AN ABSTRACT OF THE THESIS OF

<u>Caitlin E. Lawrence</u> for the degree of <u>Master of Science</u> in <u>Botany and Plant Pathology</u> presented on <u>February 18, 2015</u>

Title: <u>Responses of Pacific Northwest prairies to soil nutrient manipulations: Implications for restoration of *Castilleja levisecta* and control of invasive species</u>

Abstract approved: _____

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Pacific Northwest prairies have become significantly reduced in extent, and in the Willamette Valley of Oregon, less than one percent of native upland prairies remain. Many species have been impacted by this extreme loss of habitat, including *Castilleja levisecta* (golden paintbrush), a threatened hemiparasitic forb species endemic to the Pacific Northwest. Many of the prairie fragments that remain are poor quality, and face the threat of invasion from non-native species and a loss of biodiversity. These non-native species pose an obstacle to restoration and to the reintroduction of threatened and endangered species. Nutrient enrichment has been shown to promote increased invasion of communities, decrease success of native species, and decrease biodiversity. A way to counter these effects is through carbon addition to the soil which stimulates microbial activity and immobilizes nutrients, making them unavailable to plants. This strategy has potential as a restoration tool to improve conditions for native species, which are often outcompeted under high nutrient conditions.

We tested the efficacy of carbon addition for controlling non-native species and restoring *C. levisecta* in two Willamette Valley prairies. We created treatments of varying nutrient availability and measured the responses of the plant community and of *C. levisecta*.

Experimental plots were established in autumn 2012 with either carbon (sucrose) addition, ambient soil nutrients (controls), or nutrient addition (NPK fertilizer). Nutrient treatments were reapplied throughout the length of the experiment. Crossed with these treatments in a fully factorial design were seeding treatments of *C. levisecta* and of thirteen other native species, to increase the diversity of the plant community, providing host plants for *C. levisecta*, a hemiparasitic plant. In the two growing seasons following treatment we conducted plant community surveys and counted and measured seedlings of *C. levisecta* to determine the effects of the nutrient manipulations and seeding treatments on the community overall and on this threatened plant.

Community composition differed significantly among the different nutrient treatments (perMANOVA, p<0.001) at both study sites, and in both years of the experiment. Mean total vascular plant cover was significantly reduced by carbon addition compared to ambient nutrient availability, and increased by nutrient addition. Native grasses were unaffected by nutrient manipulation at either site, whereas non-native grasses were greatly reduced by carbon addition and increased by nutrient addition, compared to ambient soil nutrient availability. Non-native forbs were also greatly reduced by carbon addition, and were also reduced by nutrient addition by the second year of the experiment. Richness of non-native species was more affected by nutrient manipulation than richness of natives, and was either increased or unaffected by nutrient enrichment and decreased by carbon addition. Some non-native species were found to be highly affected by the nutrient manipulation, while others were not, suggesting that carbon addition may be an effective control for only certain non-native species.

At both sites, *C. levisecta* emergence was lower in carbon addition plots compared with controls. In nutrient addition plots, *C. levisecta* emergence was higher at one site but lower at the

other compared with controls, suggesting that nutrient addition may be beneficial only under certain conditions. Additionally, by the second year of the experiment, there was no difference in *C. levisecta* numbers between the control and nutrient addition plots at one site, and there were fewer in the nutrient addition plots than in controls at the other site. The seeding of additional native species increased community richness but had little effect on diversity and no effect on *C. levisecta* establishment.

Overall, these results suggest that nutrient enrichment can promote the success of non-native species and that carbon addition can be used to counter these effects on a species- and site-specific basis. However, carbon addition did not improve reintroduction success of a threatened plant species, *C. levisecta*, and the effects of nutrient addition on its success after two years were mixed.

Responses of Pacific Northwest prairies to soil nutrient manipulations: Implications for restoration of *Castilleja levisecta* and control of invasive species

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

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Caitlin E. Lawrence, Author

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Chapter 1:

Introduction

Pacific Northwest Prairies

In the U.S. Pacific Northwest (PNW), prairies have become significantly reduced in extent since European settlement, and are now considered to be an endangered ecosystem. In the Willamette Valley of Oregon, less than one percent of native upland prairies remain (Noss 1995). In addition to being greatly reduced in extent, many of the prairie fragments that remain have become highly degraded and suffer from invasion by exotic species (Chappell & Crawford 1997; Chappell et al. 2000; MacDougall et al. 2004). Reducing cover of these exotic species, therefore, is a high priority in restoration. In addition, many prairie species have become threatened as a result of this loss and degradation of native habitat (Chappell et al. 2000, Dunwiddie & Bakker 2011).

Study Species: *Castilleja levisecta*

The extreme loss of prairie habitat in the PNW has greatly impacted many native species, including *Castilleja levisecta* (golden paintbrush), a threatened hemiparasitic forb species endemic to the PNW. *Castilleja levisecta* has become completely extirpated from Oregon, and has only eleven remaining natural populations in Washington and British Columbia, Canada (USFWS 2000). Within these populations, however, there are still exceptionally high levels of genetic diversity (Godt et al. 2005), which provides a high potential for recovery of *C. levisecta,* if appropriate restoration can increase the number of viable populations of the species (USFWS 2000).

A challenge for the reintroduction of *C. levisecta* is that it is a hemiparasite. Although it does not require a host for survival, it often benefits from the presence of hosts that provide water, nutrients and secondary compounds through their roots (Lawrence & Kaye 2008). This

hemiparasitic characteristic may result in a strong relationship between the biodiversity of the community and reintroduction success of *C. levisecta.* It has been demonstrated experimentally that root hemiparasites can benefit from a high functional diversity of hosts (Joshi et al. 2000). Previous studies on *C. levisecta* have looked at some host preferences, as well as other habitat characteristics contributing to successful reintroductions (Lawrence and Kaye 2008; Lawrence and Kaye 2009) but not at biodiversity of the plant community, which could be another key component to increasing the success of reintroductions.

Since *C. levisecta* is a hemiparasitic plant, its reintroduction has the potential to alter a community by impacting the species that it parasitizes. Hemiparasitic plants can be keystone species, due to their profound effects on ecosystems (Press & Phoenix 2005). They can affect the productivity and diversity of their community (Bardgett et al. 2006). Since *Castilleja* species are usually generalist parasites (Heckard 1962; Dobbins and Kuijt 1973), they might be expected to actually increase community diversity when reintroduced by parasitizing the most dominant species in a community.

Exotic Species and Nutrient Availability

An additional threat to PNW prairies, which can have negative effects on species reintroductions, but also threatens the prairies overall, is invasion from non-native, exotic plants. Nutrient enrichment in the soil, particularly nitrogen (N), can lead to an increase in faster-growing exotic invasive species, which impede the success of native plants (Huenneke et al. 1990; Bobbink et al. 1998; Davis et al. 2000). Carbon (C) addition to the soil has been used as a method to reduce plant-available N (Morgan 1994), which may negatively affect exotics to a greater degree than natives (Alpert 2010). Previous studies (Blumenthal et al. 2003; Averett et al

2004; Prober et al. 2005) have found that C addition can reduce cover of exotic species to a greater degree than natives. This approach has been tested with some success in the PNW (Mitchell & Bakker 2011), and could be a useful restoration tool for Willamette Valley prairies, and for improving the reintroduction success of *C. levisecta*.

Research Goals and Objectives

We conducted a nutrient manipulation experiment coupled with a diversity manipulation experiment to assess the effects of these treatments on both the plant community and the reintroduction success of *C. levisecta*. Specifically we asked the following questions related to the plant community:

1. Does the average community composition, total vascular plant cover, richness or diversity differ among plots that receive different nutrient treatments?

2. Does the effect of the nutrient manipulation treatments differ by species or by functional groups of species?

3. Do species, or groups of species, have a reciprocal response to C addition versus nutrient addition (i.e. does a species whose cover increases with nutrient addition also decrease with carbon addition?)

Related to *C. levisecta*, we asked:

1. Does the mean number or size of *C. levisecta* plants differ among plots that receive different nutrient treatments?

2. Does the mean number or size of *C. levisecta* plants differ between plots that are seeded for increased diversity and those that are not?

3. Does the total cover of vascular plants, community diversity or composition differ

   between plots into which *C. levisecta* is seeded and plots without *C. levisecta*?

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Chapter 2

Plant community responses to soil nutrient manipulations in two Pacific Northwest prairies

Abstract

Destruction of habitat has greatly reduced the extent of upland prairies in the U.S. Pacific

Northwest. Many of the remaining prairie fragments are poor quality and face the threat of

invasion by non-native species and a loss of biodiversity. Nutrient enrichment has been show to

promote increased invasion of communities, decrease success of native species, and decrease

biodiversity. A potential way to counter these effects is through carbon addition to the soil which

stimulates microbial activity and immobilizes nutrients, making them unavailable to plants. This

strategy has promise as a restoration tool to improve conditions for native species, which are

often outcompeted under high nutrient conditions. We tested the efficacy of carbon addition for

controlling non-native species in two Willamette Valley prairies. We created treatments of

varying nutrient availability and monitored the plant community responses for two growing

seasons. Experimental manipulations included either carbon (sucrose) addition, ambient soil

nutrients (controls), or nutrient addition (NPK fertilizer). Community composition differed

significantly among nutrient treatments (perMANOVA, $p<0.001$) at both study sites and in both

years of the experiment. Mean total vascular plant cover was significantly reduced by carbon

addition compared to ambient nutrient availability and increased by nutrient addition. Native

grasses were unaffected by nutrient manipulation at either site, whereas non-native grasses were

greatly reduced by carbon addition and increased by nutrient addition, compared to ambient soil

nutrient availability. Non-native forbs were also greatly reduced by carbon addition, and were

also reduced by nutrient addition by the second year of the experiment. Richness of non-native

species was more affected by nutrient manipulation than richness of natives, and was either

increased or unaffected by nutrient enrichment and decreased by carbon addition. Some non-

native species were found to be highly affected by the nutrient manipulation, while others were

not, suggesting that carbon addition may be an effective control for only certain non-native species. Our results suggest that nutrient enrichments can promote the success of non-native species and that carbon addition can be used to counter these effects on a species- and site-specific basis.

Introduction

Soil nutrient availability is a driving force in structuring plant communities, affecting diversity, composition, and functioning (Vitousek and Howarth 1991; Tilman and Wedin 1991; Vitousek et al. 1997). High levels of nutrient loading in the soil, particularly nitrogen (N), frequently lead to alterations in a plant community, including decreased biodiversity (Tilman 1987; Vitousek et al. 1997; Bobbink et al. 1998), and an increase in faster-growing exotic invasive species, that impede the success of native species (Huenneke et al. 1990; Bobbink et al. 1998; Davis et al. 2000). Increased levels of N can result from a number of human sources, including agricultural inputs of fertilizer, combustion of fossil fuels, and through nitrogen fixing crops (Galloway et al. 1995, Vitousek et al. 1997; Galloway et al. 2004). Due to the tendency for elevated N to favor exotics, reducing these elevated levels as part of restoration practices may improve conditions for native species.

One method for reducing plant-available soil N is through carbon (C) addition to the soil (Morgan 1994). Carbon addition stimulates microbial activity in the soil, which leads to N immobilization, and a reduction in N availability to plants, at least temporarily (Zink & Allen 1998; Blumenthal et al. 2003; Alpert 2010). The reduction in soil N may have a direct negative effect on native plants, but also a potentially greater indirect positive effect if it more negatively affects competing, non-native species, than natives (Alpert 2010). This technique has potential

for use in restoring native vegetation in areas with highly nutrient-enriched soils, such as in restorations of ex-arable fields (Averett et al. 2004; Eschen et al. 2007) or for reducing exotic species cover in existing highly invaded plant communities (Kirkpatrick & Lubetkin 2011; Mitchell & Bakker 2011).

In many previous C addition studies, a primary goal has been to reduce the success of exotic species and increase the competitiveness of native species. Some studies have observed reductions in overall plant biomass as a result of C addition (Averett et al. 2004; Eschen et al. 2007; Blumenthal 2009); however a range of native and exotic species' responses associated with C addition have been reported, from a reduction in both invasive and native species, to no reduction in either (Perry et al. 2010). Additionally, differential and conflicting responses to C addition have been observed across functional groups. For example, Mitchell and Bakker (2011) observed a reduction in forb biomass as a result of C addition but no effect on grasses or legumes, while others have found a greater reduction in grasses than forbs as a result of C addition (Averett et al. 2004; Eschen et al. 2006; Eschen et al. 2007). One study found a reduction in both native and exotic forb biomass with C addition, but an increase in both native and exotic grass biomass (Grygiel et al. 2012). Inconsistencies in these findings may result from differences in the rate of application of C (Blumenthal et al. 2003) or the duration of application (Kirkpatrick & Lubetkin 2011; Burke et al. 2013). Here we assess the effectiveness of a moderate-high rate of C addition, applied three times per year over a two year period as a restoration method in two Pacific Northwest (PNW) prairies.

In the PNW, prairies have become significantly reduced in extent since European settlement and are now considered to be an endangered ecosystem. Within the Willamette Valley of Oregon, more than 99% of native prairies have been lost (Noss 1995). The remaining prairie

fragments are often highly invaded by exotic invasive species, and have diminished native biodiversity. Generally, a combination of burning and herbicide treatments is used and recommended for control of these exotic species (e.g. Stanley et al. 2011), however prescribed fire can also benefit non-natives (Anzinger & Radosevich 2008) and many herbicides can also have a negative impact on native plants, not just non-natives (Wagner & Nelson 2014), or other non-target species such as butterflies (Russell & Schultz 2010). C addition is a method that has the potential for reducing exotic species cover, while enhancing native species (Blumenthal et al. 2003) and has shown some potential for success in PNW prairies (e.g. Mitchell & Bakker 2011). If C addition is effective at reducing exotic species cover, while either not affecting or benefiting native species (as found by Blumenthal et al. 2003 and Prober et al. 2005), then it would have excellent potential as a restoration tool for PNW prairies.

