cool-season and warm-season plant species (Lauenroth et al., 1986). During each sampling period, eight-0.5 m^2 quadrats were randomly placed within each plot and aboveground biomass from the current year was harvested to ground level, separated by species, and collected. For shrubs, only the biomass produced during that year was collected. Vegetation was then dried to constant mass at 55˚C and weighed. Total annual aboveground production for each species was estimated by using the greater of the two mass values within each year. This method of biomass estimation may result in an underestimation of shrub productivity (Kirmse & Norton, 1985); therefore, shrub density data were also collected. Density was estimated in November 2009 within each plot by establishing 1-m belt transects (60 m in length) and counting the number of each shrub species encountered.

Statistical Analysis

Relative values for plant production (percent composition) were used to reduce heterogeneity due to variation in abiotic variables, such as precipitation totals and timing (Doerr et al., 1984). Percent composition was achieved by dividing species

production values by the total plant production for that year. Shrub density values were converted to number of individuals per square meter by dividing the total number of shrubs encountered within the belt transect by the total transect length. When necessary, values were transformed to satisfy the assumptions of normality and heterogeneity of variance using log, square, or square root transformations. Vegetation composition and shrub density were analyzed with the MIXED procedure of SAS statistical software, version 9.2 (SAS Institute, Cary, NC, USA) using block as a random variable. Tukey's Studentized Range Test (HSD) was used to explore differences between treatment means.

III. RESULTS

Total plant biomass was greater across all treatments than the reference in 2008, but no differences were observed in 2009 (Tables 3.2, 3.4). Species richness was not different among treatments or the reference in either year (Tables 3.2, 3.4). The unseeded and ES treatments exhibited significantly greater exotic vegetation relative to the LS treatment and reference in both 2008 and 2009 (Figure 3.2). Differences in exotic species are mainly due to the increased contribution of *Agropyron cristatum* (L.) Gaertn. and *Bromus tectorum* L. (Tables 3.3, 3.5).

In 2008, annual forb composition was greater in the unseeded control and early seral seeded (ES) treatment relative to the reference (Table 3.2). In 2009, annual forbs were greater across all treatments than the reference (Table 3.4). *Alyssum alyssoides* (L.) L. was the main annual forb found in these plots (Tables 3.3, 3.5). In 2008, annual grass composition was greater in the ES treatment than the late seral seeded (LS) treatment or the reference, while composition was greater in the unseeded treatment than the reference only (Table 3.2). Annual grasses were greater in the ES and unseeded treatments than the reference in 2009 (Table 3.4), and for both years can be attributed entirely to *B. tectorum* (Tables 3.3, 3.5). In 2008, perennial grass composition was greater in the LS treatment relative to all others and lower in the ES treatment than all others (Table 3.2). Differences can be attributed to increased production of *Elymus lanceolatus* (Scribn. & J. G. Sm.) A. Löve, *Hesperostipa comata* (Trin. & Rupr.) Barkworth,

and *Pascopyrum smithii* (Rydb.) A. Löve (Table 3.3). Perennial grass composition was greater in both the unseeded and LS treatments than the ES treatment in 2009 (Table 3.4). Major perennial grass species in the LS treatment were again *E. lanceolatus*, *H. comata*, and *P. smithii*. Increased perennial grasses in the unseeded treatment can mainly be attributed to the exotic perennial grass *A. cristatum* (Table 3.5).

In both 2008 and 2009, mid seral shrub composition was greater in the ES treatment than the LS treatment or reference, while the unseeded treatment was greater than the reference only (Tables 3.2, 3.4). These differences can be attributed to *Ericameria nauseosa* (Pall. ex Pursh) G.L. Nesom & Baird and *Chrysothamnus viscidiflorus* (Hook.) Nutt. in the ES treatment, but only *E. nauseosa* in the unseeded treatment (Tables 3.3, 3.5). Late seral shrub composition, mainly *A. tridentata* (Tables 3.3, 3.5), was greater in the reference than all treatments in 2008 and 2009, but was significantly lower in the unseeded treatment than the ES treatment as well (Tables 3.2, 3.4). Total shrub density in 2009 was greater in the ES treatment and reference than the LS treatment (Figure 3.1). Mid-seral shrub density was greater in the unseeded and ES treatments than either the LS treatment or reference, while late seral shrub density was greater in the reference than the unseeded, ES, or LS treatments (Figure 3.1). Mid-seral shrubs encountered were *Atriplex canascens* (Pursh) Nutt., *C. viscidiflorus*, *Ericameria nauseosus*, *Gutierrezia sarothrae* (Pursh) Britton & Rusby, and *Krascheninnikovia lanata* (Pursh) A. Meeuse & Smit. The only late-seral shrub species encountered was *A. tridentata*.

IV. DISCUSSION

The initial results (1991) of this study were previously reported (Stevenson et al., 2000). These findings showed that after seven years of development, seeding with late seral species (LS) accelerated community succession, while seeding with early seral species (ES) resulted in a community similar to that of the unseeded control. Specifically, production of perennial grasses and the late seral shrub, *A. tridentata*, were greater on the LS treatment, while annual grasses, annual forbs, and the mid seral shrubs, *C. viscidiflorus*, *E. nauseosa*, and *G. sarothrae*, were greater on the unseeded and ES treatments (Stevenson et al., 2000). These findings were similar to other studies examining seed limitation and priority effects (Drake, 1991; Belyea & Lancaster, 1999; Weiher & Keddy, 1999; Lulow, 2004; Fukami et al., 2005), where initial species establishment define future vegetation composition by promoting their progeny and limiting the establishment of species with similar niches (Young et al., 2001).

These trends continued to be seen after 25 years of ecosystem development, where the two different seeding treatments resulted in considerably different species assemblages. The ES and the unseeded treatments exhibited significantly greater mid seral shrubs (Tables 3.2, 3.4; Figure 3.2) and greater exotic species than the LS treatment (Figure 3.1), while perennial grasses dominated the LS treatment (Tables 3.2, 3.4). These differences in species composition provide further evidence of how initial colonizers can impact the trajectory of ecosystem development. Contrary to the

findings of Stevenson et al. (2000), *A. tridentata* composition and density were similar in the unseeded, ES, and LS treatments (Tables 3.3, 3.5; Figure 3.2), even though *A. tridentata* was both seeded and established from transplants in the LS treatment. This indicates that, over a 25-year period, natural recruitment and development of this climax vegetation species can occur in areas where a propagule source is available, but even following transplanting, does not reach dominance found in the reference areas (Tables 3.3, 3.5).

Plant community assembly theory, built upon early individualistic successional theories proposed by Gleason (1917), Tansley (1935), Egler (1954), and Connell and Slatyer (1977), places a strong emphasis on the controls of plant colonization, establishment, and persistence in community development (Young et al., 2001). Priority effect is a main tenant of assembly theory, where initial colonizers can alter the trajectory, rate, or endpoint of ecosystem development, resulting in the possibility of different stable states (Belyea & Lancaster, 1999; Young et al., 2005). Here, initial propagule availability affected the vegetation composition at the lifeform (Tables 3.2, 3.4) and species level (Tables 3.3, 3.5) 25 years after seeding treatments were applied. Although both the ES, unseeded, and LS treatments resembled the typical mid successional community determined for this site (McLendon & Redente, 1990; McLendon & Redente, 1991), the structure of dominant vegetation was different. Mid seral shrubs dominated the ES treatment, while perennial grasses dominated the LS treatment (Tables 3.2, 3.4).

Comparing the results of the seeded and unseeded treatments at the species level allows us to further examine this mechanism. *C. viscidiflorus*, seeded in the ES treatment, was significantly greater in the ES treatment relative to the unseeded treatment, where *E. nauseosa* was the only dominant mid seral shrub, and was generally lacking in the LS treatment (Tables 3.3, 3.5). Additionally, the perennial grasses *E. lanceolatus*, *H. comata*, and *P. smithii*, seeded in the LS treatment, were significantly greater than the unseeded treatment, where the persistent exotic perennial grass *A. cristatum* was able to establish, and were considerably lower in the ES treatment (Tables 3.3, 3.5). Community assembly has been shown to converge along functional groups, but diverge along species composition, illustrating that initial species establishment can control future community assemblage by inhibiting establishment of species with similar niches (Fukami et al., 2005). Differences in the composition by functional group and individual species as a result of seed addition suggests that the dominant lifeforms of these two communities occupy similar niches in this ecosystem, where dominant perennial grasses limit the establishment of mid seral shrubs and vice versa. In the semi-arid western US, these two lifeforms can both be characterized as stress-tolerant competitors, with similar strategies of seed production and growth rate (Grime, 2001) and high root: shoot ratios (Fernandez & Caldwell, 1975; Cheplick, 1998).

An understanding of the factors controlling community development is critical to the emerging field of restoration ecology (Bradshaw, 1990; Palmer et al., 1997). Recognizing the factors that control successional development of an ecosystem can guide management prescriptions, result in more accurate descriptions of reference

conditions, and improve chances for success (Young et al., 2001). This not only adds to our knowledge of how ecosystems develop, but can also lead to improved ecosystem management, providing an opportunity to use management objectives to guide the trajectory of ecosystem recovery toward self-regulating vegetation communities that provide desired ecosystem services (Johnstone et al.; George et al., 1992; Mayer & Rietkerk, 2004; Quetier et al., 2007). In this system, increased shrub composition and greater shrub density may result in improved wildlife habitat for common and endangered species (Yoakum, 1984; Olson et al., 2000; Watters et al., 2002), while greater composition of perennial grasses may increase forage availability for large herbivores, as well as decrease erosion potential (Tow & Lazenby, 2001). Additionally, establishment of native perennial grasses has been found to limit the contribution of the persistent exotic species *B. tectorum* and *A. cristatum* (Eckert & Evans, 1963). Developing a thorough understanding of succession and assembly in community development is critical in order to use this knowledge and the mechanisms involved to devise successful restoration strategies (Young et al., 2001).

V. CONCLUSION

Most ecological studies are of short duration, however community development occurs over much longer periods of time and insights gained from longer studies aid in our understanding of successional pathways and the mechanisms that control them (Inouye & Tilman, 1995). By revisiting this study, I was able to examine the impacts of initial colonizers on community assemblage 25 years after disturbance and relate these findings to current successional theories.

In this *A. tridentata* system, long-term community development following disturbance was significantly altered by initial seed mix. A late seral seed mix resulted in a community dominated by perennial grasses, while an early seral seed mix resulted in a community dominated by mid seral shrubs. Additionally, an unseeded control resulted in a vegetation community with significant contributions by both perennial grasses and mid seral shrubs, but community composition at the species level was considerably different than that of the seeded treatments. This illustrates how priority effect of initial colonizers and niche similarity can strongly affect subsequent community assembly.

VI. TABLES AND FIGURES

Table 3.1 Early seral and late seral seed mix by lifeform expressed as scientific and common name. Seed was applied following disturbance in the fall of 1984 at the specified application rate (kg PLS ha^{-1}) to examine the effect of initial propagule availability on long-term community composition.

Scientific Name	Common Name	Application Rate
Early Seral Seed Mix		
Grasses		
Bromus tectorum L.	cheatgrass	0.78
Elymus elymoides (Raf.) Swezey	squirreltail	2.37
Hordeum jubatum L.	foxtail barley	0.05
Forbs		
Bassia scoparia (L.) A.J. Scott	burning bush	0.32
Erigeron engelmannii A. Nelson	Engelmann's fleabane	<0.01
Packera multilobata (Torr. & A. Gray ex A. Gray) W.A. Weber & A. Löve	lobeleaf groundsel	0.02
Salsola tragus L.	prickly Russian thistle	1.40
Sphaeralcea coccinea (Nutt.) Rydb.	scarlet globemallow	0.91
Shrubs		
Chrysothamnus viscidiflorus (Hook.) Nutt.	yellow rabbitbrush	0.01
Ericameria nauseosa (Pall. ex Pursh) G.L. Nesom & Baird	rubber rabbitbrush	0.38
Gutierrezia sarothrae (Pursh) Britton & Rusby	broom snakeweed	0.06

Table 3.1 (continued).

Scientific Name	Common Name	Application Rate
Late Seral Seed Mix		
Grasses		
Achnatherum hymenoides (Roem. & Schult.) Barkworth	Indian ricegrass	0.32
Elymus lanceolatus (Scribn. & J.G. Sm.)	thickspike wheatgrass	0.29
Hesperostipa comata (Trin. & Rupr.) Barkworth	needle and thread	2.57
Koeleria macrantha (Ledeb.) Schult.	prairie Junegrass	0.26
Pascopyrum smithii (Rydb.) A. Löve	western wheatgrass	0.12
Poa secunda J. Presl	Sandberg bluegrass	0.59
Pseudoroegneria spicata (Pursh) A. Löve	bluebunch wheatgrass	0.65
Forbs		
Astragalus purshii Douglas ex Hook.	woollypod milkvetch	<0.01
Phlox hoodii Richardson	spiny phlox	<0.01
Trifolium gymnocarpon Nutt.	hollyleaf clover	0.01
Shrubs		
Artemisia tridentata Nutt.[1]	big sagebrush	0.50
Krascheninnikovia lanata (Pursh) A. Meeuse & Smit	winterfat	2.40

[1] seedlings transplanted in addition to seeding

Table 3.2 Mean and standard error of 2008 plant community composition (% of total biomass) and relevant community parameters by treatment. Variables include composition by lifeform, as well as total plant production and species richness. Treatments were addition of early seral seed, an unseeded control, addition of late seral seed, and an undisturbed reference. Means followed by different letters within rows indicate significant differences ($p<0.05$) using Tukey's HSD.