For this experimental study, we created a range of altered soil nutrient availability by applying supplemental nutrients to some plots and C to additional plots in a factorial design. Most studies that look at changes in the plant community as a result of soil nutrient manipulations test these effects in one direction only, either adding nutrients (e.g. Bobbink 1991; Gough 2000; Clark & Tilman 2008) or adding C (e.g. Averett et al. 2004; Corbin & D'Antonio 2004; Kirkpatrick & Lubetkin 2011; Mitchell & Bakker 2011). Fewer studies have created a range of conditions through fertilization and C addition (but see Blumenthal 2009 and Liira et al. 2011) , which allows simultaneous testing in a given community of effects of either a reduction or increase in soil nutrient availability.

This experiment aimed to assess effects of increased and decreased soil nutrient availability on prairie plant communities. Specifically, we asked the following questions: 1) Does community composition, total vascular plant cover, species richness or species diversity

differ among plots that receive different nutrient treatments?; 2) Does the effect of the nutrient manipulation treatments differ by species or among functional groups of species?; and 3) Does a species, or group of species, have a reciprocal response to C addition as they do to nutrient addition (i.e. does a species whose cover increases with nutrient addition also decrease with carbon addition?).

We hypothesized that we would see a divergence in the plant communities as a result of the nutrient treatments. We also expected that the communities would continue to diverge as we continued the treatments. We expected to see an increase in total vascular plant cover with increasing soil nutrient availability and predicted that these effects would be greater for some species than others. Finally we expected to see decreased species richness and diversity under an enriched nutrient regime, due to increased dominance by one or a few species.

Methods

Study Sites

This experiment was conducted at William L. Finley National Wildlife Refuge (44° 24' 42"N, 123° 19' 53"W) and Baskett Slough National Wildlife Refuge (44° 57' 45"N, 123° 15' 39"W) in the Willamette Valley of Oregon. Experimental plots at both locations were situated on well-drained upland prairie habitat. At Finley NWR the study sites were within a restored prairie with lower vascular plant species diversity than the remnant prairie used at Baskett Slough NWR. Restoration on the restored site within Finley NWR was started in 2002 by the US Fish and Wildlife Service, prior to which the land was used for the cultivation of various crops (Clark & Wilson 2005). Initial restoration efforts included a prescribed burn, drilling of native grass seeds, and herbicide treatments. Additional tilling, mowing, carbon banding, and seeding treatments followed in 2003 and 2004. Other restoration efforts, such as prescribed burns, have

since continued on the site. Elevation at the restored site is approximately 90 m above sea level, and soils are in the Hazelair complex, a composite of moderate to well-drained silt loams and clay loams (Clark & Wilson 2005). The remnant prairie site within Baskett Slough NWR was located on a hill top at approximately 116 m above sea level. It had predominantly silty clay loam soils of the Chehulpum soil series. Finley and Baskett Slough NWR will henceforth be referred to as the restored and remnant sites, respectively. Climate in the Willamette Valley is characterized by warm, dry summers, and cool wet winters, with average annual precipitation of 115 cm (PRISM Climate Group 2013).

At each of the sites, the experiment was established into existing vegetation dominated by a mixture of native and exotic grasses. At the restored site, the prairie was dominated by three exotic grasses: creeping bent grass (*Agrostis stolonifera*), sweet vernal grass (*Anthoxanthum odoratum*), and brome fescue (*Vulpia bromoides*), and two native grasses, Roemer's fescue (*Festuca idahoensis* ssp. *roemeri*), and California oatgrass (*Danthonia californica*). The most common forbs were exotics such as hairy cat's ear (*Hypochaeris radicata*). The prairie at the remnant site was more diverse but was also dominated largely by exotic grasses including bristly dogstail grass (*Cynosurus echinatus*), silver hairgrass (*Aira caryophyllea*), Kentucky bluegrass (*Poa pratensis*), brome fescue (*Vulpia bromoides*), orchardgrass (*Dactylis glomerata*), tall oatgrass (*Arrhenatherum elatius*), and common velvetgrass (*Holcus lanatus*). Native grasses include Roemer's fescue (*Festuca idahoensis* ssp. *roemeri*), California oatgrass (*Danthonia californica*), and prairie Junegrass (*Koeleria macrantha*). The dominant forbs were the exotics, hairy cat's ear (*Hypochaeris radicata*), Queen Anne's lace (*Daucus carota*), and narrowleaf plantain (*Plantago lanceolata*), and native strawberry (*Fragaria virginiana*). All species names and native status follows USDA plants (USDA 2015). Full species lists are in Appendix Tables 1

and 2. Prescribed burns were performed at each site in late October 2012, prior to the experimental setup.

Experimental Design

In November 2012 experimental plots were established at each location. An identical experimental design was maintained across both sites, which consisted of 120 1x1 m plots per site, with a 1 m walkway between plots and arranged in a blocked, factorial design. Each site had ten blocks of 12 plots and each plot within a block was randomly assigned one of 12 treatments. The treatment combinations included three levels of soil nutrient availability: ambient (control), fertilized (nutrient addition), and carbon addition (nutrient reduction). The fertilized plots received a 14-14-14 NPK slow release fertilizer (8.5% ammoniacal nitrogen, 5.5% nitrate nitrogen, P_2O_5 and K_2O). Fertilizer was applied at a rate of 90 g•m^{-2}yr^{-1} (12.6 g•m^{-2}yr^{-1} each of N, P, and K), applied twice per year, 45 g each in November 2012 and 2013 and May 2013 and 2014. Carbon was applied in the form of sucrose, with each plot receiving 1500 g•m^{-2}yr^{-1} of sucrose (630 g•m^{-2}yr^{-1} of carbon), applied in three 500 g applications in November 2012 and 2013, February 2013 and 2014, and May 2013 and 2014. These treatments were crossed with two types of seed additions as part of a larger experiment. Results of the seed addition treatments are discussed elsewhere (see Chapter 3).

Vegetation Measurements

We conducted community surveys at the peak of the growing season in 2013 and 2014 (May and June). All vascular plant species in each plot were identified and the point intercept method (Jonasson 1988) was used to estimate abundance, using 100 points spaced every 10 cm

in the 1 m^2 plots. All plant species intercepted at each point were recorded and the total numbers

of intercepts per species were tallied to estimate the cover of each species in each plot. Cover of

a single species could therefore range from zero to 100 percent. Species that were present but not

intercepted were recorded as having 0.1 percent cover. Total percent cover of a plot was the sum

of all vascular species' covers in a plot and ranged from 25 to 270. Ground-level substrates were

also recorded at each point (bare ground, litter, moss, rock or wood). Abundance data were used

to calculate the Shannon-Wiener Index of diversity (Oksanen et al. 2013).

Soil Measurements

We measured nutrient concentrations by sampling soils from a subset of the plots twice

over the course of the experiment. One plot of each nutrient treatment was sampled from each

block (n=10) for a total of 30 soil samples per site. Samples were collected in December 2012,

one month after the experiment was established, to measure short term effects of the treatments

on soil nutrient levels, and in September 2014, to measure longer term effects. The top 10 cm of

soil was collected; samples were stored at 4 C immediately after collection, and processed

within 24 hours.

For nutrient analysis, each soil sample was sieved through a 2mm mesh, mixed and sub-

sampled and extracted for BrayP and DIN (ammonium and nitrite+nitrate) the same day as

sieving. For DIN extraction, 7 g of sieved, field-moist soil were extracted with 35 mL 2M KCl.

Samples were shaken for 1 hour, allowed to settle for 30 minutes, then decanted through pre-

rinsed (2M KCl) Whatman #42 filter paper (Mulvaney 1996). Phosphorus extraction followed

the Bray-1 Dilute Acid Flouride method (Bray & Kurtz 1945). Five grams of sieved, field-moist

soil were extracted with 25 mL Bray-1 solution, shaken for one minute, then centrifuged at 3400

rpm for five minutes and immediately filtered through Whatman #42 filter paper. Extracts were

frozen until they could be analyzed. Analysis for nutrients in extracts was performed using a

Lachat QC8000 flow injection analyzer.

Volumetric water content was measured in May 2014 using a Field Scout TDR 200 soil

moisture meter.

Data Analyses

Data were analyzed using R statistical software version 3.0.3 (R Core Team 2014) and

PC-ORD version 6.03 (McCune and Mefford 2011). Soil extractable nutrient levels were

analyzed using Kruskal–Wallis one-way analysis of variance, as they did not meet the ANOVA

assumptions of normality and equal variance. When Kruskal-Wallis tests were significant

($p \leq 0.05$), Dunn's tests of multiple comparisons were conducted (Dinno 2014) to determine

which groups differed. Bonferroni corrections (three comparisons) were used to adjust the p-

values for multiple comparisons.

To explore differences in plant species composition among the plots, we used non-metric

multidimensional scaling (NMS) in PC-ORD (McCune and Mefford 2011). NMS ordinations of

plots in species space were performed first for both sites and years together, to examine site, year

and treatment differences concurrently, and also for each site and year separately to focus on the

effects of the treatments. Main matrices were constructed with plots as rows and species as

columns. Environmental matrices were constructed with plots as rows and the experimental

treatments and other environmental variables as columns. Variables included in the

environmental matrices were: treatments, covers for each species, total cumulative cover of all

species, ground cover by substrate type (bare ground, litter etc.), soil moisture, richness,

Shannon-Weiner diversity, as well as some combination variables such as pooled cover of all native or exotic, annual or perennial species, and functional groups (grasses, forbs, and legumes). Raw, unrelativized data were used for the analyses, as the effect of treatments on total cover in a plot was also a variable of interest, and relativization would remove this signal. NMS was performed using Sørenson's distance, with random starting configurations and without penalizing for ties in the distance matrix. NMS ordinations were rotated such that the nutrient treatment effect was entirely accounted for by Axis 1 for ease of interpretation.

Permutational multivariate analysis of variance (perMANOVA) was used to test whether community composition differed between the treatment types (Oksanen et al. 2013). Analyses were performed for the two sites and years separately. The model included nutrient treatment and the seeding treatment, was stratified by block, and run for 999 permutations using the Bray-Curtis (Sørenson) index.

Univariate response variables for the vegetation data were also examined, including total vascular plant cover; cover of the different functional groups, separated by native status (i.e. native and exotic grasses, native and exotic forbs); and cover of the most common species at each site. Species considered as common were those that were present in more than 25% of plots of each nutrient level (C addition, control and nutrient addition) in both 2013 and 2014, and with greater than 4% mean cover in at least one treatment. Additional univariate response variables included species richness (which was also divided into native and exotic richness), and Shannon-Weiner diversity. We used linear mixed effects models to test for nutrient and seeding treatment effects on each of the response variables (Pinheiro et al. 2013). Each year and site was modeled separately, with nutrient and seeding treatments as fixed effects in the models. As random effects, the models had separate intercepts by block. Bonferroni post-hoc corrections for multiple

comparisons (Warnes 2013) were completed when the mixed effects models showed a significant (p≤0.05) effect of nutrient treatment. Cover data for individual species were natural log transformed to meet assumption of normality and equal variances; no other data required transformation for analysis. Four outliers were removed from analysis of native forbs at the restored site as they each had much higher forb cover than was typical of the rest of the site.

Results

Soil Nutrient Availability

In December 2012, one month after initial nutrient treatments, soil ammonium levels differed significantly among treatments at both the remnant (H=14.27, 2 d.f., p<0.001) and restored (H=12.86, 2 d.f., p=0.001) sites (Figure 2.1A/B). At the remnant site the C addition plots had less ammonium than the control plots (Z=2.6, p=0.01) and nutrient addition plots (Z=3.7, p<0.001), while the nutrient addition and control plots did not differ (Z=1.1, p=0.4). At the restored site, the C addition plots had significantly lower ammonium than the nutrient addition plots (Z=3.6, p<0.001), while the nutrient addition plots tended to have marginally higher ammonium than the controls (Z=1.9, p=0.08). Phosphate levels in 2012 were not different among treatments at the remnant site (H=2.5, 2 d.f., p=0.29) or at the restored site (H=3.9, 2 d.f., p=0.14). Nitrate levels were also not different among treatments at the remnant (H=2.5, d.f.=2, p=0.28) site in 2012. At the restored site, both the C addition and control treatments had nitrate levels below the detection limit of the analyzer.

In 2014, after six C additions and four nutrient additions, there was a significant difference in soil ammonium levels among treatments at both the remnant (H=18.8, 2 d.f., p<0.001) and the restored (H=19.6, 2 d.f., p<0.001; Figure 2.1C/D) sites. Differences among

treatments were also significant in the phosphate levels at the remnant (H=22.3, 2 d.f., p<0.001) and restored (H=19.6, 2 d.f., p<0.001) sites and the nitrate levels at the remnant site (H=24.4, 2 d.f., p<0.001). In the pairwise comparisons, C addition and control plots were not significantly different in any of the extractable soil nutrients, at either site. At the remnant site, the nutrient addition plots had higher ammonium (Z=3.3, p=0.001), nitrate (Z=2.6, p=0.001) and phosphate (Z=4.7, p<0.001) than the controls. At the restored site, the nutrient addition plots also had higher ammonium (Z=4.1, p<0.001), and phosphate (Z=4.1, p<0.001) than the controls, and the nitrate in the controls and C addition plots was below the detection limit of analyses.