		Early Seral		Control		Late Seral		Reference	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Annual Forb	%	5.4a	0.8	5.6a	1.3	2.7ab	0.6	1.3b	0.5
Annual Grass	%	11.4a	3.7	9.2ab	3.0	4.3bc	1.4	1.2c	0.8
Biennial Forb	%	0.5	0.5	1.2	0.4	0.7	0.4	0.1	0.1
Perennial Forb	%	11.1	2.4	9.1	2.4	9.1	2.7	14.3	6.9
Perennial Grass	%	28.9c	4.8	52.9b	6.5	71.2a	4.2	55.6b	7.0
Mid Seral Shrub	%	35.4a	4.2	15.7ab	2.7	4.9bc	2.3	0.4c	0.1
Late Seral Shrub	%	7.0b	2.3	6.3b	1.2	6.8b	2.6	27.1a	8.7
Total Plant Biomass	$g\ m^{-2}$	115a	9	127a	7	117a	9	60b	4
Species Richness	#	19.5	1.4	22.5	1.5	19.1	1.4	21.8	0.3

Table 3.3 Mean and standard error of 2008 major species composition (% of total biomass). Major species were determined as any species greater than 5% of community composition among treatments or years. Treatments were addition of early seral seed, an unseeded control, addition of late seral seed, and an undisturbed reference. Means followed by different letters within rows indicate significant differences ($p<0.05$) using Tukey's HSD.

		Early Seral		Control		Late Seral		Reference	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Agropyron cristatum	%	4.0ab	2.0	20.3a	7.8	2.4b	2.0	2.3ab	2.2
Alyssum alyssoides	%	5.3b	0.8	4.5a	0.8	2.7ab	0.6	1.3b	0.5
Artemisia tridentata	%	7.0b	2.3	6.1b	1.2	6.8b	2.6	26.8a	8.8
Bromus tectorum	%	11.4a	3.7	9.2ab	2.9	4.3bc	1.4	1.2c	0.8
Castilleja angustifolia	%	0	0	0.3	0.3	0	0	0	0
Chrysothmnus viscidiflorus	%	13.0a	3.1	2.1b	1.5	1.0b	0.7	0.4b	0.1
Elymus lanceolatus	%	0.9	0.9	1.9	1.4	7.6	4.8	1.8	1.7
Ericameria nauseosa	%	22.4a	5.0	13.5ab	3.0	3.9b	1.8	0c	0
Hesperostipa comata	%	8.4b	2.1	12.2b	2.7	24.5a	4.1	6.4b	1.9
Koeleria macrantha	%	2.7b	1.6	1.6b	0.8	1.8b	0.7	9.0a	1.9
Linum lewisii	%	4.1	1.6	3.1	1.6	2.8	1.3	0	0
Melilotus officinalis	%	0.5	0.4	0.2	0.1	0	0	0	0
Pascopyrum smithii	%	6.3b	3.3	7.4b	1.7	20.8a	4.6	11.5ab	1.6
Phlox hoodii	%	0b	0	0b	0	0.1b	0.1	7.2a	5.4
Poa fendleriana	%	0	0	0	0	0.1	0.1	2.6	2.6
Poa secunda	%	2.8b	1.8	1.5b	0.5	3.6ab	0.8	13.5a	3.5
Pseudoroegneria spicata	%	0	0	0.3	0.3	2.4	1.4	0	0
Sphaeralcea coccinea	%	1.7	0.8	3.4	1.2	2.9	0.9	0.5	0.4

Table 3.4 Mean and standard error of 2009 plant community composition (% of total biomass) and relevant community parameters by treatment. Variables include composition by lifeform, as well as total plant production and species richness. Treatments were addition of early seral seed, an unseeded control, addition of late seral seed, and an undisturbed reference. Means followed by different letters within rows indicate significant differences ($p<0.05$) using Tukey's HSD.

		Early Seral		Control		Late Seral		Reference	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Annual Forb	%	4.4a	1.3	3.2a	1.0	2.1a	0.6	0.2b	0.1
Annual Grass	%	4.5a	1.6	5.3a	3.4	1.9ab	0.7	0.2b	0.1
Biennial Forb	%	8.4	5.4	4.4	2.6	0.4	0.2	0.3	0.2
Perennial Forb	%	12.7	3.7	19.0	5.9	13.2	3.4	12.0	3.3
Perennial Grass	%	27.8b	6.7	51.0a	9.9	72.2a	3.3	42.1ab	5.6
Mid Seral Shrub	%	23.3a	2.5	10.8ab	3.0	2.8bc	0.7	1.5c	0.9
Late Seral Shrub	%	17.6b	4.1	6.3c	2.0	7.5bc	1.8	43.7a	5.5
Total Plant Biomass	$g\ m^{-2}$	65b	5	76ab	5	79b	2	61a	3
Species Richness	#	20.3	1.4	21.4	1.2	21.1	1.2	25.8	1.7

Table 3.5 Mean and standard error of 2009 major species composition (% of total biomass). Major species were determined as any species greater than 5% of community composition among treatments or years. Treatments were addition of early seral seed, an unseeded control, addition of late seral seed, and an undisturbed reference. Means followed by different letters within rows indicate significant differences ($p<0.05$) using Tukey's HSD.

		Early Seral		Control		Late Seral		Reference	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Agropyron cristatum	%	8.1	3.3	17.2	10.0	3.3	1.4	6.8	4.4
Alyssum alyssoides	%	4.2a	1.3	2.8a	1.0	2.0a	0.6	0.2b	0.1
Artemisia tridentata	%	17.6b	4.1	6.3c	2.0	7.5bc	1.8	40.6a	7.2
Bromus tectorum	%	4.5a	1.6	5.3a	3.4	1.9ab	0.7	0.2b	0.1
Castilleja angustifolia	%	0	0	5.6	4.4	0	0	0	0
Chrysothmnus viscidiflorus	%	7.7a	1.5	2.4b	1.5	1.1b	0.5	0.2b	0.2
Elymus lanceolatus	%	0.4b	0.3	1.2b	0.6	17.1a	4.3	4.2b	1.3
Ericameria nauseosa	%	15.6a	2.8	8.4ab	2.5	1.8bc	0.7	1.0c	0.7
Hesperostipa comata	%	5.6	1.3	7.7	2.7	15.1	4.1	4.5	2.0
Koeleria macrantha	%	0.3b	0.3	1.7b	0.8	0.6b	0.3	4.6a	1.0
Linum lewisii	%	3.4ab	1.8	4.2ab	2.1	7.0a	2.9	0b	0
Melilotus officinalis	%	8.3	5.4	3.9	2.7	0	0	0	0
Pascopyrum smithii	%	3.3b	2.4	6.5b	1.8	16.9a	2.3	2.5b	0.3
Phlox hoodii	%	0b	0	0.3b	0.3	0.1b	0.1	5.7a	3.0
Poa fendleriana	%	5.1ab	3.7	1.2ab	0.5	0.1b	0.1	10.0a	4.3
Poa secunda	%	0.2ab	0.1	0.1b	0	0.2ab	0.1	0.7a	0.5
Pseudoroegneria spicata	%	1.9	1.9	5.6	2.7	13.0	3.4	5.4	5.0
Sphaeralcea coccinea	%	5.6	1.9	6.6	2.7	2.6	0.9	1.0	0.4

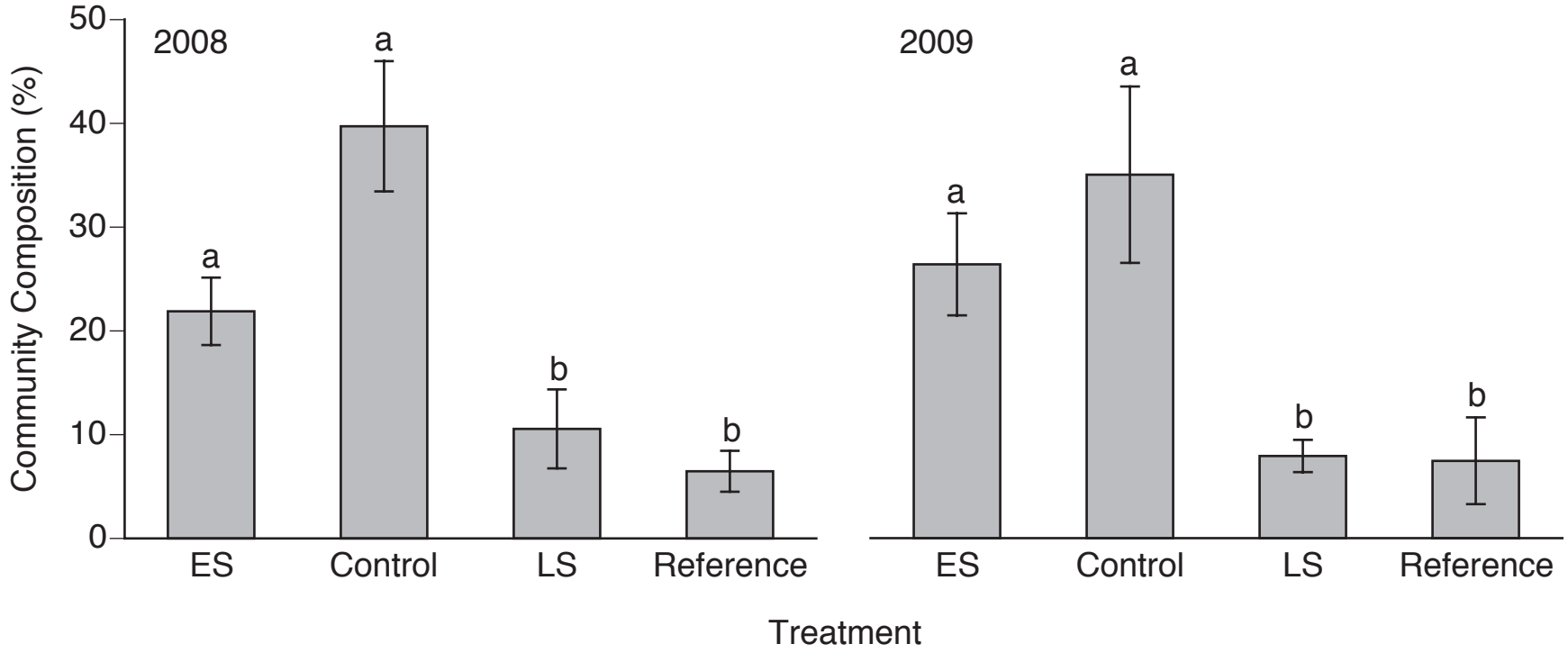

Figure 3.2 Mean and standard error for exotic biomass (%) by treatment for 2008 and 2009. Treatments were addition of early seral seed (ES), an unseeded control, addition of late seral seed (LS), and an undisturbed reference. Bars with different letters indicate significant differences ($p<0.05$) using Tukey's HSD.

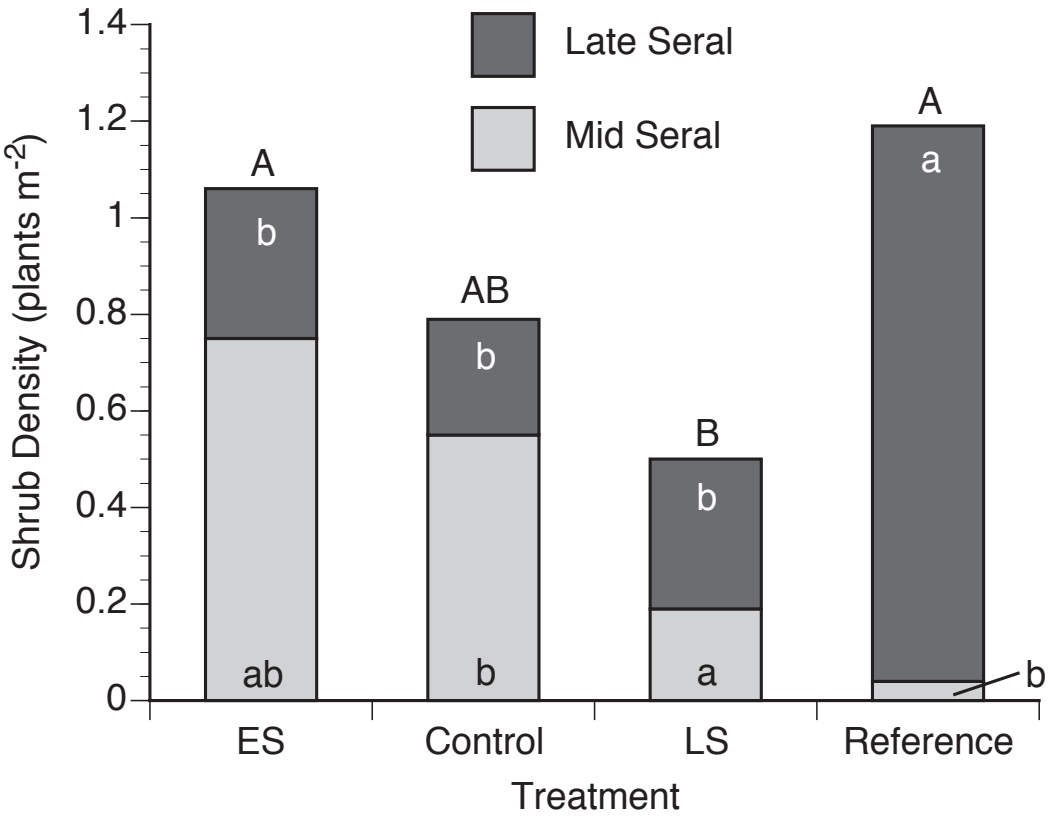

Figure 3.2 Mean of total, mid seral, and late seral shrub density (plants m^{-2}) by treatment for 2009. Treatments were early seral seed mix (ES), unseeded control, late seral seed mix (LS), and undisturbed reference. Different upper-case letters indicate significant differences in total shrub density, different white lower-case letters indicate significant differences between late seral shrub density, and different black lower-case letters indicate significant differences between mid seral shrub density using Tukey's HSD ($p<0.05$).