Community composition

Ordination of plant community data at both sites and years (Figure 2.2) resulted in a 2D solution with a final stress of 17.55. Cumulatively, the two axes explained 82% of the variation in the distance matrix. Differences in the species composition between sites are demonstrated by the clustering of the plots from the same site on Axis 2. On Axis 1, the spread between the different nutrient treatments was greater at the remnant site than at the restored site, and there was also a greater movement in the centroids of the treatments from one year to the next at the remnant site than the restored site.

Ordinations of the individual sites and years resulted in 3D solutions; the first two axes are interpreted here (Figure 2.3). Patterns were similar in 2013 and 2014 and therefore only 2014 data are presented. In 2014, ordination of community data from the remnant site had a final stress of 15.88 and the axes cumulatively explained 79% of the variation in the distance matrix (Figure 2.3A). Permutational multivariate analysis of variance (perMANOVA) showed that the differences among treatments were significant in 2013 (pseudo-F=15.54, p<0.001) and 2014

(pseudo-F=18.40, p<0.001). Nutrients, total vascular plant cover, cover of *Cynosurus echinatus*, exotics, grasses, annuals, legumes, litter, and diversity, were each positively correlated (R^2>0.25) with Axis 1 while carbon, soil moisture, and cover of bare ground were negatively correlated with Axis 1. Cover of *Arrhenatherum elatius, Rosa eglanteria, Toxicodendron diversilobum*, and perennials were positively correlated with Axis 2, and cover of *Koeleria macrantha* was negatively correlated with Axis 2. Full lists of correlated variables can be found in Appendix Table 3.

Ordination of the restored site plant community data (Figure 2.3B), resulted in a final stress of 13.44 and 87% of the variation in the distance matrix was explained. PerMANOVA indicated there were significant differences among the treatments in 2013 (pseudo-F=20.59, p<0.001) and 2014 (pseudo-F=17.69, p<0.001). Nutrients, total vascular plant cover, cover of *Anthoxanthum odoratum*, grasses, exotics, annuals and *Vulpia bromoides* were positively correlated with Axis 1 and carbon and cover of *Hypochaeris radicata* were negatively correlated with Axis 1. Cover of *Festuca idahoensis* ssp. *roemeri* and natives were positively correlated with Axis 2 and *Agrostis stolonifera* was negatively correlated with Axis 2. Full lists of correlated variables can be found in Appendix Table 4.

Vascular Plant Cover

The mean total vascular plant cover in a plot was significantly different among the nutrient treatments at both the remnant and restored sites for 2013 and 2014 (Table 2.1; Figure 2.4). At both sites, and in both years, the C addition plots had significantly less total vascular plant cover than the control plots (p<0.001). The nutrient addition plots had significantly higher vascular plant cover than the controls at both sites in both years (p<0.001). Estimates and

confidence intervals for all plant cover data are in Appendix Table 5 (remnant site) and Table 6
(restored site).

While total vascular plant cover was strongly affected by the nutrient treatments, some
groups of plants were more strongly affected than others (Figure 2.5). Mean exotic grass cover
was significantly affected by the nutrient treatments at both the remnant and restored sites in
2013 and 2014 (Table 2.1). At both sites and in both years, the mean exotic grass cover was
significantly lower in the C addition plots compared to the controls ($p<0.005$), with a greater
reduction being observed at the remnant site than the restored. At both sites, and in both years,
the nutrient addition plots had significantly greater mean exotic grass cover than the control plots
($p<0.001$). Mean native grass cover, however, was not significantly different among nutrient
treatments at either site in either year (Table 2.1; Figure 2.5).

Mean native forb cover was significantly different among nutrient treatments at the
remnant site in 2013, but not 2014 (Table 2.1: Figure 2.5A/C). At the restored site mean native
forb cover was significantly different in both 2013 and 2014 (Table 2.1; Figure 2.5B/D). At the
remnant site in 2013, only the C addition plots and nutrient addition plots were significantly
different ($p=0.003$), with the nutrient addition plots having greater mean native forb cover than
the C addition plots. At the restored site the C addition plots had lower native forb cover than the
control plots in 2013 and 2014 ($p<0.01$). The nutrient addition plots did not differ from the
controls in 2013 or 2014. The nutrient treatments also had a significant effect on mean exotic
forb cover at both the remnant and restored sites in 2013 and 2014 (Table 2.1; Figure 2.5). At
both the remnant and the restored sites the C addition plots had less exotic forb cover compared
to the controls in both in 2013 and in 2014 ($p<0.001$). At the remnant site, the mean exotic forb
cover was significantly higher in the nutrient addition plots compared to the control in 2013, but

lower in 2014 (p<0.001). At the restored site in 2013 the control and nutrient addition plots were no different in mean exotic forb cover (p=0.21), but in 2014, exotic forb cover was lower in the nutrient addition plots compared to the controls (p<0.001).

Species-specific Responses

Individual species varied in their responses to soil nutrient manipulations. Beginning with the remnant site, there were five species whose mean cover was the same across all nutrient treatments in both 2013 and 2014, including three native perennial grasses, *Danthonia californica*, *Festuca idahoensis* ssp. *roemeri* and *Koeleria macrantha*, a native perennial forb, *Fragaria virginiana,* and an exotic forb, *Plantago lanceolata* (Table 2.2; Figure 2.6A/C). The mean covers of the other four common species at the remnant site were significantly affected by the nutrient treatments (Table 2.2, Figure 2.6A/C, and see Appendix Table 7). Mean cover of *Aira caryophyllea* and *Cynosurus echinatus*, both exotic annual grasses, showed the same trend across the treatments in both years. Their covers were significantly lower in the C addition plots compared to the controls (p<0.001) in both years. They also had higher cover in the nutrient addition plots in both years, significantly so for *C. echinatus* in both 2013 and 2014 and *A. caryophyllea* in 2013 (p<0.001), but not 2014 (p=0.24). The trend in the mean cover of the two exotic forbs was also the same in both years. *Daucus carota* and *Hypochaeris radicata* both had reduced cover in the C addition plots compared to the controls in 2013 (p<0.005) and there was no significant difference between their cover in the control and nutrient addition plots (p>0.25). In 2014, cover of both forbs was lower in the C addition plots (p<0.001) and nutrient addition plots compared to the controls (p<0.001).

At the restored site, there were three species whose mean cover was not significantly different among nutrient treatments in 2013 or 2014: *Agrostis stolonifera*, an exotic perennial grass, and two native perennial grasses, *Danthonia californica* and *Festuca idahoensis* ssp. *roemeri* (Figure 2.6B/D, Table 2.2, and see also Appendix Table 8). The mean covers of the remaining four relatively common species ware significantly different across nutrient treatments. Mean cover of *A. caryophyllea* was significantly lower in the C addition plots compared to the controls in 2013 and 2014 (p<0.01) and was marginally significantly higher in the nutrient addition plots compared to the controls in 2013 (p=0.04) and 2014 (p=0.07). Mean cover of *Anthoxanthum odoratum*, an exotic perennial grass, was significantly different across treatments in 2013 and 2014. In 2013, the C addition plots had significantly less *A. odoratum* cover than the controls (p=0.01), and in 2014 there was no difference (p=0.35). In both 2013 and 2014, *A. odoratum* cover was higher in the nutrient addition plots compared to the controls (p≤0.001). Mean cover of *Hypochaeris radicata* followed the same trend as at the remnant site. Cover was lower in the C addition plots compared to the controls, in 2013 (p=0.002) and in 2014 (p=0.03). *H. radicata* cover was no different between the control and nutrient addition plots in 2013 (p=0.59) and was significantly lower in the nutrient addition plots in 2014 (p<0.001). Lastly, mean cover of *Vulpia bromoides*, an exotic annual grass, was lower in the C addition plots compared to the controls in both years (p<0.005) and higher in the nutrient addition plots compared to the controls in both years (p<0.001).

Richness and Diversity

Mean vascular species richness and Shannon-Weiner diversity were also affected by the soil nutrient treatments (Tables 2.1 and 2.3, Appendix Table 5 and 6). At the remnant site total

richness was lower in the C addition plots compared to the controls in both years ($p<0.005$). Total richness was higher in the nutrient addition plots compared to the controls in 2013 ($p<0.001$) and there was no difference in 2014 ($p=0.76$). Diversity showed the same trend, with diversity lower in the C addition plots compared to the controls in both years ($p<0.001$), and higher in the nutrient addition plots in 2013 ($p<0.001$), but not different in 2014 ($p=0.84$). Native richness did not differ among nutrient treatments in 2013 (Table 2.1; 2.3) and in 2014 it was lower in the nutrient addition plots compared to the controls ($p=0.02$). Exotic richness was lower in the C addition plots compared to the controls in both years ($p<0.001$) and higher in the nutrient addition plots compared to the controls in both years ($p\leq0.01$). At the restored site, total richness was lower in the C addition plots compared to the controls in both years ($p<0.01$). In 2013 total richness was no different in the control and nutrient addition plots ($p=0.86$) and in 2014 total richness was lower in the nutrient addition plots compared to the controls ($p=0.002$). Native richness did not differ among nutrient treatments in 2013 (Table 2.1; 2.3) and in 2014 it was lower in the C addition plots and nutrient addition plots compared to the controls ($p\leq0.001$). Exotic richness differed between the C addition and nutrient addition plots in 2013 ($p=0.003$), with the nutrient addition plots having higher richness. In 2014, exotic richness was lower in the C addition plots ($p<0.001$) and marginally lower in the nutrient addition plots compared to the controls ($p=0.05$). Finally, diversity was marginally lower in the C addition compared to the control plots in 2013 ($p=0.03$), and was significantly lower in the C addition plots in 2014 ($p<0.001$). Diversity was higher in the nutrient addition plots compared to the controls in 2013 ($p=0.002$) but not different in 2014 ($p=0.14$).

Discussion

The results of our experiment showed that additions of carbon or fertilizer to the soil can significantly alter available N, as well as the composition, total vascular plant cover, species richness and diversity of remnant and restored prairies. Different plant species and functional groups responded differently to soil nutrient manipulations, especially when comparing native and exotic species, and grasses and forbs.

Soil Nutrient Availability

Sucrose treatments were successful at reducing soil ammonium levels, but only in the short term. When soil nutrient levels were tested one month after the first addition of 500 gm^{-2} of sucrose, ammonium levels were significantly lower compared to controls at the remnant site and marginally lower at the restored site. N reduction was not detected four months after the last C application, even after two years of repeated treatments. Because sucrose is a highly labile form of C, however, it would be expected to only have a short-term effect; others have suggested the effects may last around three months (Prober et al. 2005). Additionally, in a Puget lowland prairie, Kirkpatrick and Lubetkin (2011) found that nitrate levels were reduced by sucrose addition the year after application, but not in subsequent years. Burke et al. (2013) also found nitrate was significantly reduced by sucrose addition throughout the years they applied it, however, levels rebounded the years after treatments ceased. Previous studies have found significant reductions in overall mineral N (Blumenthal 2009), in nitrate levels (Averett et al. 2004; Eschen et al. 2007; Iannone & Galatowitsch 2008; Kirkpatrick & Lubetkin 2011) and ammonium levels (Iannone & Galatowitsch 2008) with C addition. Soils at our study sites had low baseline nitrate levels, indicating rapid plant uptake and microbial immobilization, so the

effects on the plant communities we observed likely resulted from reduction in available

ammonium. While at least one previous study also observed an effect on phosphorus as well as

nitrogen (Michelson et al. 1999), we found no significant reductions in soil phosphate levels as a

result of C addition. In comparison to the control and C addition plots, the nutrient addition plots

showed significantly elevated levels for all the soil extractable nutrients by the end of the second

year of the experiment. We therefore conclude that our study design was sufficient in

establishing a range of nutrient availabilities, and the observed changes in vegetation are

associated with our soil modifications.

Plant Community Responses

One of the primary objectives of this experimental study was to assess whether plant

community composition was affected by these soil nutrient manipulations at the two prairie sites

and more specifically, what species, or functional groups of species, were most affected. We

found clear evidence that even with only one or two years of soil nutrient manipulation, there

was a significant divergence in the composition of the plant communities receiving different

nutrient treatments, but these effects were greater at the remnant site than at the restored site. For

example, ordinations showed communities in the different treatments diverged more at the

remnant site than at the restored site. At the restored site there was also a greater difference in

community composition between the nutrient addition and the control plots than there was

between the C addition plots and controls, a result similar to what Liira et al. (2012) saw in their

10 year nutrient manipulation experiment. This was not the case, though, at the remnant site,

where the C addition and nutrient addition plots both had very different compositions to the

controls. These differences between sites could relate to differences in N availability at the sites,

and the efficacy of the C addition treatments. Ammonium levels were higher in the control plots at the remnant than the restored site, and the C addition reduced ammonium levels to a greater degree at the remnant site, which could explain why the community differences between treatments were greater at the remnant site. However, other important site differences also exist. For example, species richness overall was lower at the restored site, and there were also a few species that strongly dominated the site, which would make the plots more similar to one another.