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PART FOUR

SUMMARY OF FACTORS AFFECTING ECOSYSTEM DEVELOPMENT OF A SAGEBRUSH ECOSYSTEM

Studies examining the factors that control plant and microbial succession are critical in developing a greater understanding of how ecosystems develop. This study examined plant and microbial community development in northwest Colorado in a region frequently impacted by energy extraction activities. Although most experiments of this nature occur over short time periods (Inouye & Tilman, 1995), this study revisited experiments started in the mid 1980s to understand how common restoration practices affect plant and microbial communities after two decades of ecosystem development. The restoration practices explored in these experiments included addition of limiting soil nutrients, nitrogen (N) and phosphorus (P), immobilization of N through carbon addition, and the impact of seed addition on long-term community development.

Similar to initial findings (Carpenter et al., 1990; McLendon & Redente, 1991), I found that addition of N slowed plant community development through the promotion of annual plant species, but addition of P had little effect. However, the coupled addition of N and P further impeded plant succession by encouraging the production of the invasive annual grass *Bromus tectorum* L. Additionally, the ratio of fungal: bacterial biomass in the soil, an indicator of microbial successional development (van der Wal et al., 2006; Harris, 2009), was lower in the N and N + P addition treatments, further demonstrating the impact of these nutrients on ecosystem development. These results support other studies that have shown nutrient addition slows the rate of ecosystem

succession (Foster & Gross, 1998; Zink & Allen, 1998; Holmes, 2001). Differences in plant community response between N and P addition show that this ecosystem is initially N limited, but after N addition, the ecosystem becomes P limited. Addition of N and P together further impeded community development, demonstrating that neither N nor P promote plant community succession and illustrating Liebig's Law of the Minimum (Hooker, 1917).

While one study showed that nitrogen addition decreased the rate of ecosystem succession, a second study examined how communities respond to decreased nitrogen availability. Soil nitrogen can be immobilized by stimulating soil microbial growth through frequent addition of a labile carbon source, such as sucrose (Morgan, 1994; Zink & Allen, 1998). Initial investigations found that immobilization of soil N increased the rate of plant community development (McLendon & Redente, 1992). After two decades of community development, this effect was no longer apparent. However, soil fungal development was greater as a result of this treatment, showing that the soil microbial community was affected by reductions in soil N through sucrose addition, but was not linked to plant community succession. This may be explained by considering the role of priority effect, which explains that the order and timing of seed arrival and establishment strongly influence future vegetation composition (Belyea & Lancaster, 1999; Young et al., 2001). At the beginning of this study, these research plots were seeded with a mix of early and late seral species common to the region. Soil N may have exerted a stronger selection pressure early in successional development, while the

common propagule source available across the study may have determined the vegetation community structure later in succession.

A third study further examined the concept of priority effect by observing how plant communities develop in response to different seed mixes. Following a physical disturbance, areas were seeded with either a mix of early seral species, consisting of nitrophilic grasses and forbs and mid seral shrubs, or a mix of late seral species, consisting of perennial grasses and forbs and late seral shrubs. After two decades of community development, marked differences were observed between these two treatments. The early seral seed treatment had significantly more mid seral shrubs and exotic vegetation, while perennial grasses dominated the late seral seed treatment. This provided further evidence for the role of priority effect in determining the structure of species composition.

These three studies aid in describing long-term community development and characterizing the impact of restoration practices in this region of the western US. This information can assist in the development of applied management approaches, such as development of restoration plans to promote specific ecological services, improved selection of reference areas, and development of informed regulatory guidelines, as well as more theoretical approaches such as to test current theories of community succession and development (Bradshaw, 1990; Palmer et al., 1997; Young et al., 2001). I found plant community composition to be strongly affected by initial propagule source after two decades of community development, suggesting that seed addition could be an important tool in managing vegetation communities over long time periods.

Manipulating propagule availability of desired plant species can alter the communities at both the lifeform and species level, providing an important and affordable tool to meet objectives for necessary ecosystem services, such as increased forage production, greater species richness, deceased exotic species production, decreased erosion potential, or improved habitat structure and heterogeneity. Additionally, I found elevated soil N to significantly slow the rate of succession and lead to persistence of exotic species. Addition of N should not be a technique applied to these regions unless the aim of management is to keep the community in an early seral state, as the effects are long-lived. However, if soil N levels are initially high, immobilization of N through carbon addition may increase the rate of succession and provide short-term reductions in exotic species. Coupling techniques to immobilize N and increase propagule availability of low N-use plant species (Perry et al., 2010) could be an important method to manage for stable late seral plant communities in this region.

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APPENDICES

I. PLANT PRODUCTION DATA

Appendix 1. Mean and (standard error) of production values for all species encountered in the nitrogen and phosphorus addition study for 2008. Treatments were addition of nitrogen only, nitrogen and phosphorus together, phosphorus only, an unfertilized control, and an undisturbed reference.

		Nitrogen	Nitrogen + Phosphorus	Phosphorus	Control	Reference
Achnatherum hymenoides	$g\ m^{-2}$	0 (0)	0.1 (0.1)	2.0 (1.9)	2.8 (1.5)	1.4 (0.4)
Achnatherum lettermanii	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0.3 (0.3)	0 (0)
Agropyron cristatum	$g\ m^{-2}$	0.1 (0.1)	1.6 (1.6)	10.1 (10.1)	14.4 (8.6)	1.4 (1.3)
Alyssum alyssoides	$g\ m^{-2}$	28.8 (6.8)	1.3 (0.8)	5.4 (0.8)	5.7 (2.3)	0.8 (0.4)
Artemisia frigida	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)
Arabis pendulina	$g\ m^{-2}$	0.1 (0.1)	0 (0)	0 (0)	0 (0)	0 (0)
Artemisia tridentata	$g\ m^{-2}$	20.5 (12.3)	4.7 (4.5)	11.3 (6.1)	8.2 (1.6)	17 (6.4)
Astragalus convallarius	$g\ m^{-2}$	0.3 (0.3)	0.9 (0.9)	0.2 (0.2)	0.2 (0.2)	0.6 (0.2)
Astragalus purshii	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0.2 (0.1)	0.3 (0.1)
Bromus inermis	$g\ m^{-2}$	0 (0)	0.5 (0.5)	0 (0)	1.1 (1.1)	0 (0)
Bromus tectorum	$g\ m^{-2}$	29.2 (9.2)	35.2 (8.6)	16.0 (8.3)	9.3 (4.6)	0.7 (0.5)
Castilleja angustifolia	$g\ m^{-2}$	0 (0)	0 (0)	0.3 (0.3)	0 (0)	0 (0)
Carex geyeri	$g\ m^{-2}$	0 (0)	0 (0)	0.4 (0.4)	0 (0)	0 (0)
Chrysothamnus viscidiflorus	$g\ m^{-2}$	0 (0)	0 (0)	0.8 (0.6)	4.1 (3.7)	0.2 (0.1)
Crepis accuminata	$g\ m^{-2}$	0 (0)	0 (0)	0.1 (0.1)	0 (0)	0 (0)
Cryptantha flavoculata	$g\ m^{-2}$	0 (0)	0 (0)	0.1 (0.1)	0 (0)	1.0 (0.5)

Appendix 1 (continued).

		Nitrogen	Nitrogen + Phosphorus	Phosphorus	Control	Reference
Delphinium nuttallianum	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0.1 (0.1)	0 (0)
Descurainia sophia	$g\ m^{-2}$	2.2 (2.2)	9.3 (6.4)	0 (0)	0 (0)	0 (0)
Elymus elymoides	$g\ m^{-2}$	0.2 (0.2)	0 (0)	0.2 (0.1)	0.6 (0.3)	1.2 (0.6)
Elymus lanceolatus	$g\ m^{-2}$	0 (0)	0 (0)	2.3 (2.3)	1.2 (1.2)	1.2 (1.0)
Elymus repens	$g\ m^{-2}$	1.4 (1.4)	0 (0)	0 (0)	4.6 (2.2)	0 (0)
Elymus trachycaulus	$g\ m^{-2}$	0.1 (0.1)	0 (0)	0 (0)	2.0 (2.0)	1.2 (1.2)
Erysimum asperum	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0 (0)	0.1 (0.1)
Erigeron engelmannii	$g\ m^{-2}$	0 (0)	0 (0)	0.6 (0.6)	0.2 (0.1)	1.2 (0.3)
Ericameria nauseosa	$g\ m^{-2}$	29.6 (17.1)	3.6 (3.1)	13.5 (5.0)	19.5 (5.0)	0 (0)
Festuca brevipila	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0.8 (0.8)	1.0 (1.0)
Gutierrezia sarothrae	$g\ m^{-2}$	0 (0)	0 (0)	0.7 (0.4)	1.1 (0.8)	0.1 (0.1)
Hedysarum boreale	$g\ m^{-2}$	0 (0)	0 (0)	0.3 (0.3)	1.2 (1.2)	0.2 (0.2)
Hesperostipa comata	$g\ m^{-2}$	8.7 (3.5)	0 (0)	3.3 (2.1)	18 (3.1)	3.7 (1.1)
Hordeum jubatum	$g\ m^{-2}$	1.0 (1.0)	0 (0)	0 (0)	0.4 (0.4)	0.5 (0.3)
Ipomopsis aggregata	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0.1 (0.1)	0 (0)
Koeleria macrantha	$g\ m^{-2}$	0 (0)	0 (0)	1.2 (0.7)	3.2 (2.3)	5.2 (1.1)
Lappula occidentalis	$g\ m^{-2}$	0.1 (0.1)	0.2 (0.1)	0 (0)	0 (0)	0 (0)
Lactuca serriola	$g\ m^{-2}$	0 (0)	0.3 (0.3)	0 (0)	0 (0)	0 (0)
Linum lewisii	$g\ m^{-2}$	3.4 (2.8)	0 (0)	1.0 (0.8)	5.3 (4.1)	0 (0)
Machaeranthera canescens	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	1.5 (0.9)	0 (0)

Appendix 1 (continued).

		Nitrogen	Nitrogen + Phosphorus	Phosphorus	Control	Reference
Machaeranthera grindelioides	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0.1 (0.1)	0.2 (0.1)
Medicago sativa	$g\ m^{-2}$	0 (0)	0.1 (0.1)	0 (0)	0 (0)	0 (0)
Opuntia polyacantha	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0.1 (0.1)	0.1 (0.1)
Packera multilobata	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)
Pascopyrum smithii	$g\ m^{-2}$	29.3 (14.6)	18.4 (18.4)	13.5 (7.9)	13.7 (4.7)	6.9 (1.2)
Phlox hoodii	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0 (0)	3.7 (2.5)
Pinus edulis	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0 (0)	0.1 (0.1)
Poa species	$g\ m^{-2}$	0.1 (0.1)	0 (0)	0 (0)	0 (0)	0 (0)
Poa fenderliana	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0 (0)	1.7 (1.7)
Poa secunda	$g\ m^{-2}$	0.4 (0.2)	0 (0)	0.9 (0.7)	2.9 (1.4)	7.8 (1.9)
Pseudoroegneria spicata	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	1.0 (1.0)	0 (0)
Schoencrambe linifolia	$g\ m^{-2}$	0 (0)	0 (0)	0.1 (0.1)	0.1 (0.1)	0 (0)
Sisymbrium altissimum	$g\ m^{-2}$	2.8 (1.7)	52.7 (30.5)	10.1 (10.1)	0 (0)	0 (0)
Sphaeralcea coccinea	$g\ m^{-2}$	5.8 (1.2)	2.2 (1.0)	5.6 (1.5)	3.8 (0.7)	0.3 (0.2)
Taraxacum officinale	$g\ m^{-2}$	0.3 (0.3)	0.1 (0.1)	0 (0)	0.1 (0.1)	0 (0)
Thinopyrum intermedium	$g\ m^{-2}$	5.3 (5.3)	2.5 (2.5)	0 (0)	0 (0)	0.2 (0.2)
Tragopogon dubius	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0.4 (0.4)	0 (0)
Trifolium gymnocarpon	$g\ m^{-2}$	0.2 (0.1)	0.1 (0.1)	0.1 (0.1)	0.2 (0.1)	0 (0)
unknown forb 3	$g\ m^{-2}$	0 (0)	0 (0)	0.1 (0.1)	0 (0)	0 (0)
unknown grass 3	$g\ m^{-2}$	0 (0)	0 (0)	0.1 (0.1)	0 (0)	0 (0)

Appendix 1 (continued).

		Nitrogen	Nitrogen + Phosphorus	Phosphorus	Control	Reference
Verbascum thapsus	$g\ m^{-2}$	0.2 (0.2)	0 (0)	2.6 (2.6)	0 (0)	0 (0)
Total Plant Biomass	$g\ m^{-2}$	89 (9)	112 (24)	64 (5)	74 (3)	61 (3)
Litter	$g\ m^{-2}$	no data collected				

Appendix 2. Mean and (standard error) of production values for all species encountered in the nitrogen and phosphorus addition study for 2009. Treatments were addition of nitrogen only, nitrogen and phosphorus together, phosphorus only, an unfertilized control, and an undisturbed reference.

		Nitrogen	Nitrogen + Phosphorus	Phosphorus	Control	Reference
Achnatherum hymenoides	$g\ m^{-2}$	0 (0)	0 (0)	0.6 (0.1)	0.3 (0.2)	0.5 (0.3)
Achnatherum lettermanii	$g\ m^{-2}$	0.1 (0.1)	0 (0)	0 (0)	0.2 (0.2)	0 (0)
Agropyron cristatum	$g\ m^{-2}$	1 (0.8)	2.4 (1.9)	6.0 (3.9)	6.4 (3.9)	3.8 (2.4)
Alyssum alyssoides	$g\ m^{-2}$	9.8 (3.2)	1.1 (1.0)	2.4 (0.8)	1.7 (0.4)	0.1 (0)
Artemisia tridentata	$g\ m^{-2}$	7.5 (4)	9.7 (4.4)	12.7 (4.7)	3.5 (2.1)	25.4 (5.1)
Astragalus convallarius	$g\ m^{-2}$	0.3 (0.2)	0.7 (0.7)	0.2 (0.1)	0.2 (0.2)	0.3 (0.2)
Astragalus purshii	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0 (0)	0.6 (0.4)
Bromus tectorum	$g\ m^{-2}$	10.7 (5.5)	49.5 (9.3)	9.3 (8.5)	2.0 (1.0)	0.1 (0.1)
Castilleja angustifolia	$g\ m^{-2}$	0 (0)	0 (0)	0.5 (0.5)	1.6 (1.5)	0 (0)
Chaetopappa ericoides	$g\ m^{-2}$	0 (0)	0 (0)	0.4 (0.4)	0 (0)	0 (0)
Chrysothamnus viscidiflorus	$g\ m^{-2}$	0 (0)	2.0 (2.0)	1.3 (0.7)	3.3 (2.0)	0.1 (0.1)
Crepis accuminata	$g\ m^{-2}$	0 (0)	0 (0)	0.1 (0.1)	0 (0)	0.1 (0.1)
Cryptantha flavoculata	$g\ m^{-2}$	0 (0)	0 (0)	0.3 (0.3)	0 (0)	0.8 (0.4)
Descurainia pinnata	$g\ m^{-2}$	2.0 (1.9)	0.3 (0.3)	0 (0)	0 (0)	0 (0)
Elymus elymoides	$g\ m^{-2}$	0.3 (0.2)	0.2 (0.2)	0 (0)	1.6 (1.6)	0.7 (0.4)
Elymus lanceolatus	$g\ m^{-2}$	1.8 (1.8)	13.5 (13.5)	0.6 (0.4)	0.4 (0.2)	2.6 (0.8)
Elymus repens	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	7.5 (4.2)	0.1 (0.1)
Elymus trachycaulus	g m-2	0 (0)	0 (0)	0 (0)	0 (0)	0.6 (0.5)
Erysimum asperum	g m-2	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.1)

Appendix 2 (continued).

		Nitrogen	Nitrogen + Phosphorus	Phosphorus	Control	Reference
Erigeron eatonii	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0 (0)	0.1 (0)
Erigeron engelmannii	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0 (0)	0.3 (0.1)
Ericameria nauseosa	$g\ m^{-2}$	9.5 (4.9)	2.9 (2.2)	3.7 (2.3)	8.6 (2.9)	0.6 (0.4)
Festuca brevipila	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0 (0)	0.1 (0.1)
Gutierrezia sarothrae	$g\ m^{-2}$	0 (0)	0 (0)	1.2 (1.1)	0.9 (0.4)	0.2 (0.1)
Hedysarum boreale	$g\ m^{-2}$	0.2 (0.2)	0 (0)	0.4 (0.4)	0 (0)	0 (0)
Hesperostipa comata	$g\ m^{-2}$	5.8 (3.8)	0 (0)	1.8 (1.4)	8.4 (3.1)	2.8 (1.3)
Juniperus scopulorum	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0 (0)	0.1 (0.1)
Koeleria macrantha	$g\ m^{-2}$	0 (0)	0 (0)	0.7 (0.7)	1.9 (0.9)	2.8 (0.6)
Lactuca serriola	$g\ m^{-2}$	0 (0)	6.6 (6.6)	0 (0)	0 (0)	0 (0)
Linum lewisii	$g\ m^{-2}$	0.1 (0.1)	0 (0)	1.3 (1)	3.2 (2.9)	0 (0)
Machaeranthera canescens	$g\ m^{-2}$	0 (0)	0.4 (0.4)	0 (0)	0 (0)	0 (0)
Melilotus officinalis	$g\ m^{-2}$	0 (0)	0.2 (0.2)	0.4 (0.4)	0 (0)	0 (0)
Nassella viridula	$g\ m^{-2}$	0 (0)	0 (0)	0.7 (0.6)	2.7 (1.9)	0.2 (0.2)
Opuntia polyacantha	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0 (0)	0.8 (0.3)
Packera multilobata	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0 (0)	0.4 (0.4)
Pascopyrum smithii	$g\ m^{-2}$	30.4 (14.8)	11.6 (9.5)	12.9 (7.3)	5.8 (2.0)	1.6 (0.2)
Penstemon fremontii	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)
Phlox hoodii	*g m-2*	0 (0)	0 (0)	0.2 (0.2)	0.5 (0.5)	3.5 (1.9)
Pinus edulis	*g m-2*	0 (0)	0 (0)	0 (0)	0 (0)	0.8 (0.8)

Appendix 2 (continued).

		Nitrogen	Nitrogen + Phosphorus	Phosphorus	Control	Reference
Poa fenderliana	$g\ m^{-2}$	0 (0)	0 (0)	0.1 (0.1)	1.4 (0.5)	6.5 (3.0)
Poa pratensis	$g\ m^{-2}$	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)
Poa secunda	$g\ m^{-2}$	0 (0)	0 (0)	0.4 (0.4)	0.1 (0.1)	0.4 (0.3)
Pseudoroegneria spicata	$g\ m^{-2}$	3.9 (2.7)	0 (0)	0.1 (0.1)	5.8 (3.4)	2.9 (2.7)
Sarcobatus vermiculatus	$g\ m^{-2}$	0 (0)	2.0 (2.0)	0 (0)	0 (0)	0.2 (0.2)
Sisymbrium altissimum	$g\ m^{-2}$	0.2 (0.2)	3.4 (1.4)	0 (0)	0 (0)	0 (0)
Sphaeralcea coccinea	$g\ m^{-2}$	4.0 (2.5)	5.5 (1.6)	5.1 (2.4)	5.1 (1.8)	0.6 (0.3)
Taraxacum officinale	$g\ m^{-2}$	0.2 (0.1)	0.2 (0.1)	0 (0)	0 (0)	0 (0)
Tragopogon dubius	$g\ m^{-2}$	0.1 (0.1)	0.2 (0.1)	0 (0)	0.7 (0.6)	0 (0)
Trifolium gymnocarpon	$g\ m^{-2}$	0.1 (0.1)	0 (0)	0.1 (0.1)	0.5 (0.2)	0.1 (0.1)
Verbascum thapsus	$g\ m^{-2}$	0.4 (0.4)	0 (0)	0 (0)	0 (0)	0 (0)
Total Plant Biomass	$g\ m^{-2}$	89 (9)	112 (24)	64 (5)	74 (3)	61 (3)
Litter	$g\ m^{-2}$	72 (16)	56 (16)	50 (6)	55 (5)	76 (17)

Appendix 3. Mean and (standard error) of production values for all species encountered in the nitrogen immobilization study for 2008. Treatments were addition of nitrogen, an unamended control, and addition of sucrose.

		Nitrogen	Control	Sucrose
Achnatherum hymenoides	$g\ m^{-2}$	0.3 (0.3)	0.9 (0.5)	5.3 (3.4)
Achnatherum lettermanii	$g\ m^{-2}$	0 (0)	0 (0)	1.2 (1.2)
Agropyron cristatum	$g\ m^{-2}$	14.3 (6.5)	2.2 (1.8)	9.8 (6.3)
Alyssum alyssoides	$g\ m^{-2}$	14.3 (4.4)	5.1 (1.4)	9.3 (2.4)
Arabis pendulina	$g\ m^{-2}$	0 (0)	0 (0)	0.1 (0.1)
Artemisia tridentata	$g\ m^{-2}$	3.6 (2.1)	4.7 (1.7)	5.5 (2.3)
Astragalus chamaeleuce	$g\ m^{-2}$	0 (0)	0.3 (0.2)	0 (0)
Astragalus convallarius	$g\ m^{-2}$	0 (0)	0.1 (0.1)	0.6 (0.6)
Bromus inermis	$g\ m^{-2}$	7.3 (7.3)	0.4 (0.4)	1.1 (1.1)
Bromus tectorum	$g\ m^{-2}$	24.5 (8.3)	10.2 (4.7)	11.6 (2.8)
Castilleja angustifolia	$g\ m^{-2}$	0 (0)	0.7 (0.7)	0 (0)
Carex geyeri	$g\ m^{-2}$	0 (0)	0 (0)	0.1 (0.1)
Castilleja linariifolia	$g\ m^{-2}$	0 (0)	5.4 (5.0)	0.3 (0.2)
Chrysothamnus viscidiflorus	$g\ m^{-2}$	0.5 (0.5)	1.1 (0.7)	2.4 (2.4)
Crepis accuminata	$g\ m^{-2}$	0 (0)	0.8 (0.6)	0 (0)
Cryptantha flavoculata	$g\ m^{-2}$	0.2 (0.2)	0 (0)	0 (0)
Descurainia sophia	$g\ m^{-2}$	1.9 (1.9)	0 (0)	0.1 (0.1)
Elymus lanceolatus	*g m-2*	0.1 (0.1)	0 (0)	1.1 (0.6)
Elymus repens	*g m-2*	0 (0)	0.1 (0.1)	0 (0)

Appendix 3 (continued).

		Nitrogen	Control	Sucrose
Elymus trachycaulus	$g\ m^{-2}$	0 (0)	0.5 (0.4)	0 (0)
Erodium cicutarium	$g\ m^{-2}$	0.1 (0.1)	0 (0)	0 (0)
Erigeron engelmannii	$g\ m^{-2}$	0 (0)	0.1 (0.1)	0.1 (0.1)
Ericameria nauseosa	$g\ m^{-2}$	17.5 (11.8)	12.2 (7.6)	14.8 (10.5)
Eriogonum umbellatum	$g\ m^{-2}$	0 (0)	5.3 (5.3)	0.1 (0.1)
Gutierrezia sarothrae	$g\ m^{-2}$	0.4 (0.4)	0 (0)	0.2 (0.2)
Hedysarum boreale	$g\ m^{-2}$	0 (0)	0 (0)	0.3 (0.3)
Hesperostipa comata	$g\ m^{-2}$	0.7 (0.7)	4.6 (2.8)	0.6 (0.4)
Hordeum jubatum	$g\ m^{-2}$	0.3 (0.1)	0.4 (0.4)	0.3 (0.3)
Koeleria macrantha	$g\ m^{-2}$	0 (0)	0 (0)	0.7 (0.7)
Lappula occidentalis	$g\ m^{-2}$	0.2 (0.1)	0 (0)	0 (0)
Linum lewisii	$g\ m^{-2}$	3.5 (2.8)	4.3 (0.9)	6.0 (2.2)
Machaeranthera canescens	$g\ m^{-2}$	0.1 (0.1)	0 (0)	0.7 (0.7)
Melilotus officinalis	$g\ m^{-2}$	0 (0)	1.6 (1.4)	0 (0)
Pascopyrum smithii	$g\ m^{-2}$	10.0 (4.0)	4.1 (1.9)	6.3 (2.5)
Phlox hoodii	$g\ m^{-2}$	0 (0)	0 (0)	0.5 (0.5)
Poa secunda	$g\ m^{-2}$	0.3 (0.2)	2.0 (1.3)	0.8 (0.6)
Pseudoroegneria spicata	$g\ m^{-2}$	0.1 (0.1)	0 (0)	0 (0)
Purshia tridentata	*g m-2*	0.1 (0.1)	0 (0)	0 (0)
Sisymbrium altissimum	*g m-2*	9.5 (8.6)	0.1 (0.1)	0.2 (0.2)

Appendix 3 (continued).

		Nitrogen	Control	Sucrose
Sphaeralcea coccinea	$g\ m^{-2}$	3.1 (1.1)	4.6 (1.0)	3.9 (1.7)
Taraxacum officinale	$g\ m^{-2}$	0.1 (0.1)	0 (0)	0 (0)
Tragopogon dubius	$g\ m^{-2}$	1.2 (1.2)	0.3 (0.3)	0.2 (0.1)
Trifolium gymnocarpon	$g\ m^{-2}$	0.1 (0.1)	0.6 (0.5)	0.2 (0.2)
unknown forb 2	$g\ m^{-2}$	0 (0)	0 (0)	0.1 (0.1)
unknown grass 4	$g\ m^{-2}$	0 (0)	0 (0)	0.2 (0.2)
Total Plant Biomass	$g\ m^{-2}$	114 (27)	73 (13)	85 (3)
Litter		no data collected		

Appendix 4. Mean and (standard error) of production values for all species encountered in the nitrogen immobilization study for 2009. Treatments were addition of nitrogen, an unamended control, and addition of sucrose.

		Control	Nitrogen	Sucrose
Achnatherum hymenoides	$g\ m^{-2}$	1.7 (0.8)	0.3 (0.2)	1.9 (1.4)
Agropyron cristatum	$g\ m^{-2}$	0.5 (0.5)	7.7 (4.5)	8.5 (4.6)
Alyssum alyssoides	$g\ m^{-2}$	1.1 (0.4)	4.5 (1.9)	3.8 (0.6)
Ambrosia psilostachya	$g\ m^{-2}$	0 (0)	0 (0)	0.7 (0.7)
Artemisia tridentata	$g\ m^{-2}$	12.1 (3.7)	4.1 (2.3)	5.5 (2.7)
Astragalus convallarius	$g\ m^{-2}$	0.1 (0.1)	0 (0)	0.1 (0.1)
Bromus inermis	$g\ m^{-2}$	1.3 (1.3)	0 (0)	1.1 (1.1)
Bromus tectorum	$g\ m^{-2}$	1.5 (0.9)	10.3 (4.5)	1.8 (0.7)
Castilleja angustifolia	$g\ m^{-2}$	0.5 (0.4)	0.4 (0.3)	2.4 (1.1)
Chrysothamnus viscidiflorus	$g\ m^{-2}$	0.3 (0.2)	0.1 (0.1)	1.1 (0.6)
Crepis accuminata	$g\ m^{-2}$	0 (0)	0 (0)	0.1 (0.1)
Cryptantha flavoculata	$g\ m^{-2}$	0 (0)	0.2 (0.1)	0.6 (0.5)
Elymus elymoides	$g\ m^{-2}$	0.4 (0.1)	0.5 (0.3)	0.2 (0.1)
Elymus lanceolatus	$g\ m^{-2}$	1.9 (1.6)	0 (0)	0.8 (0.5)
Elymus trachycaulus	$g\ m^{-2}$	1.8 (1.7)	0 (0)	0 (0)
Erodium cicutarium	$g\ m^{-2}$	0 (0)	0.1 (0.1)	0 (0)
Ericameria nauseosa	$g\ m^{-2}$	2.8 (0.6)	8.3 (2.7)	2.3 (0.6)
Eriogonum umbellatum	*g m-2*	4.5 (4.5)	0 (0)	0.3 (0.3)
Gutierrezia sarothrae	*g m-2*	0.1 (0.1)	0.2 (0.2)	0 (0)

Appendix 4 (continued).