Our second objective was to determine which functional groups, and species, if any, were driving the community response to nutrient manipulations. Other studies have frequently observed an effect of C addition on total plant cover or biomass (Averett et al. 2004; Eschen et al. 2007; Blumenthal 2009) but the distribution of these effects among lifeforms and natives versus exotics have been mixed. When adding a sugar/sawdust combination, Eschen et al. (2007) saw a reduction in grass cover and an increase in forb and legume cover. In contrast, Mitchell and Bakker (2011) saw a reduction in forb biomass but not grasses or legumes with C addition. Averett et al. (2004) saw a reduction in both grasses and forbs, but the native grasses and exotics were reduced to a greater extent than the native forbs. In general, previous studies have also found a greater effect of C addition on exotic species than natives (Averett et al. 2004; Prober et al. 2005; Eschen et al 2007), or even a positive effect on natives (Blumenthal et al. 2003) but others have found no significant benefit to natives (Corbin and D'Antonio 2004; Huddleston & Young 2005). Additionally, Grygiel et al. (2012) observed a trend only related to functional group, where forb biomass was reduced by C addition but grass biomass was increased, regardless of whether they were native or exotic.

In our study, our ordination results (species' and functional groups' correlations with individual axes) and univariate mixed models highlighted key species and functional groups important in determining community responses. The ordination results are consistent with the univariate analyses in a number of ways. First, consistent across both sites, we saw a trend of lowest total cover in the C addition plots, intermediate levels in the controls and the highest cover in the nutrient addition plots, but a differential effect of the treatments on different functional groups and species. We saw no response to nutrient manipulation from the native grasses at either site. Two of the biggest contributors to this, *Danthonia californica* and *Festuca idahoensis* ssp*. roemeri,* native perennial grasses, were present at both sites, and although they had very different abundances at the two sites, they showed a consistent lack of response to the treatments at both sites. The responses of forbs, both exotic and native, were also similar across sites. Native forb cover was not significantly different across treatments at the remnant site by the second year of study, and while there were differences at the restored site, cover of native forbs was low and differences by treatment likely not biologically significant. Exotic forbs were also similarly affected by the treatments across sites, being more strongly affected than native forbs, such that exotic forb cover was significantly lower in C addition plots at both sites. Exotic forb cover was also lower in the nutrient addition plots by the second year, likely due to competition with the exotic grasses. The common exotic forbs contributing to this trend were *Daucus carota* which was present at the remnant site and *Hypochaeris radicata,* which was present at both sites. These forbs are a biennial and a perennial, and showed the same trend. A similar response by *H. radicata* was found in a Puget lowland prairie (Kirkpatrick and Lubetkin 2011), where cover was reduced by C addition. However, in their study the effect only lasted two years after cessation of treatments.

While we observed many responses that were consistent across both our sites, one inconsistency was the effect on exotic grasses, which were positively affected by nutrient enrichment at both sites, and negatively affected by C addition, but to different degrees. Within the literature, varying results have been observed related to the efficacy of C addition, which could be due to rates of C addition (Mangold & Sheley 2008), type of C applied (Burke et al. 2013) or duration of application (Kirkpatrick & Lubetkin 2011); however, in our study, we observed some differences between our sites, which received the same treatments. This could suggest site- and species-specific responses to nutrient manipulations. One difference might be between annuals and perennials; the three most common exotic annual grasses at our sites, *Aira caryophyllea*, *Cynosurus echinatus*, and *Vulpia bromoides*, generally increased in abundance with increasing nutrient availability, and the reduction in *V. bromoides* with C addition is consistent with another PNW prairie study (Mitchell and Bakker 2011). The dominant exotic grasses at the restored site, which did not show a significant response to the nutrient treatments, were *Agrostis stolonifera*, and *Anthoxanthum odoratum,* exotic perennial grasses. It appears that at least with only two years of treatment, C addition was not effective at controlling these two grasses. A different bentgrass, *Agrostis capillaris*, has also been found to be only marginally reduced in cover by C addition, and it rebounded greatly in C treated plots after treatments ceased (Kirkpatrick and Lubetkin 2011). This could be because C addition has a more immediate effect on annuals as opposed to perennials, although at least one study (Michelson et al. 1999) did find an effect on perennials as well, and some of our perennial forbs were affected by C addition. In the greenhouse, Eschen et al. (2006) also saw that total biomass of both perennials and annuals were similarly affected by C addition, which could imply species-specific differences are more important.

Lastly, our results comparing species richness and diversity across the plots of different nutrient treatments varied at the two sites. We saw a reduction in overall richness in the nutrient addition plots at the restored site by the second year of the study, as we expected based on previous work (Bobbink 1991; Wedin and Tilman 1996; Gough et al 2000; Suding et al. 2005). Others (e.g. Gross et al. 2005) have seen an initial positive effect of nutrient addition on richness, as we did at the remnant site, but a negative or neutral effect later (by the third year for Gross et al.). This initial increase in richness could have resulted from the added resources allowing more rare species to access nutrients, however in later years competition becomes more intense. We would expect to see additional species lost in the nutrient enriched plots with continued treatment (Wedin and Tilman 2008; Patrick et al. 2008). With C addition we had some species loss by the second year, as in other studies (Mitchell & Bakker 2011); however, at least at the remnant site it was more exotic species that were lost than native, suggesting that C addition may reduce exotic richness to a greater degree than it reduces native richness. Longer term studies have seen different results; in their 10 year nutrient manipulation experiment, Liira et al. (2012) found the C addition and control plots to be equal in richness throughout the study, while the nutrient treatments had lower richness. It is likely that in this short term we have only begun to lose some of the rare species and the effects may change over time.

Management Implications

Overall, our results suggest that nutrient enrichment increases the abundance of exotic plants compared to native plants, primarily grasses, and that C addition can be an effective way to counter these effects. Additionally, we found C addition to be effective at reducing plant cover, specifically exotics, at two sites with very different site histories. However, we did not

find that all exotic species were affected by the treatments, indicating that the success of C addition may depend on the target species. Some exotic species do not have a stronger response to changes in available N than natives (Lowe et al. 2002, Blumenthal et al. 2003, Corbin and D'Antonio 2004), and site factors, species present, and seed bank composition probably influence effectiveness of C addition treatments. The results of this and other studies show that at least in PNW prairies (Mitchell and Bakker 2011), if the system is dominated by exotic annual grasses, or exotic forbs, C addition is more likely to be successful than if exotic perennial grasses are the problem.

Questions about the persistence of C addition remain. In many studies, C is only applied one year (Averett et al. 2004; Kirkpatrick & Lubetkin 2011; Mitchell & Bakker 2011) or for a few years (Prober et al. 2005; Eschen et al. 2007), and even when applied long term, the soil and community effects do not persist long post-treatment (Burke et al. 2013). Our study suggested that effects on communities persist with repeated treatments; however, further research is needed on the maintenance of effects. Other studies (e.g. Prober et al. 2005) have found effects on plant communities can be maintained when treatments are maintained. However, in another study, C was applied only in the first year, and a marked recovery of the introduced species occurred post-treatment (Kirkpatrick & Lubetkin 2011). This suggests that although C addition can initially be effective, once the treatments cease there is a risk of the community returning to pretreatment or even higher levels of exotic cover. After 8 years of continued C treatments, Burke et al. (2013) observed a post-treatment soil N level of 250% the controls in their sugar treated plots the year following the cessation of treatments. Given that soil N may not remain reduced, and that communities may not remain lower in exotic cover post-treatment with C, seeding or planting of native species at this time may be a way to diminish the reinvasion of exotics. Carbon addition

may also be useful in combination with other treatments, such as herbicides, in areas where exotic annual and perennial grasses dominate.

An additional issue with C treatments involves applying them to larger scale restorations (Perry et al. 2010). Our results combined with other studies suggest that high levels and frequent treatments may be necessary to achieve the desired results. At the rates we applied, 15 000 $\text{kgha}^{-1}\text{yr}^{-1}$ would be required to achieve similar results to ours in PNW prairies. If applied in well-timed increments, lower rates would probably be effective; however, further research should explore ways to scale these treatments up to larger scales.

While there are still some obstacles to using C addition in managing exotic species, it has shown great potential in many regions and should remain as a tool that we continue to test and learn how to best apply in restoration practices.

Table 2.1: Results of linear mixed effects models testing the effect of the soil nutrient manipulations on total vascular plant cover, cover of native grasses, exotic grasses, native forbs, and exotic forbs; and species richness and Shannon-Weiner diversity.

| | Remnant | | | | Restored | | | |
| | 2013 | | 2014 | | 2013 | | 2014 | |
	F	p	F	p	F	p	F	p
Total Cover	154.94	<0.001	202.09	<0.001	219.29	<0.001	80.20	<0.001
Native Grass	0.02	0.977	1.64	0.200	1.61	0.206	1.16	0.317
Exotic Grass	181.64	<0.001	137.52	<0.001	137.08	<0.001	126.79	<0.001
Native Forb	5.47	0.006	2.05	0.134	15.34	<0.001	5.69	0.005
Exotic Forb	74.21	<0.001	59.26	<0.001	15.45	<0.001	25.43	<0.001
Total Richness	43.54	<0.001	29.08	<0.001	6.19	<0.001	15.25	<0.001
Native Richness	0.43	0.655	4.59	0.012	0.86	0.427	10.07	<0.001
Exotic Richness	60.09	<0.001	51.70	<0.001	5.58	0.005	10.48	<0.001
Diversity	40.99	<0.001	36.26	<0.001	17.48	<0.001	7.34	<0.001

*Degrees of freedom for each model was 2, 99 except for the exotic forbs at the restored site which had 2, 95

Table 2.2: Results of linear mixed effects models testing the effect of the soil nutrient manipulations on the most common species at each site.

	Remnant				Restored			
	2013		2014		2013		2014	
	F	p	F	p	F	p	F	p
Agrostis stolonifera	--	--	--	--	0.02	0.980	0.38	0.682
Aira caryophyllea	32.48	<0.001	35.18	<0.001	16.54	<0.001	4.12	0.019
Anthoxanthum odoratum	--	--	--	--	33.11	<0.001	66.22	<0.001
Cynosurus echinatus	45.93	0.0001	86.15	<0.001	--	--	--	--
Danthonia californica	0.21	0.809	1.89	0.156	0.12	0.838	0.36	0.7011
Daucus carota	19.53	<0.001	20.15	<0.001	--	--	--	--
Festuca idahoensis ssp. *roemeri*	2.08	0.131	0.18	0.837	0.66	0.517	0.32	0.730
Fragaria virginiana	0.18	0.839	0.35	0.705	--	--	--	--
Hypochaeris radicata	9.19	<0.001	17.65	<0.001	9.69	<0.001	39.66	<0.001
Koeleria macrantha	3.52	0.033	3.36	0.039	--	--	--	--
Plantago lanceolata	1.85	0.163	1.22	0.301	--	--	--	--
Vulpia bromoides	--	--	--	--	161.74	<0.001	171.86	<0.001

*Degrees of freedom for each model was 2, 99

Table 2.3: Mean Richness and Diversity of Vascular Plants (± 1 SE)

	Remnant			Restored		
	Carbon	Control	Nutrients	Carbon	Control	Nutrients
		2013			2013	
Total Richness	23.3 (0.53)[a]	25.4 (0.58)[b]	29.2 (0.57)[c]	15.5 (0.50)[a]	16.7 (0.55)[b]	16.8 (0.51)[b]
Native Richness	10.3 (0.55)[a]	10.4 (0.50)[a]	10.7 (0.46)[a]	7.5 (0.45)[a]	7.8 (0.53)[a]	7.6 (0.48)[a]
Exotic Richness	13.0 (0.46)[a]	15.0 (0.42)[b]	18.5 (0.45)[c]	8.2 (0.30)[a]	8.9 (0.27)[ab]	9.2 (0.26)[b]
Diversity (H')	2.28 (0.03)[a]	2.49 (0.03)[b]	2.65 (0.03)[c]	1.72 (0.03)[a]	1.80 (0.03)[a]	1.92 (0.03)[b]
		2014			2014	
Total Richness	23.5 (0.73)[a]	28.7 (0.72)[b]	28.9 (0.60)[b]	15.3 (0.43)[a]	18.4 (0.67)[b]	16.4 (0.57)[a]
Native Richness	10.9 (0.54)[ab]	12.0 (0.62)[a]	10.7 (0.51)[b]	6.7 (0.40)[a]	8.1 (0.46)[b]	6.9 (0.41)[a]
Exotic Richness	12.6 (0.65)[a]	16.6 (0.46)[b]	18.2 (0.45)[c]	8.6 (0.27)[a]	10.3 (0.34)[b]	9.6 (0.31)[ab]
Diversity (H')	2.16 (0.04)[a]	2.53 (0.04)[b]	2.52 (0.03)[b]	1.70 (0.03)[a]	1.85 (0.04)[b]	1.79 (0.04)[ab]

*Significant differences ($p < 0.016$ – Bonferroni corrected for three comparisons) between treatments within a year and site are indicated by different letters.
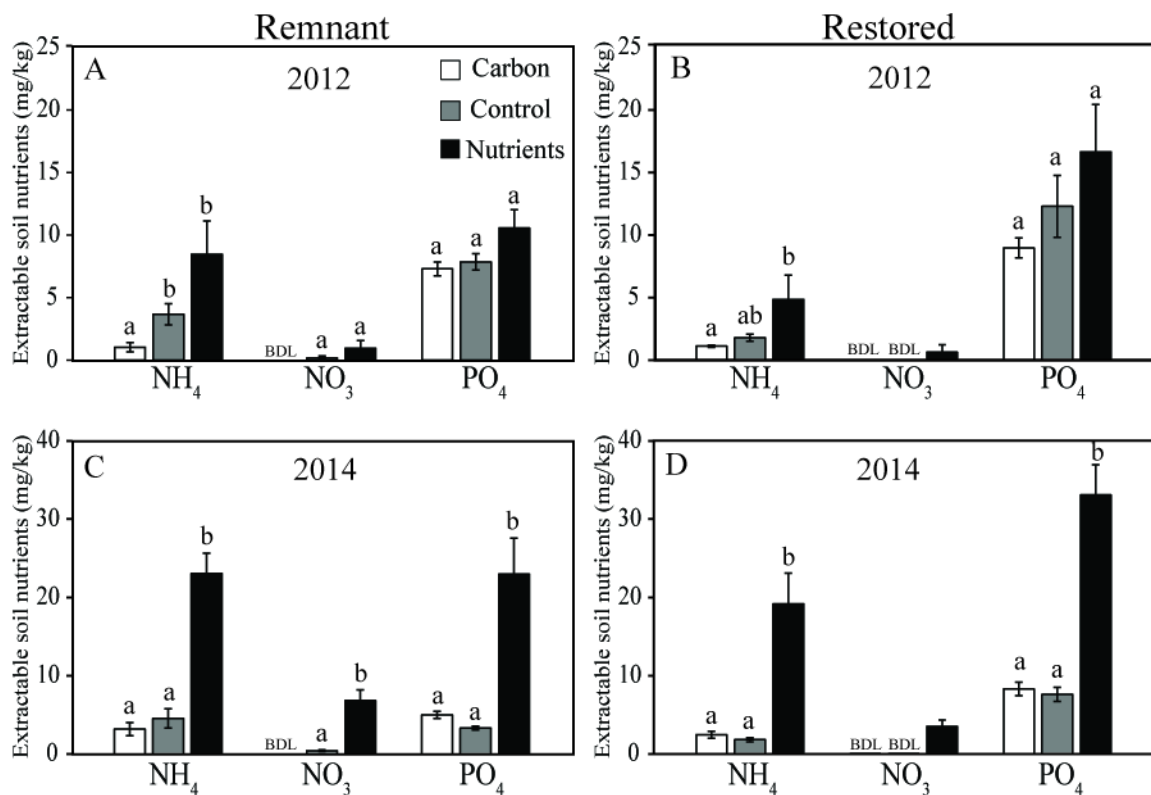
Figure 2.1: Average KCl-extractable NH_4-N and NO_3-N and Bray-P from December 2012 and September 2014. Data are means with standard error bars. Different letters above bars indicate significant differences in the amount of a given nutrient at $p<0.05$ (after Bonferroni correction). "BDL" indicates the nutrient was below the detection limit of the analyzer. Note that the two years are presented on different scales on the y-axis.
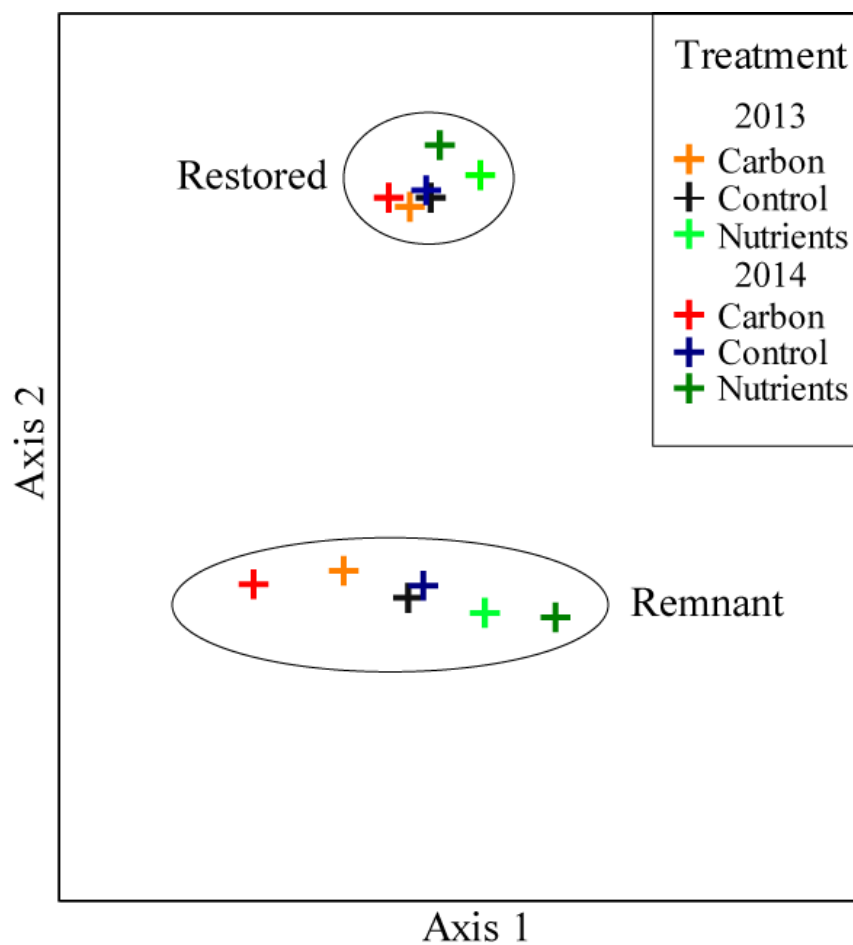
Figure 2.2: NMS Ordination of plots in species space at the remnant and restored sites. Points are the centroids (averages) of each of the three nutrient treatments for both years. Circles were drawn manually to designate the two sites.
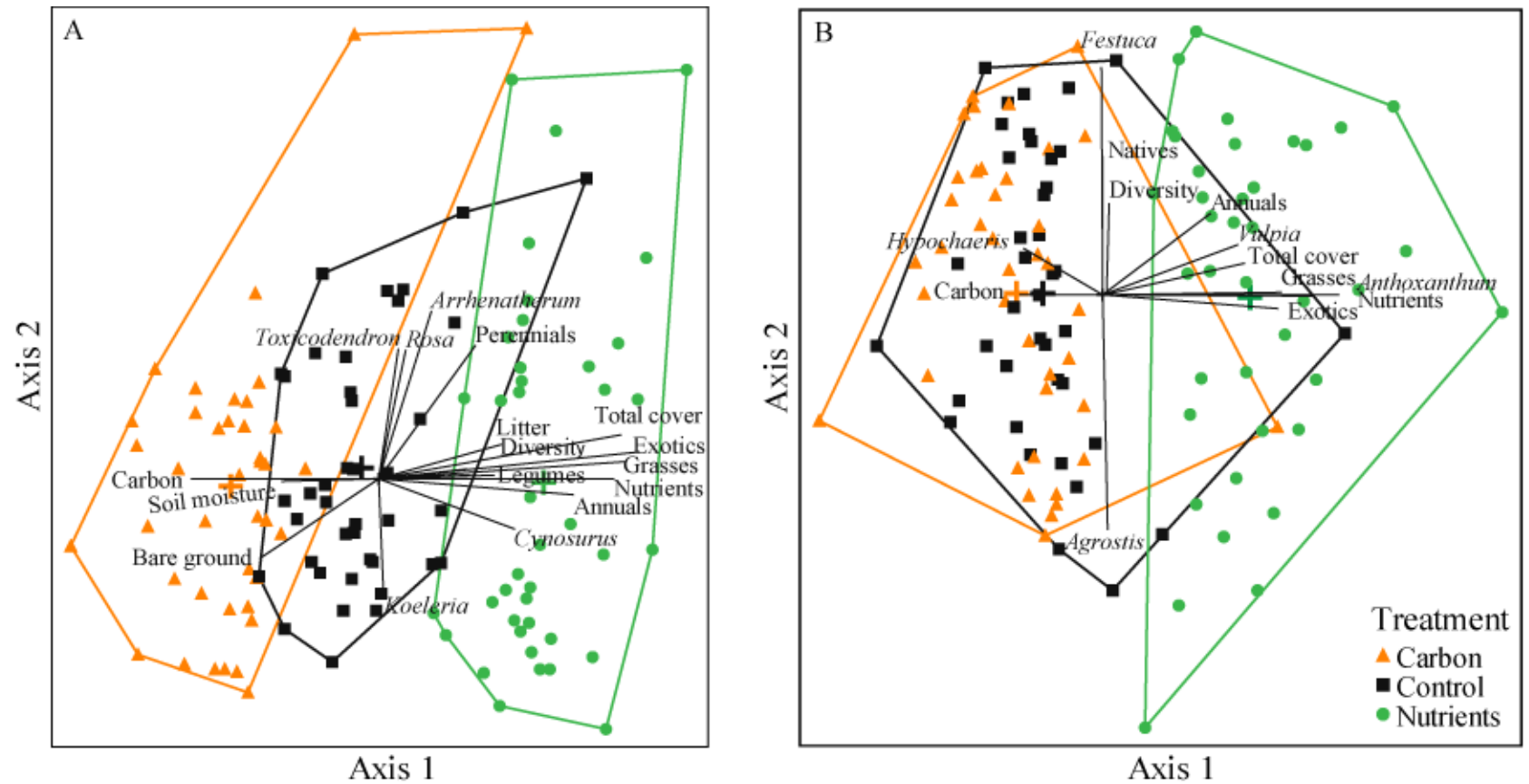
Figure 2.3: NMS ordinations of sample units in species space for the remnant (panel A) and restored (panel B) sites in 2014. Points represent each individual plot (n=120 per site) and centroids of each treatment are indicated by the cross symbol. Joint plot overlays indicate strength and direction of variables with a greater than 0.25 R^2 correlation with one of the axes. Carbon and Nutrients refer to the correlation of those treatments with the axes. Covers of species (indicated by genus names) and functional groups of species correlated with axes are also shown. Diversity refers to calculated Shannon-Weiner diversity and litter and bare ground refer to the ground level cover in a plot.
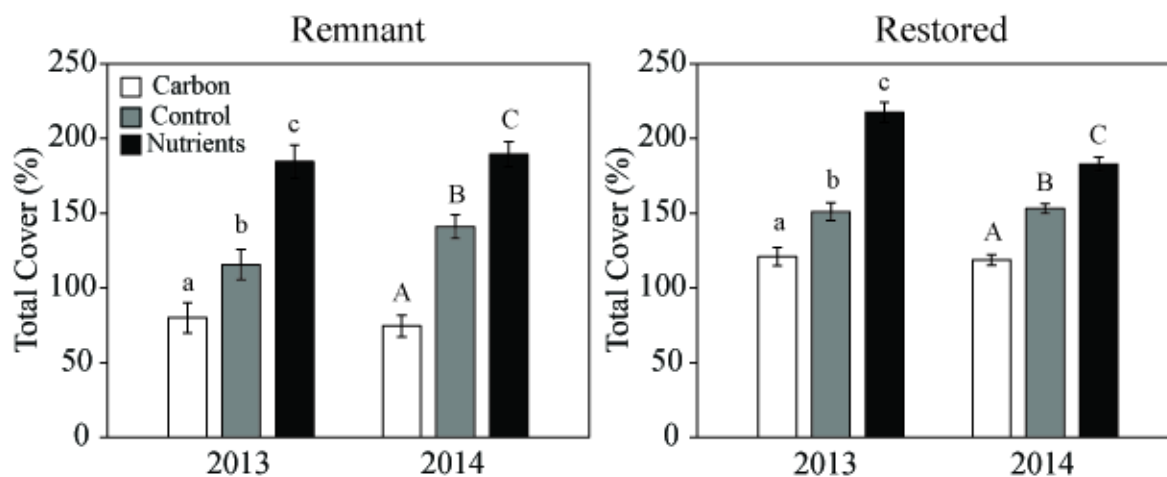
Figure 2.4: Total vascular plant cover in 2013 and 2014. Data shown are means across 40 plots of each nutrient treatment and standard error bars. Different letters over the bars indicate significant differences among treatments within a year at $p<0.016$ (Bonferroni corrected for three comparisons).
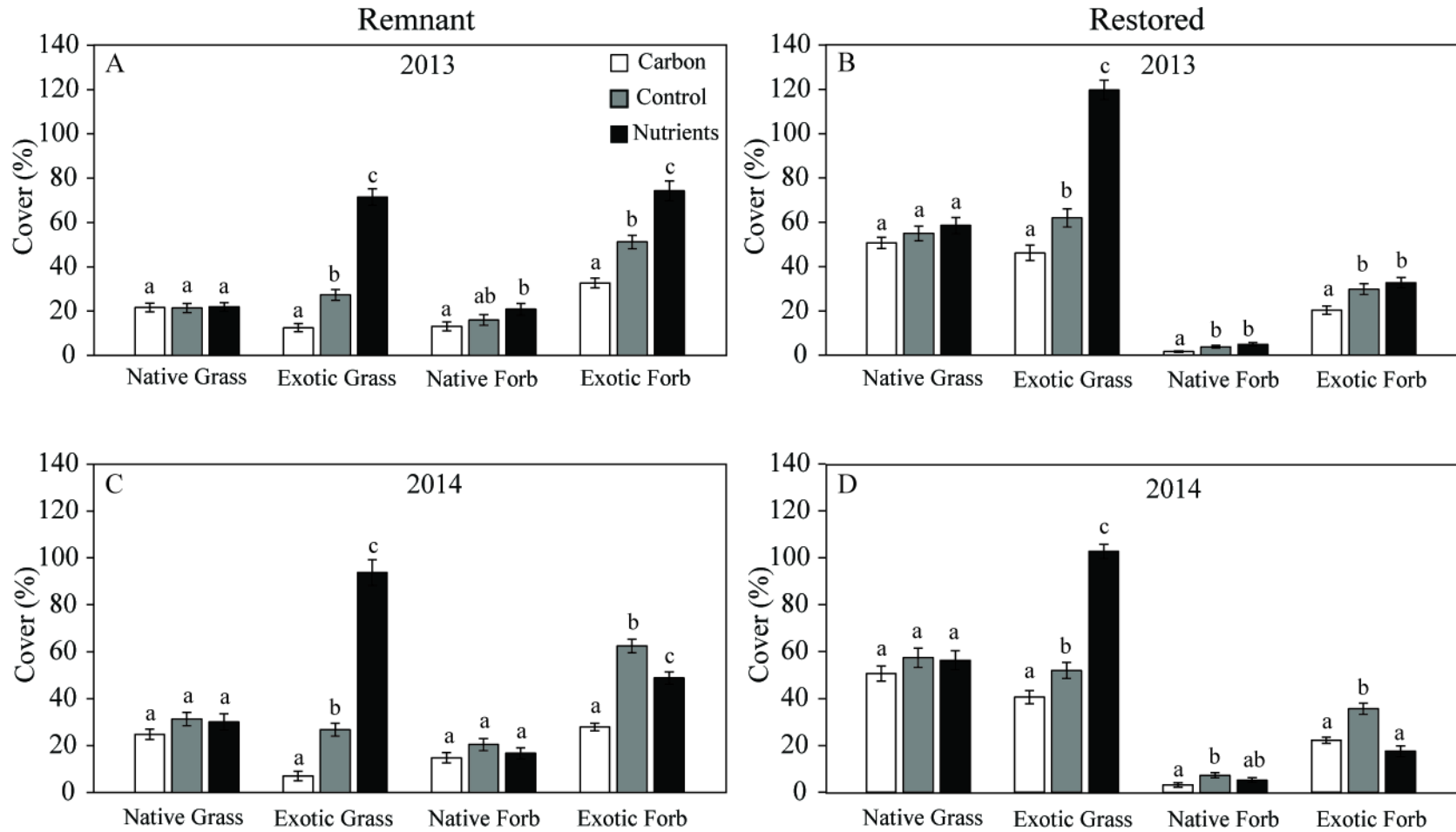
Figure 2.5: Vascular plant cover in 2013 and 2014, divided by functional group, native status and treatment. Data shown are means across 40 plots of each nutrient treatment and standard error bars. Different letters indicate significant differences among treatments within a functional group and year at p<0.016 (Bonferroni corrected for three comparisons).
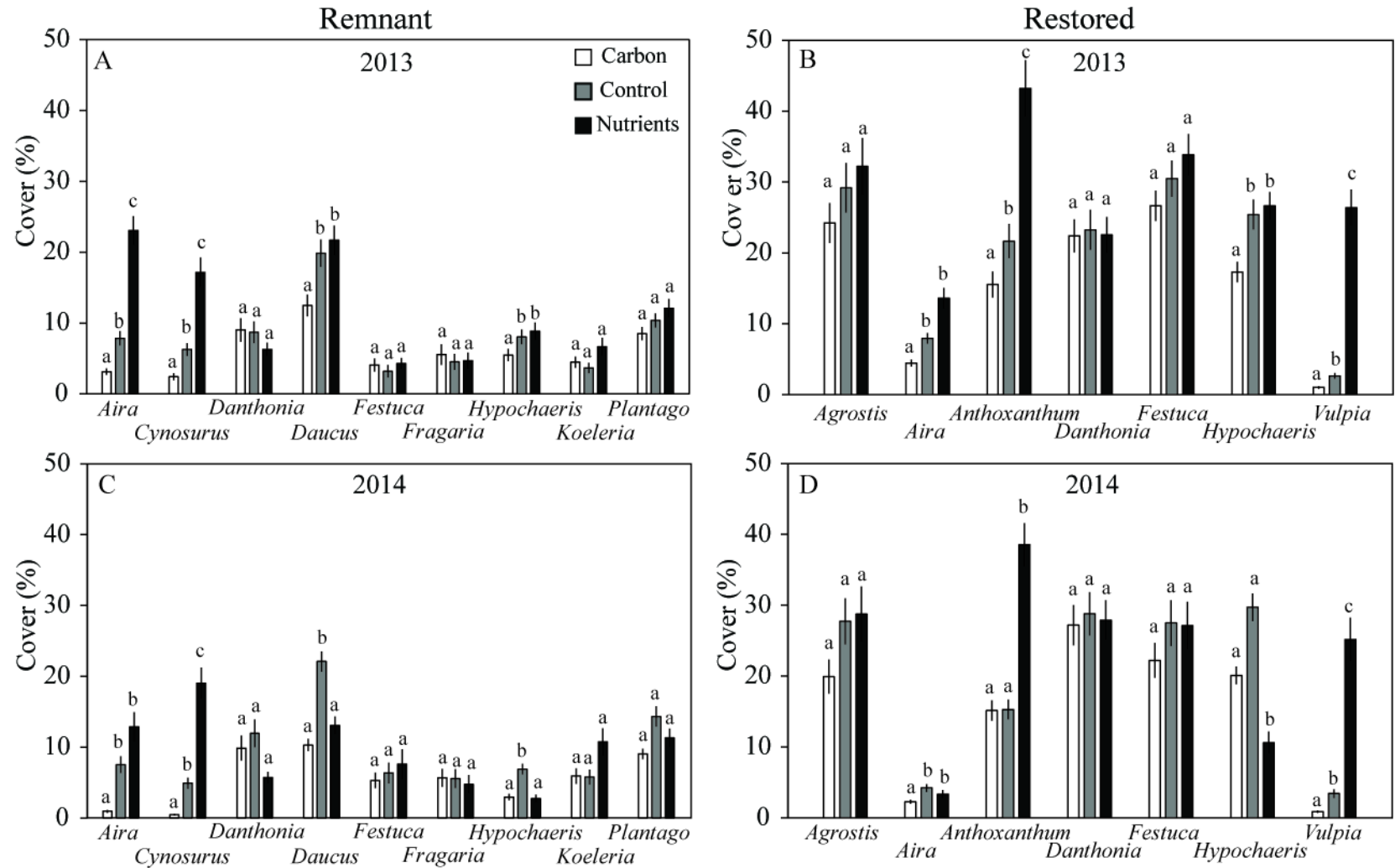
Figure 2.6: Mean cover of the most common species (listed by genus name) at each site in 2013 and 2014. Data shown are means across 40 plots of each nutrient treatment and standard error bars. Different letters indicate significant differences among treatments within a species and year at $p<0.016$ (Bonferroni corrected for three comparisons).