		Control	Nitrogen	Sucrose
Hesperostipa comata	$g\ m^{-2}$	2.3 (2.1)	4.0 (3.3)	3.1 (1.7)
Koeleria macrantha	$g\ m^{-2}$	0.6 (0.6)	0 (0)	0 (0)
Lactuca serriola	$g\ m^{-2}$	0 (0)	0.2 (0.2)	0 (0)
Linum lewisii	$g\ m^{-2}$	4.7 (2.2)	8.9 (6.2)	1.8 (0.8)
Machaeranthera canescens	$g\ m^{-2}$	0 (0)	0.1 (0)	0 (0)
Melilotus officinalis	$g\ m^{-2}$	0 (0)	0 (0)	0.7 (0.7)
Medicago sativa	$g\ m^{-2}$	0.2 (0.1)	0 (0)	0.2 (0.2)
Nassella viridula	$g\ m^{-2}$	0 (0)	0.2 (0.2)	0 (0)
Pascopyrum smithii	$g\ m^{-2}$	3.3 (1.6)	14.3 (8.0)	2.1 (0.9)
Phlox hoodii	$g\ m^{-2}$	0 (0)	0 (0)	0.3 (0.3)
Pinus edulis	$g\ m^{-2}$	0 (0)	0 (0)	0.4 (0.4)
Poa fenderliana	$g\ m^{-2}$	0 (0)	0.1 (0)	0.2 (0.2)
Poa secunda	$g\ m^{-2}$	0 (0)	0 (0)	0.2 (0.2)
Pseudoroegneria spicata	$g\ m^{-2}$	0 (0)	0 (0)	2.1 (2.1)
Purshia tridentata	$g\ m^{-2}$	0 (0)	0.3 (0.3)	0 (0)
Sphaeralcea coccinea	$g\ m^{-2}$	2.8 (0.9)	4.1 (1.5)	3.3 (1.1)
Taraxacum officinale	$g\ m^{-2}$	0 (0)	0.3 (0.3)	0 (0)
Tragopogon dubius	$g\ m^{-2}$	0.4 (0.3)	0.7 (0.5)	0.1 (0)
Trifolium gymnocarpon	*g m-2*	0.2 (0.2)	0 (0)	0 (0)

Appendix 4 (continued).

		Control	Nitrogen	Sucrose
Total Plant Biomass	$g\ m^{-2}$	45 (10)	70 (11)	46 (8)
Litter	$g\ m^{-2}$	30 (10)	47 (3)	35 (4)

Appendix 5. Mean and (standard error) of production values for all species encountered in the fumigation and seed mix study for 2008. Treatments at the first level of the split-block design were fumigated and non-fumigated. Treatments at the second level were addition of early seral seed, an unseeded control, and addition of late seral seed. An undisturbed reference was also included.

		Fumigated			Non-fumigated			Undisturbed Reference
		Early Seral	Control	Late Seral	Early Seral	Control	Late Seral	
Achnatherum hymenoides	$g\ m^{-2}$	0.1 (0.1)	0.4 (0.4)	0.2 (0.2)	3.6 (1.0)	2.8 (1.5)	0.8 (0.2)	1.4 (0.4)
Achnatherum lettermanii	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0 (0)	0.3 (0.3)	0 (0)	0 (0)
Agropyron cristatum	$g\ m^{-2}$	0.6 (0.6)	35.6 (14.2)	0.9 (0.5)	9.1 (4.0)	14.4 (8.6)	6.3 (6.3)	1.4 (1.3)
Alyssum alyssoides	$g\ m^{-2}$	6.5 (2.2)	5.8 (0.3)	2.7 (1.2)	6.0 (1.5)	5.7 (2.3)	3.7 (0.8)	0.8 (0.4)
Artemisia filifolia	$g\ m^{-2}$	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Artemisia frigida	$g\ m^{-2}$	0 (0)	0.1 (0.1)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)
Arabis pendulina	$g\ m^{-2}$	0.2 (0.2)	0 (0)	0.4 (0.4)	0 (0)	0 (0)	0.2 (0.2)	0 (0)
Artemisia tridentata	$g\ m^{-2}$	13.1 (4.3)	7.5 (2.8)	9.1 (5.0)	3.2 (1.1)	8.2 (1.6)	6.8 (3.3)	17.0 (6.4)
Astragalus chamaeleuce	$g\ m^{-2}$	0 (0)	0 (0)	2.2 (2.2)	0.5 (0.5)	0 (0)	0 (0)	0 (0)
Astragalus convallarius	$g\ m^{-2}$	0.1 (0.1)	0 (0)	0 (0)	1.1 (0.9)	0.2 (0.2)	0.1 (0.1)	0.6 (0.2)
Astragalus purshii	$g\ m^{-2}$	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0.2 (0.1)	0 (0)	0.3 (0.1)
Bromus inermis	$g\ m^{-2}$	0 (0)	5.7 (3.4)	0 (0)	0 (0)	1.1 (1.1)	0 (0)	0 (0)
Bromus tectorum	$g\ m^{-2}$	11.9 (5.5)	12.5 (5.0)	4.0 (2.1)	14.2 (6.5)	9.3 (4.6)	6.7 (3.8)	0.7 (0.5)
Castilleja angustifolia	$g\ m^{-2}$	0 (0)	1.0 (0.9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Castilleja linariifolia	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0.3 (0.2)	0 (0)	0 (0)	0 (0)
Chrysothamnus viscidiflorus	$g\ m^{-2}$	12.9 (4.0)	1.2 (0.7)	0.5 (0.4)	16.8 (5.9)	4.1 (3.7)	1.4 (1.4)	0.2 (0.1)
Crepis accuminata	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0.1 (0.1)	0 (0)	0 (0)	0 (0)
Cryptantha flavoculata	$g\ m^{-2}$	0 (0)	0.1 (0.1)	0 (0)	0.5 (0.4)	0 (0)	0.1 (0.1)	1.0 (0.5)

Appendix 5 (continued).

		Fumigated			Non-fumigated			Undisturbed Reference
		Early Seral	Control	Late Seral	Early Seral	Control	Late Seral	
Delphinium nuttallianum	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0.1 (0.1)	0.1 (0.1)	0 (0)	0 (0)
Descurainia pinnata	$g\ m^{-2}$	0 (0)	0.1 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Descurainia sophia	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.1 (0.1)	0 (0)
Elymus elymoides	$g\ m^{-2}$	0 (0)	0.9 (0.4)	0 (0)	0 (0)	0.6 (0.3)	0 (0)	1.2 (0.6)
Elymus glaucus	$g\ m^{-2}$	0 (0)	0 (0)	0.6 (0.6)	0.8 (0.8)	0 (0)	0 (0)	0 (0)
Elymus lanceolatus	$g\ m^{-2}$	2.5 (2.5)	3.0 (3.0)	17.3 (9.5)	0 (0)	1.2 (1.2)	0.3 (0.2)	1.2 (1.0)
Elymus repens	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	1.5 (1.5)	4.6 (2.2)	2.0 (1.9)	0 (0)
Elymus trachycaulus	$g\ m^{-2}$	0 (0)	0.7 (0.5)	11.8 (7.1)	1.4 (1.3)	2.0 (2.0)	3.2 (1.9)	1.2 (1.2)
Erysimum asperum	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.1 (0.1)
Erigeron engelmannii	$g\ m^{-2}$	0.1 (0.1)	0 (0)	0 (0)	0.4 (0.2)	0.2 (0.1)	0 (0)	1.2 (0.3)
Ericameria nauseosa	$g\ m^{-2}$	36.1 (9.8)	14.5 (5.0)	3.2 (2.6)	17.6 (6.0)	19.5 (5.0)	5.5 (2.5)	0 (0)
Festuca brevipila	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0.1 (0.1)	0.8 (0.8)	0 (0)	1 (1)
Grindelia squarrosa	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0.1 (0.1)	0 (0)	0 (0)	0 (0)
Gutierrezia sarothrae	$g\ m^{-2}$	3.9 (1.9)	1.0 (0.5)	0.5 (0.5)	2.4 (1.0)	1.1 (0.8)	0.7 (0.3)	0.1 (0.1)
Hedysarum boreale	$g\ m^{-2}$	0 (0)	1.1 (1.1)	1.7 (1.7)	0 (0)	1.2 (1.2)	0.2 (0.2)	0.2 (0.2)
Hesperostipa comata	$g\ m^{-2}$	11.9 (4.8)	12.3 (5.2)	22.2 (5.1)	8.2 (3.3)	18.0 (3.1)	32.8 (8.0)	3.7 (1.1)
Hordeum jubatum	$g\ m^{-2}$	0.6 (0.5)	0.5 (0.5)	0 (0)	0.9 (0.8)	0.4 (0.4)	0 (0)	0.5 (0.3)
Ipomopsis aggregata	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0 (0)	0.1 (0.1)	0 (0)	0 (0)
Koeleria macrantha	$g\ m^{-2}$	3.7 (2.3)	1.4 (0.9)	1.7 (0.8)	1.3 (1.2)	3.2 (2.3)	2.3 (1.2)	5.2 (1.1)

Appendix 5 (continued).

		Fumigated			Non-fumigated			Undisturbed Reference
		Early Seral	Control	Late Seral	Early Seral	Control	Late Seral	
Lappula occidentalis	$g\ m^{-2}$	0 (0)	0.5 (0.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Lactuca serriola	$g\ m^{-2}$	0 (0)	0.1 (0.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Leymus cinereus	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1.1 (1.1)	0 (0)
Lepidium perfoliatum	$g\ m^{-2}$	0 (0)	0.1 (0.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Linum lewisii	$g\ m^{-2}$	3.3 (2.1)	3.1 (1.8)	3.6 (1.6)	5.5 (3.2)	5.3 (4.1)	3.3 (3.3)	0 (0)
Machaeranthera canescens	$g\ m^{-2}$	0.1 (0.1)	0.6 (0.5)	0.7 (0.7)	0 (0)	1.5 (0.9)	0 (0)	0 (0)
Machaeranthera grindelioides	$g\ m^{-2}$	0 (0)	0 (0)	0.1 (0.1)	0 (0)	0.1 (0.1)	0 (0)	0.2 (0.1)
Melilotus officinalis	$g\ m^{-2}$	1.1 (1)	0.5 (0.4)	0 (0)	0.1 (0.1)	0 (0)	0.1 (0.1)	0 (0)
Opuntia polyacantha	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0 (0)	0.1 (0.1)	0 (0)	0.1 (0.1)
Packera multilobata	$g\ m^{-2}$	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0.2 (0.2)
Pascopyrum smithii	$g\ m^{-2}$	1.6 (1.5)	6.0 (1.7)	18.8 (7.6)	13.1 (8.7)	13.7 (4.7)	29.4 (9.6)	6.9 (1.2)
Penstemon fremontii	$g\ m^{-2}$	0 (0)	0 (0)	0.1 (0.1)	0 (0)	0 (0)	0 (0)	0 (0)
Phlox hoodii	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	3.7 (2.5)
Pinus edulis	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.1 (0.1)
Poa species	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)
Poa fenderliana	$g\ m^{-2}$	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)	1.7 (1.7)
Poa secunda	$g\ m^{-2}$	3.7 (2.5)	1.3 (0.7)	4.4 (1.7)	1.7 (1.0)	2.9 (1.4)	4.7 (2.1)	7.8 (1.9)
Pseudoroegneria spicata	$g\ m^{-2}$	0 (0)	0 (0)	3.8 (3.3)	0 (0)	1.0 (1.0)	2.7 (2.4)	0 (0)
Schoencrambe linifolia	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0 (0)	0.1 (0.1)	0 (0)	0 (0)

Appendix 5 (continued).

		Fumigated			Non-fumigated			Undisturbed Reference
		Early Seral	Control	Late Seral	Early Seral	Control	Late Seral	
Sisymbrium altissimum	*g m*$^{-2}$	0 (0)	2.1 (2.1)	0 (0)	0.1 (0.1)	0 (0)	0 (0)	0 (0)
Sphaeralcea coccinea	*g m*$^{-2}$	0.5 (0.3)	5.2 (3.2)	1.0 (0.7)	3.3 (1.3)	3.8 (0.7)	5.6 (1.0)	0.3 (0.2)
Taraxacum officinale	*g m*$^{-2}$	0.1 (0)	0 (0)	0 (0)	0 (0)	0.1 (0.1)	0 (0)	0 (0)
Thinopyrum intermedium	*g m*$^{-2}$	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)
Tragopogon dubius	*g m*$^{-2}$	0 (0)	0 (0)	0.1 (0.1)	0 (0)	0.4 (0.4)	0.5 (0.5)	0 (0)
Trifolium gymnocarpon	*g m*$^{-2}$	0 (0)	0 (0)	0 (0)	0.5 (0.2)	0.2 (0.1)	0.4 (0.2)	0 (0)
unknown grass 1	*g m*$^{-2}$	0 (0)	0.1 (0.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
unknown grass 2	*g m*$^{-2}$	0 (0)	0 (0)	0.1 (0.1)	0 (0)	0 (0)	0 (0)	0 (0)
unknown grass 4	*g m*$^{-2}$	0 (0)	0 (0)	0 (0)	0.5 (0.5)	0 (0)	0 (0)	0 (0)
Total Plant Biomass	*g m*$^{-2}$	115 (15)	125 (9)	112 (9)	115 (11)	129 (12)	121 (16)	60 (4)
Litter		no data collected						

Appendix 6. Mean and (standard error) of production values for all species encountered in the fumigation and seed mix study for 2009. Treatments at the first level of the split-block design were fumigated and non-fumigated. Treatments at the second level were addition of early seral seed, an unseeded control, and addition of late seral seed. An undisturbed reference was also included.