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Chapter 3:

Restoring the endangered golden paintbrush (*Castelleja levisecta*): Effects of community diversity and nutrient availability on reintroduction success

Abstract

Currently, over twenty thousand species are listed as threatened by the International Union for the Conservation of Nature and Natural Resources, and, as such, improving the success of species reintroductions has become increasingly important. In the U.S. Pacific Northwest, upland prairie habitats have become greatly reduced in extent, and with that many species have become threatened, including *Castilleja levisecta* (golden paintbrush), a hemiparasitic forb. We tested the effects of soil nutrient availability and plant community diversity on the reintroduction success of *C. levisecta*. Experimental plots were established with either carbon (sucrose) addition, ambient soil nutrients (controls), or nutrient addition (NPK fertilizer). Crossed with these treatments in a fully factorial design were seeding treatments of *C. levisecta* and thirteen native species, to increase the diversity of the plant community, providing host plants for *C. levisecta*. For two growing seasons, plant community surveys were conducted, and *C. levisecta* plants were counted and measured. At both sites, *C. levisecta* counts were lower in carbon addition plots compared to controls. In nutrient addition plots, *C. levisecta* counts were higher at one site but no different at the other, compared with controls, in the first year, and by the second year of the experiment there was either a negative or neutral effect of nutrient addition on *C. levisecta* numbers, compared with controls. The seeding of additional native species increased community richness but had little effect on diversity and no effect on *C. levisecta* establishment. However, *C. levisecta* numbers were correlated with measured levels of community diversity at one site. Our results suggest that neither carbon additions nor nutrient additions were beneficial to the reintroduction success of *C. levisecta*. Highly diverse communities, however, did correlate with higher *C. levisecta* numbers, and therefore seeding into diverse sites may increase reintroduction success of this threatened plant.

Introduction

Currently, over twenty thousand species are listed as threatened by the International Union for the Conservation of Nature and Natural Resources (IUCN 2014). This is in large part a result of the substantial alterations humans have made to the ecosystems and processes of the earth. We have transformed between thirty and fifty percent of the total land surface for uses such as agriculture, industry, urban development, and recreation (Vitousek et al. 1997). We have altered global biogeochemical cycles, changed the composition of the atmosphere, and facilitated the transport of biota to novel habitats (Chapin et al. 2000). With the threat of extinction existing for so many species, the field of restoration ecology has been growing rapidly and, with that, the question of how to improve the success of species reintroductions has become increasingly important (Godefroid et al. 2011).

In the U.S. Pacific Northwest (PNW), prairies are a critically endangered ecosystem (Noss 1995). The extreme habitat loss that has occurred has greatly affected many native prairie species, such as golden paintbrush (*Castilleja levisecta*), a threatened forb species endemic to the PNW. *Castilleja levisecta* has been completely extirpated from Oregon, and has only eleven remaining natural populations in Washington and British Columbia, Canada (USFWS 2000). Although only a small number of populations remain, there is still exceptionally high genetic diversity within these populations (Godt et al. 2005), providing a high potential for delisting as an endangered species (USFWS 2000).

An interesting restoration challenge for *C. levisecta* is that it is a hemiparasitic plant. While it does not require a host for survival, it benefits from an array of host species, which provide water, nutrients and secondary compounds through haustorial connections that *C. levisecta* makes with their roots (Lawrence & Kaye 2008). This hemiparasitic characteristic may

result in a strong relationship between the biodiversity of the community and reintroduction

success of *C. levisecta*. Experimental work has demonstrated that root hemiparasites benefit from

a high functional diversity of hosts (Joshi et al. 2000). Previous studies on *C. levisecta* have

evaluated particular host preferences and other habitat characteristics contributing to successful

reintroductions (Lawrence and Kaye 2008; Lawrence and Kaye 2009) but not biodiversity of the

plant community, which could be another key component to increase the success of

reintroductions.

In addition to the threat of severe habitat loss, threatened species, including *C. levisecta,*

also face competition from exotic species, which pose a major obstacle for restoration. Exotic

species are the second most common threat to vascular plant species, after habitat loss (Wilcove

and Master 2005) and they can be a major hindrance to species reintroductions (Pavlik et al.

1993; Jusaitis et al. 2004). Previous work on *C. levisecta* (Lawrence and Kaye 2009) found that

its performance decreased with increasing abundance of exotic plants, and suggested that *C.

levisecta* is a poor competitor with tall exotic grasses that out-compete it for light. Its survival

and fitness can be lower at sites dominated by exotic annuals (Lawrence and Kaye 2009),

suggesting that the reduction of these species could be beneficial for reintroductions.

Soil nutrient availability may also impact the success of reintroductions, by affecting both

the diversity and invasibility of the community. Increased nitrogen (N) loading in the soil can

contribute to the decline in biodiversity of a community (e.g. Bobbink et al. 1998; Tilman 1987;

Vitousek et al. 1997) and result in increased invasion of a community, especially by annual

grasses (Huenneke et al. 1990; Bobbink et al. 1998), both of which have potential negative

ramifications for the success of *C. levisecta* reintroductions. One way to potentially counter these

negative effects is by reducing the availability of nutrients to plants through carbon (C) addition

to the soil, which stimulates microbial activity and temporarily immobilizes N (Zink & Allen 1998; Blumenthal et al. 2003; Alpert 2010). Previous studies have often seen a reduction in exotic species cover with C addition (Averett et al. 2004; Prober et al. 2005; Eschen et al. 2007), suggesting that this could be a useful tool to increase success of *C. levisecta* reintroductions.

Additionally, due to its ability to parasitize neighboring plants, *C. levisecta* could also have an effect on communities to which it is reintroduced. Hemiparasitic plants are considered keystone species, due to their profound effect on ecosystems (Press & Phoenix 2005); they benefit from the presence of neighbors by drawing water and nutrients from their roots (Lawrence & Kaye 2008). Through this parasitism, they can affect the overall success and fitness of neighboring species. It has been experimentally shown that the presence of the root hemiparasite *Rhinanthus* reduces the above-ground biomass of neighboring species to between 40-60% of the biomass in control plots without *Rhinanthus* (Ameloot et al. 2005). Aboveground, hemiparasites also compete with their neighbors for light (Mudrak & Leps 2010). Clearly, these parasites influence competitive interactions within a community and could also increase diversity by lessening the competitive advantage of any one species. Their effects on species interactions may be more pronounced in low productivity systems (Watson 2009), where competition for resources is higher. Therefore, the reintroduction of *C. levisecta* may have a positive effect on the community at large by reducing competitive dominance of the species that it parasitizes.