		Fumigated			Non-fumigated			Undisturbed Reference
		Early Seral	Control	Late Seral	Early Seral	Control	Late Seral	
Achnatherum hymenoides	$g\ m^{-2}$	0.9 (0.6)	1.1 (0.7)	0.6 (0.4)	1.0 (0.7)	0.3 (0.2)	2.0 (1.1)	0.5 (0.3)
Achnatherum lettermanii	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)
Agropyron cristatum	$g\ m^{-2}$	6.5 (3.6)	23.9 (18.2)	3.2 (2.3)	5.0 (3.6)	6.4 (3.9)	2.1 (1.0)	3.8 (2.4)
Agoseris glauca	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0.1 (0.1)	0 (0)	0.1 (0.1)	0 (0)
Alyssum alyssoides	$g\ m^{-2}$	2.3 (1.1)	2.2 (1.1)	0.9 (0.4)	2.8 (0.9)	1.7 (0.4)	2.3 (0.6)	0.1 (0)
Artemisia tridentata	$g\ m^{-2}$	8.1 (2.7)	6.4 (2.9)	4.9 (2.4)	13.6 (3.9)	3.5 (2.1)	6.8 (1.5)	25.4 (5.1)
Astragalus convallarius	$g\ m^{-2}$	0 (0)	1.0 (1.0)	0 (0)	1.0 (0.6)	0.2 (0.2)	0 (0)	0.3 (0.2)
Astragalus purshii	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.6 (0.4)
Bromus inermis	$g\ m^{-2}$	0.2 (0.2)	0 (0)	0.5 (0.5)	0 (0)	0 (0)	0 (0)	0 (0)
Bromus tectorum	$g\ m^{-2}$	3.4 (1.5)	4.6 (3.9)	1.3 (0.6)	2.0 (0.8)	2.0 (1.0)	1.7 (0.9)	0.1 (0.1)
Castilleja angustifolia	$g\ m^{-2}$	0 (0)	6.5 (5.9)	0 (0)	0 (0)	1.6 (1.5)	0 (0)	0 (0)
Chrysothamnus viscidiflorus	$g\ m^{-2}$	3.5 (1.1)	0.2 (0.2)	1.3 (0.8)	6.2 (1.5)	3.3 (2.0)	0.4 (0.3)	0.1 (0.1)
Crepis accuminata	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.1 (0.1)
Cryptantha flavoculata	$g\ m^{-2}$	0.1 (0.1)	0 (0)	0 (0)	0.1 (0.1)	0 (0)	0.8 (0.8)	0.8 (0.4)
Elymus elymoides	$g\ m^{-2}$	0.1 (0.1)	0.1 (0.1)	0 (0)	0 (0)	1.6 (1.6)	0 (0)	0.7 (0.4)
Elymus lanceolatus	$g\ m^{-2}$	0.1 (0.1)	1.8 (1.0)	17 (6.4)	0.3 (0.2)	0.4 (0.2)	10.4 (3.4)	2.6 (0.8)
Elymus repens	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0.1 (0.1)	7.5 (4.2)	0 (0)	0.1 (0.1)
Elymus trachycaulus	$g\ m^{-2}$	0 (0)	0 (0)	1.1 (0.5)	0 (0)	0 (0)	3.9 (2.2)	0.6 (0.5)

Appendix 6 (continued).

		Fumigated			Non-fumigated			Undisturbed Reference
		Early Seral	Control	Late Seral	Early Seral	Control	Late Seral	
Erysimum asperum	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.1 (0.1)	0.2 (0.1)
Erigeron eatonii	$g\ m^{-2}$	0 (0)	0.1 (0.1)	0 (0)	0 (0)	0 (0)	0.1 (0.1)	0.1 (0)
Erigeron engelmannii	$g\ m^{-2}$	0.1 (0.1)	0.1 (0)	0.1 (0.1)	0.2 (0.2)	0 (0)	0.2 (0.2)	0.3 (0.1)
Ericameria nauseosa	$g\ m^{-2}$	12.4 (2.7)	3.5 (0.7)	0.4 (0.3)	7.1 (2.0)	8.6 (2.9)	2.4 (0.9)	0.6 (0.4)
Festuca brevipila	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.1 (0.1)
Gutierrezia sarothrae	$g\ m^{-2}$	1.0 (0.7)	0.4 (0.4)	1.2 (0.7)	1.2 (0.5)	0.9 (0.4)	0.1 (0.1)	0.2 (0.1)
Hedysarum boreale	$g\ m^{-2}$	0 (0)	0 (0)	0.4 (0.4)	0 (0)	0 (0)	0.6 (0.6)	0 (0)
Hesperostipa comata	$g\ m^{-2}$	5.0 (1.8)	2.5 (1.3)	13.5 (5.2)	2.6 (1.2)	8.4 (3.1)	9.5 (3.3)	2.8 (1.3)
Juniperus scopulorum	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.1 (0.1)
Koeleria macrantha	$g\ m^{-2}$	0.4 (0.3)	0.6 (0.4)	0.4 (0.3)	0 (0)	1.9 (0.9)	0.6 (0.6)	2.8 (0.6)
Linum lewisii	$g\ m^{-2}$	1.7 (1.0)	3.2 (1.7)	6.6 (3.7)	3.1 (2.8)	3.2 (2.9)	4.1 (2.7)	0 (0)
Machaeranthera canescens	$g\ m^{-2}$	0 (0)	0 (0)	0.1 (0.1)	0 (0)	0 (0)	0 (0)	0 (0)
Machaeranthera grindelioides	$g\ m^{-2}$	0 (0)	0 (0)	0.7 (0.7)	0 (0)	0 (0)	0.3 (0.3)	0 (0)
Melilotus officinalis	$g\ m^{-2}$	11.2 (6.7)	6.2 (3.6)	0 (0)	0.4 (0.3)	0 (0)	0 (0)	0 (0)
Medicago sativa	$g\ m^{-2}$	0.2 (0.1)	0.5 (0.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Nassella viridula	$g\ m^{-2}$	0 (0)	1.2 (1.1)	0.6 (0.5)	0 (0)	2.7 (1.9)	0.6 (0.3)	0.2 (0.2)
Opuntia polyacantha	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.8 (0.3)
Packera multilobata	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.4 (0.4)
Pascopyrum smithii	$g\ m^{-2}$	0.1 (0.1)	4.4 (2.6)	10.9 (1.9)	4.0 (3.0)	5.8 (2.0)	15.8 (2.8)	1.6 (0.2)

Appendix 6 (continued).

		Fumigated			Non-fumigated			Undisturbed Reference
		Early Seral	Control	Late Seral	Early Seral	Control	Late Seral	
Penstemon fremontii	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)
Phlox hoodii	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0 (0)	0.5 (0.5)	0.1 (0.1)	3.5 (1.9)
Pinus edulis	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.8 (0.8)
Poa fenderliana	$g\ m^{-2}$	5.7 (5.6)	0.4 (0.3)	0.1 (0)	1.7 (1.2)	1.4 (0.5)	0.2 (0.1)	6.5 (3.0)
Poa secunda	$g\ m^{-2}$	0 (0)	0 (0)	0.2 (0.1)	0.2 (0.1)	0.1 (0.1)	0.2 (0.1)	0.4 (0.3)
Pseudoroegneria spicata	$g\ m^{-2}$	0 (0)	3.7 (3.7)	9.3 (5.2)	1.6 (1.6)	5.8 (3.4)	11.5 (3.0)	2.9 (2.7)
Sarcobatus vermiculatus	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)
Sphaeralcea coccinea	$g\ m^{-2}$	1.6 (0.8)	3.8 (3)	1.1 (0.4)	5.4 (2.4)	5.1 (1.8)	2.9 (1.3)	0.6 (0.3)
Taraxacum officinale	$g\ m^{-2}$	0.1 (0.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Thinopyrum intermedium	$g\ m^{-2}$	1.1 (1.1)	0 (0)	0 (0)	0.1 (0.1)	0 (0)	0 (0)	0 (0)
Townsendia incana	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0.1 (0.1)	0 (0)	0 (0)	0 (0)
Tragopogon dubius	$g\ m^{-2}$	0 (0)	0.1 (0.1)	0.2 (0.1)	0 (0)	0.7 (0.6)	0.2 (0.1)	0 (0)
Trifolium gymnocarpon	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0.2 (0.1)	0.5 (0.2)	0.3 (0.1)	0.1 (0.1)
unknown grass 5	$g\ m^{-2}$	1.9 (1.9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
unknown grass 6	$g\ m^{-2}$	1.0 (1.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Verbascum thapsus	$g\ m^{-2}$	0 (0)	0.1 (0.1)	0.1 (0.1)	0 (0)	0 (0)	0 (0)	0 (0)
Zigadenus paniculatus	$g\ m^{-2}$	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.1 (0.1)	0 (0)
Total Plant Biomass	$g\ m^{-2}$	69 (6)	79 (10)	77 (4)	60 (8)	74 (3)	81 (3)	61 (3)

Appendix 6 (continued).

		Fumigated			Non-fumigated			Undisturbed Reference
		Early Seral	Control	Late Seral	Early Seral	Control	Late Seral	
Litter	$g\ m^{-2}$	79 (22)	64 (22)	49 (4)	57 (8)	55 (5)	58 (11)	76 (17)

II. SHRUB DENSITY DATA

Appendix 7. Mean and (standard error) of shrub density values for all species encountered in the nitrogen and phosphorus addition study for data collected in November 2009. Treatments were addition of nitrogen only, nitrogen and phosphorus together, phosphorus only, an unfertilized control, and an undisturbed reference.

		Nitrogen	Nitrogen + Phosphorus	Phosphorus	Control	Reference
Artemisia tridentata	plants m^{-2}	0.11 (0.05)	0.14 (0.08)	0.22 (0.10)	0.22 (0.04)	1.15 (0.33)
Atriplex canescens	plants m^{-2}	0 (0)	0 (0)	0 (0)	0 (0)	0.01 (0.01)
Chrysothamnus viscidiflorus	plants m^{-2}	0.24 (0.14)	0.05 (0.02)	0.44 (0.18)	0.60 (0.16)	0.03 (0.01)
Ericameria nauseosa	plants m^{-2}	0.09 (0.06)	0.05 (0.04)	0.02 (0.01)	0.20 (0.16)	0 (0)
Gutierrezia sarothrae	plants m^{-2}	0.02 (0.02)	0 (0)	0.02 (0.02)	0.02 (0.02)	0 (0)
Sarcobatus vermiculatus	plants m^{-2}	0 (0)	0.01 (0.01)	0 (0)	0 (0)	0 (0)

Appendix 8. Mean and (standard error) of shrub density values for all species encountered in the nitrogen immobilization study for data collected in November 2009. Treatments were addition of nitrogen, an unamended control, and addition of sucrose.

		Nitrogen	Control	Sucrose
Artemisia tridentata	plants m^{-2}	0.09 (0.02)	0.31 (0.11)	0.23 (0.07)
Chrysothamnus viscidiflorus	plants m^{-2}	0.13 (0.04)	0.16 (0.06)	0.27 (0.07)
Ericameria nauseosa	plants m^{-2}	0.02 (0.02)	0.04 (0.02)	0.02 (0.02)
Gutierrezia sarothrae	plants m^{-2}	0 (0)	0.03 (0.02)	0.05 (0.03)

Appendix 9. Mean and (standard error) of shrub density values for all species encountered in the fumigation and seed mix study for data collected in November 2009. Treatments at the first level of the split-block design were fumigated and non-fumigated. Treatments at the second level were addition of early seral seed, an unseeded control, and addition of late seral seed. An undisturbed reference was also included.

		Fumigated			Non-fumigated			Undisturbed Reference
		Early Seral	Control	Late Seral	Early Seral	Control	Late Seral	
Artemisia tridentata	plants m^{-2}	0.38 (0.14)	0.25 (0.05)	0.31 (0.09)	0.24 (0.1)	0.22 (0.04)	0.30 (0.03)	1.15 (0.33)
Atriplex canescens	plants m^{-2}	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.01 (0.01)
Chrysothamnus viscidiflorus	plants m^{-2}	0.47 (0.13)	0.20 (0.07)	0.13 (0.07)	0.58 (0.27)	0.60 (0.16)	0.15 (0.10)	0.03 (0.01)
Ericameria nauseosa	plants m^{-2}	0.08 (0.06)	0.05 (0.03)	0 (0)	0.16 (0.06)	0.20 (0.16)	0.03 (0.02)	0 (0)
Gutierrezia sarothrae	plants m^{-2}	0.20 (0.12)	0.01 (0.01)	0.05 (0.05)	0.02 (0.02)	0.02 (0.02)	0.03 (0.01)	0 (0)
Kraschenninikovia lanata	plants m^{-2}	0.01 (0.01)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

III. SOIL NUTRIENT DATA

Appendix 10. Mean and (standard error) of soil parameters in the nitrogen and phosphorus addition study for data collected in May 2009. Treatments were addition of nitrogen only, nitrogen and phosphorus together, phosphorus only, an unfertilized control, and an undisturbed reference.