Our experiment was designed to incorporate the reintroduction of *C. levisecta* with an evaluation of its effect on the plant community. We aimed to test whether alterations to soil nutrient availability or plant community diversity would influence reintroduction success of *C. levisecta*, while also examining whether its reintroduction changed the plant community. Specifically, we asked the following questions: 1) Does the mean number and size of *C. levisecta*

seedlings differ among plots that receive different nutrient treatments?; 2) Does the mean number and size of *C. levisecta* differ between plots that are seeded for increased diversity and those that are not?; 3) Does the total cover of vascular plants, community diversity or composition differ between plots to which *C. levisecta* is seeded and plots without *C. levisecta*?

From these questions we hypothesized that C addition would improve *C. levisecta* establishment by reducing cover of annual exotic species and increasing community diversity. A previous fertilization study with *C. levisecta* (Dunwiddie et al. 2013) found no effect of fertilization on seedling survival; however, we expected that with higher levels and longer durations of nutrient addition, establishment might be negatively affected as other species out-compete *C. levisecta*. We also hypothesized that increasing diversity of the community would increase *C. levisecta* establishment, by providing a greater functional diversity of hosts. Finally, we expected that *C. levisecta* would decrease vascular plant cover through its parasitism, and that community diversity would increase with the reintroduction of *C. levisecta*, as a result of it preferentially parasitizing the most dominant species in the community. To address these questions, we conducted our experiment at two Willamette Valley prairies, a priority region for the reintroduction of *C. levisecta* (USFWS 2000).

Methods

Study Sites

This experiment was conducted at William L. Finley National Wildlife Refuge (44° 24' 42"N, 123° 19' 53"W) and Baskett Slough National Wildlife Refuge (44° 57' 45"N, 123° 15' 39"W). Plots at both study sites were situated on well drained upland prairie habitat, at elevations ranging from approximately 90 – 116 m above sea level. The prairie at Finley NWR is located on

moderate to well-drained silt loams and clay loams of the Hazelair complex. The prairie at

Baskett Slough NWR is located on predominately silty clay loam soils of the Chehulpum series.

Climate in the Willamette Valley is characterized by warm, dry summers, and cool wet winters,

with average annual precipitation of 115 cm (PRISM Climate Group 2013). The plots at Finley

NWR were established in a restored prairie with lower plant diversity than Baskett Slough NWR,

where plots were in a remnant prairie fragment (henceforth referred to as the restored and

remnant sites, respectively). Details of the restoration at Finley NWR can be found in Chapter 2

and Clark & Wilson (2005). At each of the sites, the experiment was established into existing

vegetation dominated by a mixture of native and exotic grasses. More detailed descriptions of the

resident community can be found in Chapter 2 and full species lists can be found in Appendix

Tables 1 and 2. Prescribed burns were performed at each site in late October 2012.

Experimental Design

Experimental plots were established in November 2012. A common design was

maintained across both sites, consisting of 120 1x1 m plots per site, with a 1 m walkway between

plots and arranged in a blocked, factorial design. Each site had 10 blocks of 12 plots and each

plot within a block was randomly assigned one of 12 treatments. The treatment combinations

included three levels of soil nutrient availability: ambient (control), fertilized (nutrient addition),

and C addition plots (nutrient reduction). The nutrient treatments were crossed with four seeding

treatments, seeding of *C. levisecta*, seeding of 13 native species with the goal of increasing

diversity, seeding both *C. levisecta* and the other native species, or no seeding. The fertilized

plots received a 14-14-14 NPK slow release fertilizer (8.5% ammoniacal nitrogen, 5.5% nitrate

nitrogen, P_2O_5 and K_2O) at a rate of 90 g•m^{-2}yr^{-1} (12.6 g•m^{-2}yr^{-1} each of N, P, and K), applied

twice per year, 45g each in November 2012, 2013 and May 2013, 2014. Carbon was applied in the form of sucrose, with each plot receiving 1500 g•m^{-2}yr^{-1} of sucrose (630 g•m^{-2}yr^{-1} of C), applied in three 500 g applications per year; November 2012, 2013, February 2013, 2014, and May 2013, 2014. *C. levisecta* plots received 1 g of seed in November 2012 and an additional 1 g in December 2012. Plots seeded for increased diversity received 0.5 g of each of the following 13 species, seeded once in November 2012: *Achillea millefolium, Clarkia amoena, Danthonia californica, Elymus glaucus, Eriophyllum lanatum, Festuca idahoensis* ssp. *roemeri, Koeleria macrantha, Lomatium nudicaule , Plectritis congesta, Potentilla gracilis, Prunella vulgaris, Ranunculus occidentalis,* and *Sidalcea malviflora* ssp. *virgata.*

Vegetation Measurements

We conducted community surveys at the peak of the growing season in 2013 and 2014 (May and June). All vascular plant species in each plot were identified and the point intercept method was used to estimate abundance, using 100 points spaced every 10 cm in the 1 m^2 plots (Jonasson 1988). Abundance data were used to calculate the Shannon-Wiener Index of diversity (Oksanen et al. 2013). Plants of *C. levisecta* were counted and measured in May of 2013 and 2014. The size of each *C. levisecta* plant was measured as the number of stems and the height of each stem. Small seedlings (0-5 cm) were all counted as 2.5 cm.

Soil Measurements

Soil samples were collected from a subset of the plots twice over the course of the experiment to be analyzed for nutrient levels to determine whether the treatments had an effect on those levels. The procedure and results are presented in Chapter 2.

Data Analyses

Data were analyzed using R statistical software version 3.0.3 (R Core Team 2014). *C. levisecta* count data were analyzed using negative binomial generalized linear models (Venables & Ripley 2002). Each site and year was modelled separately. Model variables included the nutrient treatments, diversity seeding treatment, and block. To also test for a correlation between existing community diversity and *C. levisecta* counts, counts were also modeled as a function of nutrient treatment, the measured Shannon-Weiner diversity, and block. Linear mixed effects models were used to analyze the effect of the diversity seeding treatment and the *C. levisecta* seeding treatment on the total cover, richness and diversity of the plant community (Pinheiro 2013). Each site and year was modeled separately, with nutrient treatment, diversity seeding treatment and *C. levisecta* seeding treatment as fixed effects and block as a random effect. Permutational multivariate analysis of variance (perMANOVA) tests were used to test whether community composition was affected by either the paintbrush seeding or the diversity seeding (Oksanen et al. 2013). Analyses were performed for the two sites and years separately. The model included nutrient treatment and the seeding treatments, was stratified by block, and run for 999 permutations using the Bray-Curtis (Sørenson) index.

Results

Soil Nutrient availability

Soil nutrient availability was significantly increased by the nutrient addition treatments at both sites by the end of the study. Nutrient availability was decreased in the short term (1 month after treatment) with C addition at the remnant site, and only marginally reduced at the restored site. Full results are discussed in Chapter 2.

Effects of Nutrient Manipulation

Total counts of *C. levisecta* were significantly different among nutrient treatments at both sites and in both years (Table 3.1; Figure 3.1), however, the magnitude and direction of the effects varied by site and year. At both sites, and in both years, there were fewer *C. levisecta* in the C addition plots compared with the controls (p<0.001). At the remnant site in 2013 there were more *C. levisecta* in the nutrient addition plots compared to the controls (p<0.01). In 2014, there was no difference between the controls and nutrient addition plots (p=0.85). At the restored site in 2013 there was no difference between the nutrient addition and control plots (p=0.77), and in 2014, there were fewer *C. levisecta* in the nutrient addition plots compared to the controls (p<0.01). Estimates and confidence intervals can be found in Appendix Tables 9 and 10.

The size of *C. levisecta* seedlings was significantly different among nutrient treatments at the remnant site in 2013, but not in 2014 or at the restored site (Figure 3.2, Table 3.2). At the remnant site in 2013 seedlings in the nutrient addition plots were larger than in control plots and carbon addition plots (p<0.001).

Effects of Diversity Seeding

The diversity seeding treatments did not have an effect on total cover at either site or in either year (Tables 3.3; 3.4). The seeding treatment significantly increased the richness of the community compared to the unseeded plots at both sites and in both years (Table 3.3; 3.4). Shannon-Weiner diversity was increased significantly by the seeding treatment at the remnant site in 2014 and marginally in 2013, but was not affected at the restored site (Table 3.3; 3.4). Overall community composition was significantly affected by the diversity seeding treatment at the remnant site in 2013 (pseudo-F=1.33, p=0.04) but not in 2014 (pseudo-F=0.93, p=0.27).

Community composition was not significantly affected at the restored site in 2013 (pseudo-F=1.22, p=0.14) or 2014 (pseudo-F=0.90, p=0.28).

While the seeding treatments did have some minor effects on the community, there was no significant effect of the seeding treatments on *C. levisecta* counts in either year, at either site (Table 3.1). There was, however, a relationship between measured community diversity and *C. levisecta* counts, which often depended on soil nutrient availability (Table 3.5). At the remnant site, there was an interaction between the effect of nutrient treatment and community diversity on *C. levisecta* counts, significantly in 2014 and marginally in 2013 (Figure 3.3; Table 3.5). The relationship between community diversity and *C. levisecta* counts was only positive in the nutrient addition plots. At the restored site, there was a marginally significant interaction in 2014, but not in 2013. In all nutrient conditions, *C. levisecta* counts increased with increasing community diversity, however, the rate of increase differed by nutrient treatment.

Effects of C. levisecta on the Community

Seeding of *C. levisecta* did not significantly affect total cover at either site in either year (Table 3.3; 3.4). Seeding of *C. levisecta* increased richness compared to unseeded plots by about one species at the remnant site in 2013 but had no significant effect on richness in 2014 or at the restored site in either year (Table 3.3; 3.4). Shannon-Weiner diversity of the plant community was no different between *C. levisecta* seeded and unseeded plots at either site or in either year (Table 3.3; 3.4). *C. levisecta* seeding also did not affect community composition at the remnant site in 2013 (pseudo-F=1.1, p=0.10) or 2014 (pseudo-F=0.67, p=0.52) or at the restored site in 2013 (pseudo-F=0.52, p=0.60) or 2014 (pseudo-F=0.29, p=0.88).

Discussion

      Our experiment determined that soil nutrient manipulations had significant effects on *C. levisecta* numbers, but less of an effect on the mean size of the plants. Additionally, we found that seeding for increased diversity had only minimal effects on the plant community within two years after seeding and that this did not provide a benefit for *C. levisecta* establishment. Counts of *C. levisecta* were, however, positively correlated with community diversity at the restored site, and the strength of the relationships depended on nutrient treatment. Finally, we did not detect an effect on the plant community as a result of reintroduction *C. levisecta* within two years.

Effects of Nutrient Availability

      We predicted that C addition would have a positive effect on *C. levisecta* establishment; we found, however, that it negatively impacted *C. levisecta*. Our prediction was based on previous work (Lawrence & Kaye 2009) that showed a negative correlation between success of *C. levisecta* and the abundance of non-native plants. Others have also observed negative effects of non-native species on the success of reintroductions (Pavlik et al. 1993; Jusaitis et al. 2004), suggesting that reducing these species could be beneficial. C addition has often been successful in reducing cover of non-native species (e.g. Blumenthal et al. 2003; Prober et al 2005; Eschen et al. 2007), and we did see some reductions in non-native cover with C addition (see Chapter 2), however, these did not benefit establishment of *C. levisecta*. It is likely that the reduction in available nutrients caused by C additions outweighed the positive effects of reduced competition from non-native species, and therefore did not provide a benefit to *C. levisecta*. Seeding following the cessation of C addition may yield better results; if a window of opportunity for

establishment can be created with C addition, by reducing non-native cover, then perhaps timing seeding after C treatments cease would result in a benefit to *C. levisecta*.

Our nutrient addition treatments had a positive effect on *C. levisecta* counts at the remnant site in the short term; however, the effect was either negative or neutral by the second year of study at both sites. In a previous fertilization study, *C. levisecta* survival did not differ between fertilized and control plots (Dunwiddie et al. 2013). This difference in results could have been due to our higher dose and longer term duration of application, but additionally the different results observed between our two sites and their site suggests also that the existing plant community may play an important role in the response of *C. levisecta* to nutrient addition. Since increased nutrient loading in the soil can lead to decreased biodiversity (Tilman 1987; Vitousek et al. 1997; Bobbink et al. 1998), and an increase in exotic species (Huenneke et al. 1990; Bobbink et al. 1998), we would expect the effect of fertilization on *C. levisecta* to become or remain negative over time.

Effects of Plant Community Diversity

Our second treatment aimed at increasing the reintroduction success of *C. levisecta* was to experimentally increase the diversity of the community, providing *C. levisecta* a greater diversity of hosts to parasitize. While the seeding treatments did increase the richness of our communities, the seeded plants were small and not abundant so had a negligible effect on Shannon-Weiner diversity and did not result in a significantly different community composition over two years. We did not see an effect of these treatments on the establishment of *C. levisecta*. While we were unsuccessful at experimentally increasing community diversity to a biologically meaningful degree, some plots were naturally more diverse than others so we also tested for

correlation between the measured diversity of the community at each site and *C. levisecta* counts. Previous work on the root hemiparasite *Rhinanthus alectorolophus* found biomass and inflorescence length to be positively affected by increases in functional diversity of hosts, establishment was marginally affected and survival was actually lower with more functional diversity (Joshi et al. 2000). We found positive correlations between diversity and *C. levisecta* counts across nutrient treatments at the restored site, our lower diversity site, although the relative effect varied by nutrient level. At the remnant site there was an interaction between diversity and nutrient treatment, and higher diversity was positively correlated with *C. levisecta* counts only in the nutrient addition plots. Although these results are correlative, they suggest that seeding into more diverse communities may provide greater chance of reintroduction success of *C. levisecta*. More *C. levisecta* established at our more diverse site; while this likely also relates to other site differences, there was also a within-site relationship between diversity and plant counts at the less diverse site. This relationship may suggest a threshold of diversity above which further increases no longer benefit *C. levisecta*; at the remnant site diversity was higher across almost the entire site than at the restored site and it was at the remnant site that we observed little to no relationship between diversity and *C. levisecta* counts.