		Nitrogen	Nitrogen + Phosphorus	Phosphorus	Control	Reference
Organic Matter	%	2.7 (0.1)	2.5 (0.1)	2.4 (0.3)	2.0 (0.1)	2.2 (0.1)
Total Nitrogen	%	0.12 (0.01)	0.12 (0.01)	0.10 (0.01)	0.10 (0.01)	0.13 (0.02)
Nitrate-N	mg kg^{-1}	4.0 (0.4)	4.5 (0.5)	2.5 (0.5)	2.5 (0.3)	2.5 (0.3)
Ammonium-N	mg kg^{-1}	2.6 (0.5)	3.6 (1)	1.0 (0)	1.7 (0.4)	2.2 (0.5)
Potential Net Nitrogen Mineralization	mg kg^{-1}	1.08 (0.18)	1.11 (0.14)	0.45 (0.08)	0.46 (0.05)	0.46 (0.04)
Phosphorus	mg kg^{-1}	6.3 (1.0)	111.8 (47.2)	35.0 (10.4)	5.0 (0.7)	6.5 (0.9)
Potassium	mg kg^{-1}	152 (15)	123 (24)	116 (5)	132 (10)	129 (7)
Calcium	mg kg^{-1}	4240 (210)	3760 (500)	4230 (250)	4030 (480)	3820 (330)
Magnesium	mg kg^{-1}	274 (38)	279 (54)	278 (36)	251 (21)	233 (18)
Sodium	mg kg^{-1}	18.5 (3.9)	15.5 (2.2)	19.8 (3.2)	12.8 (0.6)	15.5 (0.6)
Sulfur	mg kg^{-1}	11.3 (1.9)	8.5 (1.5)	8.8 (0.9)	6.0 (1.8)	7.0 (0.6)
Zinc	mg kg^{-1}	0.48 (0.14)	0.38 (0.03)	0.45 (0.12)	0.15 (0.03)	0.25 (0.03)
pH		7.9 (0.1)	7.7 (0.2)	8.0 (0.1)	8.0 (0.1)	8.0 (0.1)
CEC		24.0 (0.8)	21.5 (2.4)	23.9 (1.5)	22.7 (2.3)	21.4 (1.5)

Appendix 11. Mean and (standard error) of soil parameters in the nitrogen immobilization study for data collected in May 2009. Treatments were addition of nitrogen, an unamended control, and addition of sucrose.

		Nitrogen	Control	Sucrose
Organic Matter	%	2.5 (0.1)	2.1 (0.1)	2.3 (0.2)
Total Nitrogen	%	0.12 (0.01)	0.1 (0.01)	0.11 (0)
Nitrate-N	mg kg^{-1}	3.0 (0.7)	3 (0.7)	2.8 (0.5)
Ammonium-N	mg kg^{-1}	2.5 (0.5)	1.5 (0.2)	1.3 (0.1)
Potential Net Nitrogen Mineralization	mg kg^{-1}	0.93 (0.17)	0.34 (0.06)	0.54 (0.06)
Phosphorus	mg kg^{-1}	6.3 (1.3)	5.0 (0.4)	6.0 (0.4)
Potassium	mg kg^{-1}	121 (14)	138 (27)	129 (18)
Calcium	mg kg^{-1}	317 (59)	260 (37)	314 (69)
Magnesium	mg kg^{-1}	3510 (390)	4300 (300)	3490 (470)
Sodium	mg kg^{-1}	6.3 (1.5)	11.0 (2.0)	9.3 (2.3)
Sulfur	mg kg^{-1}	0.33 (0.19)	0.40 (0.18)	0.35 (0.16)
Zinc	mg kg^{-1}	16.8 (1.4)	29.3 (5.2)	17.5 (2.5)
pH		7.9 (0.2)	8.2 (0.1)	7.9 (0.2)
CEC		20.6 (1.6)	24.2 (1.6)	20.5 (2.1)

Appendix 12. Mean and (standard error) of soil parameters in the fumigation and seed mix study for data collected in May 2009. Treatments at the first level of the split-block design were fumigated and non-fumigated. Treatments at the second level were addition of early seral seed, an unseeded control, and addition of late seral seed. An undisturbed reference was also included.

		Fumigated			Non-fumigated			Undisturbed Reference
		Early Seral	Control	Late Seral	Early Seral	Control	Late Seral	
Organic Matter	%	1.9 (0.1)	2.0 (0.1)	2.1 (0.1)	2.2 (0.1)	2 (0.1)	1.9 (0.1)	2.2 (0.1)
Nitrate-N	mg kg^{-1}	2.3 (0.3)	2.8 (0.3)	2.0 (0)	2.8 (0.5)	2.5 (0.3)	3.3 (0.5)	2.5 (0.3)
Ammonium-N	mg kg^{-1}	1.4 (0.3)	1.3 (0.2)	1.2 (0.2)	1 (0.1)	1.7 (0.4)	2.6 (1.5)	2.2 (0.5)
Total Nitrogen	%	0.10 (0)	0.10 (0.01)	0.09 (0.01)	0.10 (0.01)	0.1 (0.01)	0.09 (0)	0.13 (0.02)
Potential Net Nitrogen Mineralization	mg kg^{-1}	0.25 (0.1)	0.36 (0.04)	0.45 (0.13)	0.44 (0.10)	0.46 (0.05)	0.37 (0.02)	0.46 (0.04)
Phosphorus	mg kg^{-1}	5.5 (0.6)	4.3 (0.5)	4.5 (0.5)	5.0 (0.4)	5.0 (0.7)	4.8 (0.6)	6.5 (0.9)
Potassium	mg kg^{-1}	1267 (17)	142 (21)	112 (14)	137 (11)	132 (10)	127 (9)	129 (7)
Calcium	mg kg^{-1}	253 (26)	333 (61)	243 (51)	251 (31)	251 (21)	255 (9)	233 (18)
Magnesium	mg kg^{-1}	4030 (400)	4090 (390)	4300 (240)	3970 (340)	4030 (480)	3740 (500)	3820 (330)
Sodium	mg kg^{-1}	7.0 (1.3)	7.5 (1.3)	8.8 (1.3)	8.5 (0.9)	6.0 (1.8)	6.5 (1.9)	7.0 (0.6)
Sulfur	mg kg^{-1}	0.20 (0.04)	0.23 (0.03)	0.13 (0.03)	0.18 (0.05)	0.15 (0.03)	0.23 (0.09)	0.25 (0.03)
Zinc	mg kg^{-1}	20.3 (4.6)	20.8 (5.1)	23.3 (6.9)	15.8 (1.5)	12.8 (0.6)	12.5 (0.6)	15.5 (0.6)
pH		8.2 (0.1)	8.1 (0.1)	8.2 (0.1)	8.2 (0.1)	8.0 (0.1)	7.9 (0.2)	8.0 (0.1)
CEC		22.7 (1.7)	23.7 (2.1)	23.9 (1.2)	22.4 (1.7)	22.7 (2.3)	21.2 (2.4)	21.4 (1.5)

IV. PLANT TISSUE NUTRIENT DATA

Appendix 13. Mean and (standard error) of plant tissue analysis (total carbon, total nitrogen, and total phosphorus) in the nitrogen and phosphorus addition study for plant species collected in July 2009 Plant species were *Artemisia tridentata* Nutt., *Bromus tectorum* L., *Pascopyrum smithii* Rydb. A. Löve, and *Sphaeralcea coccinea* Nutt. Rydb. Treatments were addition of nitrogen only, nitrogen and phosphorus together, phosphorus only, an unfertilized control, and an undisturbed reference.

Analysis	Plant species	Nitrogen	Nitrogen + Phosphorus	Phosphorus	Control	Reference
Carbon (%)	*A. tridentata*	49 (0)	48 (0)	49 (0)	49 (0)	49 (0)
	B. tectorum	44 (0)	43 (1)	42 (1)	43 (0)	42 (1)
	P. smithii	44 (0)	44 (0)	44 (0)	43 (0)	43 (0)
	S. coccinea	42 (1)	42 (0)	40 (1)	52 (10)	40 (1)
Nitrogen (%)	*A. tridentata*	2.6 (0.1)	2.5 (0.1)	2.1 (0.1)	2.0 (0.1)	1.6 (0)
	B. tectorum	0.6 (0.2)	0.4 (0.2)	1.0 (0.5)	0.7 (0.2)	0.5 (0.1)
	P. smithii	1.3 (0.2)	1.3 (0.3)	0.9 (0.2)	0.9 (0.3)	1.0 (0)
	S. coccinea	2.2 (0)	2.1 (0.1)	1.7 (0.1)	2.1 (0.3)	1.5 (0)
Phosphorus ($mg\ kg^{-1}$)	*A. tridentata*	4810 (320)	5890 (220)	5360 (240)	4820 (380)	4750 (120)
	B. tectorum	1260 (130)	1130 (70)	1930 (30)	1670 (100)	1210 (70)
	P. smithii	1330 (190)	2050 (170)	1520 (120)	1270 (220)	1370 (20)
	S. coccinea	2100 (130)	2790 (450)	3030 (340)	2110 (430)	1670 (50)

Appendix 14. Mean and (standard error) of plant tissue analysis (total carbon, total nitrogen, and total phosphorus) in the nitrogen immobilization study for plant species collected in July 2009 Plant species were *Artemisia tridentata* Nutt., *Bromus tectorum* L., *Pascopyrum smithii* Rydb. A. Löve, and *Sphaeralcea coccinea* Nutt. Rydb. Treatments were addition of nitrogen, an unamended control, and addition of sucrose.

Analysis	Plant Species	Nitrogen	Control	Sucrose
Carbon (%)	*A. tridentata*	49 (0)	49 (0)	49 (0)
	B. tectorum	43 (0)	41 (1)	42 (1)
	P. smithii	44 (0)	44 (0)	43 (0)
	S. coccinea	43 (0)	41 (0)	42 (0)
Nitrogen (%)	*A. tridentata*	2.4 (0.1)	2.0 (0.1)	2.0 (0.1)
	B. tectorum	0.7 (0.2)	0.7 (0.2)	0.8 (0.2)
	P. smithii	1.4 (0.2)	1.0 (0.1)	1.0 (0.1)
	S. coccinea	2.4 (0.1)	1.6 (0.1)	1.7 (0.1)
Phosphorus ($mg\ kg^{-1}$)	*A. tridentata*	4690 (510)	4890 (410)	4510 (400)
	B. tectorum	1130 (160)	1690 (60)	1570 (250)
	P. smithii	1270 (160)	1300 (170)	1360 (180)
	S. coccinea	1700 (120)	1740 (150)	1990 (240)

Appendix 15. Mean and (standard error) of plant tissue analysis (total carbon, total nitrogen, and total phosphorus) in the fumigation and seed mix study for plant species collected in July 2009. Plant species were *Artemisia tridentata* Nutt., *Bromus tectorum* L., *Pascopyrum smithii* Rydb. A. Löve, and *Sphaeralcea coccinea* Nutt. Rydb. Treatments at the first level of the split-block design were fumigated and non-fumigated. Treatments at the second level were addition of early seral seed, an unseeded control, and addition of late seral seed. An undisturbed reference was also included.

		Fumigated			Non-fumigated			Undisturbed Reference
		Early Seral	Control	Late Seral	Early Seral	Control	Late Seral	
Carbon (%)	*A. tridentata*	49 (0)	49 (0)	48 (0)	49 (0)	49 (0)	49 (0)	49 (0)
	B. tectorum	43 (1)	42 (0)	43 (1)	43 (0)	43 (0)	43 (0)	42 (1)
	P. smithii	43 (0)	43 (0)	43 (0)	43 (0)	43 (0)	43 (0)	43 (0)
	S. coccinea	41 (0)	41 (1)	42 (0)	42 (0)	52 (10)	41 (0)	40 (1)
Nitrogen (%)	*A. tridentata*	2.0 (0.1)	1.9 (0.1)	2.0 (0)	1.9 (0.1)	2.0 (0.1)	1.9 (0.1)	1.6 (0)
	B. tectorum	0.8 (0.6)	0.5 (0.2)	0.6 (0.1)	0.6 (0.1)	0.7 (0.2)	0.8 (0.2)	0.5 (0.1)
	P. smithii	1.3 (0.1)	1.0 (0.1)	1.0 (0.1)	0.9 (0.2)	0.9 (0.3)	0.8 (0.2)	1.0 (0)
	S. coccinea	1.5 (0.1)	1.6 (0.1)	1.7 (0.1)	1.7 (0.1)	2.1 (0.3)	1.6 (0)	1.5 (0)
Phosphorus ($mg\ kg^{-1}$)	*A. tridentata*	5010 (400)	5000 (550)	5210 (400)	4550 (340)	4820 (380)	4940 (180)	4750 (120)
	B. tectorum	1490 (190)	1500 (100)	1390 (110)	1450 (140)	1670 (100)	1420 (150)	1210 (70)
	P. smithii	1670 (370)	1180 (190)	1170 (150)	1150 (150)	1270 (220)	1350 (140)	1370 (20)
	S. coccinea	1660 (180)	1820 (150)	1720 (80)	1820 (60)	2110 (430)	1820 (160)	1670 (50)

V. SOIL MICROBIAL DATA

Appendix 16. Mean and (standard error) of microbial parameters in the nitrogen and phosphorus addition study for soil collected in May 2009. Analyses were microbial biomass (fumigation extraction method without subtracting non-fumigated control), mycorrhizal infection by structure (mycorrhizal inoculum potential method), and fatty acid analysis (ester linked-fatty acid methyl ester method). Treatments were addition of nitrogen only, nitrogen and phosphorus together, phosphorus only, an unfertilized control, and an undisturbed reference.

		Nitrogen	Nitrogen + Phosphorus	Phosphorus	Control	Reference
Microbial Biomass Carbon	µg g^{-1}	1100 (80)	1250 (330)	950 (130)	885 (180)	1060 (110)
Microbial Biomass Nitrogen	µg g^{-1}	140 (7)	145 (11)	109 (15)	102 (19)	113 (9)
Arbuscular Infection	%	0.27 (0.18)	0.03 (0.02)	0.04 (0.04)	0.02 (0.01)	0.10 (0.10)
Hyphal Infection	%	3.74 (0.70)	2.41 (0.63)	2.72 (0.39)	1.96 (0.23)	1.77 (0.33)
Vesicle Infection	%	0.27 (0.09)	0.02 (0.01)	0.08 (0.03)	0.02 (0)	0.08 (0.01)
12:0	nmol g^{-1} dry soil	0 (0)	0 (0)	2 (2)	0 (0)	0 (0)
14:0	nmol g^{-1} dry soil	7 (2)	10 (3)	10 (3)	6 (1)	7 (1)
15:0 3OH	nmol g^{-1} dry soil	4 (3)	1 (1)	0 (0)	0 (0)	4 (2)
15:0 ANTEISO	nmol g^{-1} dry soil	8 (2)	13 (4)	16 (5)	7 (2)	5 (1)
15:0 ISO	nmol g^{-1} dry soil	9 (2)	15 (5)	16 (5)	9 (3)	7 (1)
16:0	nmol g^{-1} dry soil	37 (10)	57 (18)	65 (21)	35 (11)	28 (4)
16:0 ISO	nmol g^{-1} dry soil	9 (2)	14 (5)	15 (5)	8 (3)	6 (1)
16:1 2OH	nmol g^{-1} dry soil	6 (2)	5 (2)	5 (2)	5 (3)	3 (0)
16:1 ISO H	nmol g^{-1} dry soil	0 (0)	0 (0)	3 (3)	0 (0)	0 (0)

Appendix 16 (continued).

		Nitrogen	Nitrogen + Phosphorus	Phosphorus	Control	Reference
16:1 ω5c	nmol g^{-1} dry soil	22 (6)	18 (7)	12 (4)	18 (7)	16 (3)
16:1 ω7c	nmol g^{-1} dry soil	22 (5)	26 (8)	25 (9)	20 (7)	14 (1)
17:0 ANTEISO	nmol g^{-1} dry soil	2 (2)	6 (2)	7 (2)	3 (2)	3 (0)
17:0 CYCLO	nmol g^{-1} dry soil	0 (0)	2 (2)	5 (2)	0 (0)	1 (1)
17:0 ISO	nmol g^{-1} dry soil	0 (0)	1 (1)	4 (2)	1 (1)	1 (1)
10Me 17:0	nmol g^{-1} dry soil	0 (0)	5 (2)	4 (2)	0 (0)	1 (1)
ISO 17:1 G	nmol g^{-1} dry soil	14 (4)	16 (6)	15 (5)	10 (3)	9 (1)
17:1 ω8c	nmol g^{-1} dry soil	7 (2)	9 (3)	10 (3)	7 (2)	5 (1)
18:0	nmol g^{-1} dry soil	7 (2)	9 (3)	10 (4)	6 (2)	5 (1)
18:0 2OH	nmol g^{-1} dry soil	6 (1)	8 (2)	9 (3)	3 (1)	7 (1)
10Me 18:0	nmol g^{-1} dry soil	0 (0)	6 (3)	6 (3)	1 (1)	3 (1)
18:1 ω9c	nmol g^{-1} dry soil	42 (12)	48 (15)	47 (16)	36 (13)	26 (2)
18:2 ω6c	nmol g^{-1} dry soil	15 (5)	22 (7)	22 (7)	16 (7)	12 (2)
19:0 CYCLO c11-12	nmol g^{-1} dry soil	0 (0)	5 (2)	5 (2)	1 (1)	1 (1)
Sum In Feature 8	nmol g^{-1} dry soil	17 (5)	22 (8)	22 (8)	16 (6)	11 (1)

Appendix 17. Mean and (standard error) of microbial parameters in the nitrogen immobilization study for soil collected in May 2009. Analyses were microbial biomass (fumigation extraction method without subtracting non-fumigated control), mycorrhizal infection by structure (mycorrhizal inoculum potential method), and fatty acid analysis (ester linked-fatty acid methyl ester method). Treatments were addition of nitrogen, an unamended control, and addition of sucrose.

		Nitrogen	Control	Sucrose
Microbial Biomass Carbon	µg g^{-1}	1140 (340)	903 (130)	911 (130)
Microbial Biomass Nitrogen	µg g^{-1}	123 (19)	107 (13)	110 (16)
Arbuscular Infection	%	0.02 (0.02)	0.01 (0.01)	0.03 (0.03)
Hyphal Infection	%	1.27 (0.17)	1.44 (0.26)	3.28 (0.49)
Vesicular Infection	%	0.05 (0.02)	0.07 (0.02)	0.11 (0.05)
14:0	nmol g^{-1} dry soil	8 (3)	5 (2)	9 (3)
15:0 3OH	nmol g^{-1} dry soil	0 (0)	4 (3)	6 (3)
15:0 ANTEISO	nmol g^{-1} dry soil	13 (5)	8 (3)	18 (7)
15:0 ISO	nmol g^{-1} dry soil	14 (6)	10 (3)	17 (6)
15:1 ISO G	nmol g^{-1} dry soil	0 (0)	0 (0)	3 (2)
16:0	nmol g^{-1} dry soil	52 (19)	38 (11)	68 (24)
16:0 ISO	nmol g^{-1} dry soil	13 (5)	10 (3)	18 (7)
16:1 2OH	nmol g^{-1} dry soil	6 (4)	4 (3)	7 (4)
16:1 ISO G	nmol g^{-1} dry soil	3 (3)	0 (0)	3 (3)
16:1 ISO H	nmol g^{-1} dry soil	0 (0)	0 (0)	3 (3)
16:1 ω5c	nmol g-1 dry soil	16 (6)	26 (9)	24 (9)
16:1 ω7c	nmol g-1 dry soil	23 (8)	26 (8)	38 (14)

Appendix 17 (continued).

		Nitrogen	Control	Sucrose
17:0 ANTEISO	nmol g^{-1} dry soil	5 (2)	1 (1)	8 (3)
17:0 CYCLO	nmol g^{-1} dry soil	2 (2)	0 (0)	0 (0)
17:0 ISO	nmol g^{-1} dry soil	2 (2)	0 (0)	4 (2)
10Me 17:0	nmol g^{-1} dry soil	5 (2)	0 (0)	4 (2)
ISO 17:1 G	nmol g^{-1} dry soil	14 (5)	14 (4)	20 (8)
17:1 ω8c	nmol g^{-1} dry soil	8 (3)	8 (3)	12 (4)
18:0	nmol g^{-1} dry soil	8 (3)	6 (2)	11 (4)
18:0 2OH	nmol g^{-1} dry soil	8 (3)	4 (2)	7 (3)
10Me 18:0	nmol g^{-1} dry soil	6 (3)	1 (1)	5 (3)
18:1 ω9c	nmol g^{-1} dry soil	42 (16)	45 (14)	119 (38)
18:2 ω6c	nmol g^{-1} dry soil	21 (8)	19 (6)	64 (22)
19:0 CYCLO c11-12	nmol g^{-1} dry soil	5 (3)	0 (0)	2 (2)
Sum In Feature 4	nmol g^{-1} dry soil	0 (0)	1 (1)	0 (0)
Sum In Feature 8	nmol g^{-1} dry soil	19 (7)	19 (6)	23 (8)

Appendix 18. Mean and (standard error) of microbial parameters in the fumigation and seed mix study for soil collected in May 2009. Analyses were microbial biomass (fumigation extraction method without subtracting non-fumigated control), mycorrhizal infection by structure (mycorrhizal inoculum potential method), and fatty acid analysis (ester linked-fatty acid methyl ester method). Treatments at the second level were addition of early seral seed, an unseeded control, and addition of late seral seed. An undisturbed reference was also included.

		Fumigated			Non-fumigated			Undisturbed Reference
		Early Seral	Control	Late Seral	Early Seral	Control	Late Seral	
Microbial Biomass Carbon	µg g^{-1}	730 (80)	710 (80)	840 (100)	1270 (370)	890 (180)	700 (100)	1060 (110)
Microbial Biomass Nitrogen	µg g^{-1}	84 (9)	87 (10)	99 (13)	126 (18)	102 (19)	94 (13)	113 (9)
Arbuscular Infection	%	0.01 (0)	0.02 (0.01)	0 (0)	0.02 (0.01)	0.02 (0.01)	0 (0)	0.1 (0.1)
Hyphal Infection	%	2.19 (0.67)	3.07 (1.12)	2.38 (0.58)	1.96 (0.23)	1.96 (0.23)	2.37 (0.54)	1.77 (0.33)
Vesicular Infection	%	0.20 (0.09)	0.43 (0.13)	0.31 (0.04)	0.02 (0)	0.02 (0)	0.46 (0.19)	0.08 (0.01)
12:0	nmol g^{-1} dry soil	0 (0)	0 (0)	3 (3)	0 (0)	0 (0)	0 (0)	0 (0)
14:0	nmol g^{-1} dry soil	6 (2)	6 (2)	34 (26)	7 (1)	7 (2)	8 (3)	7 (1)
15:0 3OH	nmol g^{-1} dry soil	1 (1)	4 (2)	1 (1)	0 (0)	4 (3)	5 (3)	4 (2)
15:0 ANTEISO	nmol g^{-1} dry soil	6 (2)	8 (2)	10 (2)	9 (2)	8 (2)	8 (3)	5 (1)
15:0 ISO	nmol g^{-1} dry soil	8 (3)	10 (3)	13 (2)	11 (2)	9 (2)	10 (3)	7 (1)
16:0	nmol g^{-1} dry soil	30 (10)	33 (10)	52 (10)	43 (10)	37 (10)	42 (15)	28 (4)
16:0 ISO	nmol g^{-1} dry soil	7 (2)	9 (3)	11 (2)	11 (3)	9 (2)	9 (3)	6 (1)
16:1 2OH	nmol g^{-1} dry soil	2 (1)	6 (2)	5 (2)	6 (1)	6 (2)	6 (2)	3 (0)
16:1 ω5c	nmol g-1 dry soil	20 (7)	20 (6)	33 (9)	21 (5)	22 (6)	21 (8)	16 (3)
16:1 ω7c	nmol g-1 dry soil	17 (5)	21 (7)	38 (9)	25 (6)	22 (5)	27 (10)	14 (1)

Appendix 18 (continued).

		Fumigated			Non-fumigated			Undisturbed Reference
		Early Seral	Control	Late Seral	Early Seral	Control	Late Seral	
17:0 ANTEISO	nmol g^{-1} dry soil	1 (1)	2 (1)	4 (2)	1 (1)	2 (2)	2 (2)	3 (0)
17:0 CYCLO	nmol g^{-1} dry soil	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)
17:0 ISO	nmol g^{-1} dry soil	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)
10Me 17:0	nmol g^{-1} dry soil	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)
ISO 17:1 G	nmol g^{-1} dry soil	10 (3)	12 (3)	16 (3)	16 (4)	14 (4)	14 (5)	9 (1)
17:1 ω8c	nmol g^{-1} dry soil	7 (2)	8 (2)	10 (2)	9 (2)	7 (2)	7 (3)	5 (1)
18:0	nmol g^{-1} dry soil	5 (2)	5 (2)	12 (4)	8 (2)	7 (2)	8 (3)	5 (1)
18:0 2OH	nmol g^{-1} dry soil	5 (2)	2 (2)	6 (3)	7 (1)	6 (1)	3 (2)	7 (1)
10Me 18:0	nmol g^{-1} dry soil	1 (1)	0 (0)	3 (2)	0 (0)	0 (0)	2 (2)	3 (1)
18:1 ω9c	nmol g^{-1} dry soil	31 (9)	37 (12)	77 (18)	47 (12)	42 (12)	61 (27)	26 (2)
18:2 ω6c	nmol g^{-1} dry soil	14 (4)	14 (5)	27 (5)	21 (5)	15 (5)	22 (9)	12 (2)
19:0CYCLO c11-12	nmol g^{-1} dry soil	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (3)	1 (1)
Sum In Feature 4	nmol g^{-1} dry soil	0 (0)	0 (0)	8 (8)	21 (11)	0 (0)	4 (4)	0 (0)
Sum In Feature 8	nmol g^{-1} dry soil	15 (5)	17 (5)	23 (4)	21 (6)	17 (5)	19 (7)	11 (1)

Appendix 19. List of all microbial fatty acids found in soils collected in May 2009 using the ester linked-fatty acid methyl ester analysis linked by microbial indicator with citation.

Fatty Acid Marker	Microbial Indicator	Citations
10Me 17:0	Bacteria (Actinomycetes)	Drenovsky et al., 2004
10Me 18:0	Bacteria (Actinomycetes)	Drenovsky et al., 2004
16:1 ω7c	Bacteria (Gram Negative)	Frostegard & Baath, 1996; Zelles, 1997
17:0 CYCLO	Bacteria (Gram Negative)	Frostegard & Baath, 1996; Zelles, 1997
19:0 CYCLO c11-12	Bacteria (Gram Negative)	Frostegard & Baath, 1996; Zelles, 1997
15:0 ANTEISO	Bacteria (Gram Positive)	Frostegard & Baath, 1996; Zelles, 1997
15:0 ISO	Bacteria (Gram Positive)	Frostegard & Baath, 1996; Zelles, 1997
16:0 ISO	Bacteria (Gram Positive)	Frostegard & Baath, 1996; Zelles, 1997
17:0 ANTEISO	Bacteria (Gram Positive)	Frostegard & Baath, 1996; Zelles, 1997
17:0 ISO	Bacteria (Gram Positive)	Frostegard & Baath, 1996; Zelles, 1997
16:1 ω5c	Fungi (Arbuscular Mycorrhizal)	Olsson, 1999
18:1 ω9c	Fungi (Saprophytic)	Frostegard & Baath, 1996; Stahl & Klug, 1996; Zelles, 1997
18:2 ω6c	Fungi (Saprophytic)	Frostegard & Baath, 1996; Stahl & Klug, 1996; Zelles, 1997
16:0	Ubiquitous	Denef et al., 2009
12:0	Unclassified	
14:0	Unclassified	
15:0 3OH	Unclassified	
16:0 ISO G	Unclassified	
16:0 ISO H	Unclassified	
16:1 2OH	Unclassified	

Appendix 19 (continued).

Fatty Acid Marker	Microbial Indicator	Citation
17:1 ω8c	Unclassified	
ISO 17:1 G	Unclassified	
18:0	Unclassified	
18:0 2OH	Unclassified	
Sum In Feature 4	Unclassified	
Sum In Feature 8	Unclassified	

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