Effects of C. levisecta

Our last objective was to study the effects of reintroducing *C. levisect*a on plant community cover, composition, and diversity. Numerous studies have examined the interactions of parasitic plants with the ecosystem, and have found them to have significant effects, making them keystone species in a plant community (Press & Phoenix 2005). Many studies have found that parasitic plants reduce community productivity (Bardgett et al. 2006; Mudrák & Lepš 2010;

Borowicz & Armstrong 2012). Additionally, studies have found both a positive (Pennings & Callaway 1996; Callaway & Pennings 1998; Westbury & Dunnett 2000; Bardgett et al. 2006) and more rarely, negative, (Gibson &Watkinson 1989) effect on diversity as a result of parasitic plants, depending on whether the host species are dominant in the system or rare. We predicted that *C. levisecta* would reduce the total plant cover and increase diversity, because *Castilleja* species are usually generalist parasites (Heckard 1962; Dobbins and Kuijt 1973), which might be likely to preferentially parasitize the most dominant species in the community. In two years, however, we saw no significant changes in total cover, Shannon-Weiner diversity or community composition with the addition of *C. levisecta*, and effects did not depend on nutrient level. In the first year of study, most of the plants remained relatively small, less than 5 cm in height, which could explain why they did not have effects on the communities. In the second year of the experiment, *C. levisecta* numbers were much lower, and although the plants were generally larger, they still did not have detectable effects on communities. Some studies that have found large effects of parasites on their communities have been removal studies (e.g. Mudrák & Lepš 2010; Borowicz & Armstrong 2012), but effects have also been observed where the parasite was seeded into an experimental community (Joshi et al. 2000). Joshi et al. (2000) also found, however, that the reduction in biomass of the community was greater in monocultures than in more functionally diverse communities. Therefore, the high diversity of our communities could contribute to why we saw no effect. Additionally, Joshi et al. (2000) had a maximum mean density of 161 seedlings per m^2, which was much higher than we observed, suggesting the number of individuals in our study may not have been sufficient to affect the host community.

Conclusions

Overall, this experiment suggests that C addition does not have any immediate positive effects on *C. levisecta* success, and that nutrient addition may have short term positive effects, but these effects are not sustained in the long term. Our results also suggest that reintroduction may be more successful when the host community is more diverse, and that nutrient availability may affect the extent to which diversity is important. In two years, *C. levisecta* also did not alter the community composition at the densities with which we established it. Future work should examine whether seeding *C. levisecta* after the cessation of C addition would yield different results than observed here. The longer-term role of *C. levisecta* in structuring its host community is another area that could be explored, to test whether, over the longer term, the community is affected, or whether higher densities and larger plants of *C. levisecta* may have effects. Our results suggest that, while nutrient manipulations may not facilitate reintroductions of *C. levisecta,* seeding into highly diverse plant communities may enhance establishment.

Table 3.1 Results of generalized linear models testing the effect of the soil nutrient manipulations and diversity seeding treatments on *C. levisecta* counts

			Df	Deviance	Resid Df	Resid. Dev	Pr (>Chi)
Remnant	2013	Nutrient treatment	2	120.79	57	124.55	<0.001
		Diversity treatment	1	0.05	56	124.50	0.83
		Block	9	61.42	47	63.08	<0.001
		Nutrient*Diversity	2	1.76	45	61.33	0.42
	2014	Nutrient treatment	2	31.62	57	112.54	<0.001
		Diversity treatment	1	0.42	56	112.12	0.52
		Block	9	37.50	47	74.61	<0.001
		Nutrient*Diversity	2	2.65	45	71.97	0.27
Restored	2013	Nutrient treatment	2	27.92	57	95.91	<0.001
		Diversity treatment	1	0.42	56	95.49	0.51
		Block	9	32.24	47	63.25	<0.001
		Nutrient*Diversity	2	0.34	45	62.91	0.85
	2014	Nutrient treatment	2	30.89	57	118.35	<0.001
		Diversity treatment	1	2.87	56	115.48	0.09
		Block	9	45.20	47	70.28	<0.001
		Nutrient*Diversity	2	4.97	45	65.31	0.08

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Table 3.2 Results of linear mixed models testing the effect of the nutrient treatment and diversity seeding treatment on *C. levisecta* heights

Remnant				Restored			
2013		2014		2013		2014	
F	p	F	p	F	p	F	p
Effect of Nutrient Treatment (2, 45 d.f.)				Effect of Nutrient Treatment (2, 45 d.f.)			
17.27	<0.001	2.27	0.12	2.51	0.09	1.78	0.18
Effect of Diversity Seeding (1, 45 d.f.)				Effect of Diversity Seeding (1, 45 d.f.)			
1.66	0.20	3.03	0.09	2.74	0.11	0.49	0.49

Table 3.3 Results of linear mixed models testing the effect of the diversity and *C.levisecta* seeding treatments on total cover, community richness and Shannon-Weiner diversity

	Remnant				Restored			
	2013		2014		2013		2014	
	F	p	F	p	F	p	F	p
	Effect of Diversity Seeding				Effect of Diversity Seeding			
Total cover	1.64	0.20	0.39	0.53	0.01	0.93	0.06	0.81
Richness	48.14	<0.001	29.30	<0.001	207.83	<0.001	78.32	<0.001
Diversity	3.57	0.06	5.10	0.03	0.00	0.98	0.002	0.97
	Effect of *C. levisecta* Seeding				Effect of *C. levisecta* Seeding			
Total cover	0.36	0.55	0.09	0.77	0.05	0.82	2.39	0.13
Richness	4.87	0.03	0.58	0.45	1.07	0.30	1.25	0.27
Diversity	0.19	0.66	0.80	0.37	0.10	0.75	0.65	0.42

*For each comparison there was 1, 99 df

Table 3.4 Mean total vascular plant cover, richness, and Shannon-Weiner diversity by seeding treatment

	Remnant				Restored			
	2013		2014		2013		2014	
	Control	Seeded	Control	Seeded	Control	Seeded	Control	Seeded
	Effect of Diversity Seeding				Effect of Diversity Seeding			
Total cover	125	128	135	135	164	162	150	153
Richness	24	28	25	29	14	19	15	19
Diversity	2.44	2.51	2.35	2.44	1.81	1.81	1.78	1.78
	Effect of *C. levisecta* Seeding				Effect of *C. levisecta* Seeding			
Total cover	126	127	135	135	163	163	150	153
Richness	25	27	27	27	16	16	16	17
Diversity	2.48	2.47	2.42	2.38	1.82	1.81	1.79	1.76

Table 3.5 Results of generalized linear models testing the effect of the soil nutrient manipulations and Shannon-Weiner diversity of the plant community on *C. levisecta* counts

			Df	Deviance	Resid Df	Resid. Dev	Pr (>Chi)
Remnant	2013	Nutrient treatment	2	136.00	57	139.74	<0.001
		Community diversity	1	1.19	56	138.55	0.28
		Block	9	72.28	47	66.27	<0.001
		Nutrient*Diversity	2	4.97	45	61.30	0.08
	2014	Nutrient treatment	2	37.34	57	129.24	<0.001
		Community diversity	1	3.24	56	126.01	0.07
		Block	9	43.90	47	82.10	<0.001
		Nutrient*Diversity	2	8.15	45	73.95	0.02
Restored	2013	Nutrient treatment	2	31.20	57	106.51	<0.001
		Community diversity	1	21.02	56	85.50	<0.001
		Block	9	21.03	47	64.46	0.01
		Nutrient*Diversity	2	2.24	45	62.22	0.33
	2014	Nutrient treatment	2	41.64	57	148.14	<0.001
		Community diversity	1	52.76	56	95.38	<0.001
		Block	9	18.97	47	76.41	0.025
		Nutrient*Diversity	2	5.20	45	71.21	0.07

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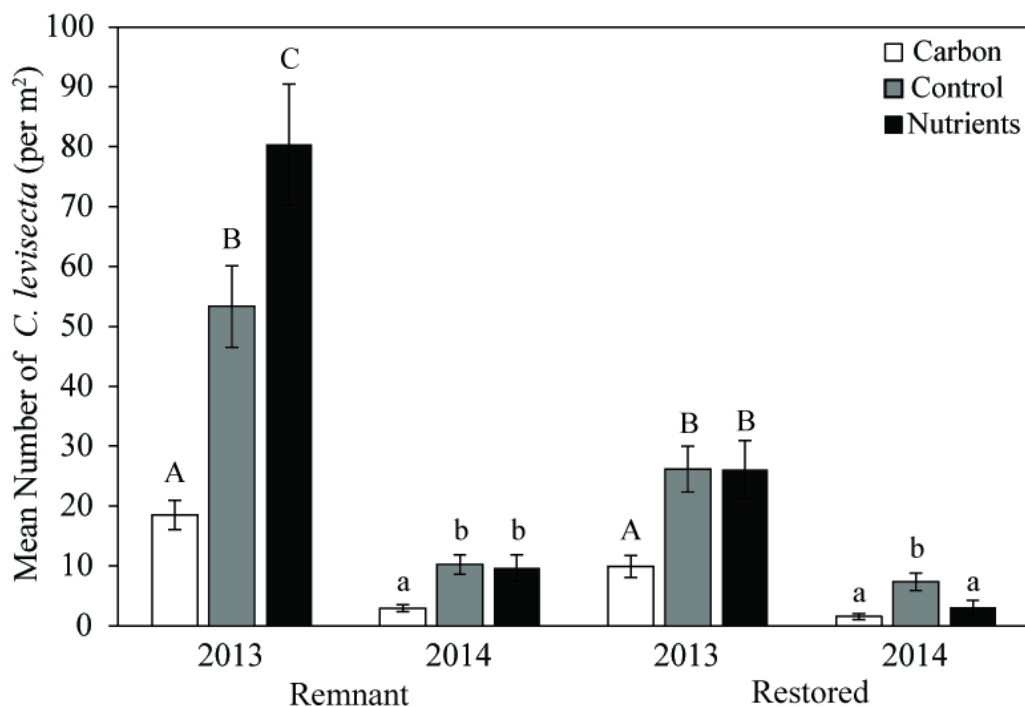
Figure 3.1: Mean *C. levisecta* counts at the remnant and restored sites in 2013 and 2014. Data are shown as means across 20 plots of each nutrient level and standard error. Different letters within a site and year were significantly different at p<0.016 (Bonferroni corrected for three comparisons).
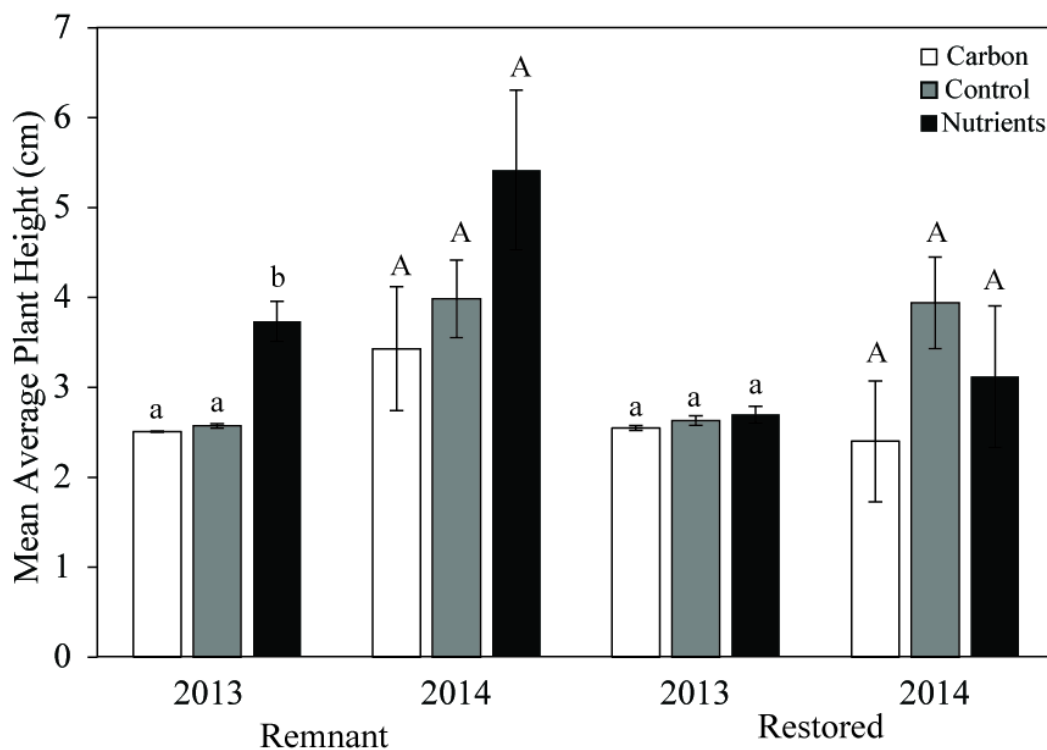
Figure 3.2: Mean average *C. levisecta* height at the remnant and restored sites in 2013 and 2014. Data are shown as means across 20 plots of each nutrient level and standard error. Different letters within a site and year were significantly different at p<0.016 (Bonferroni corrected for three comparisons).
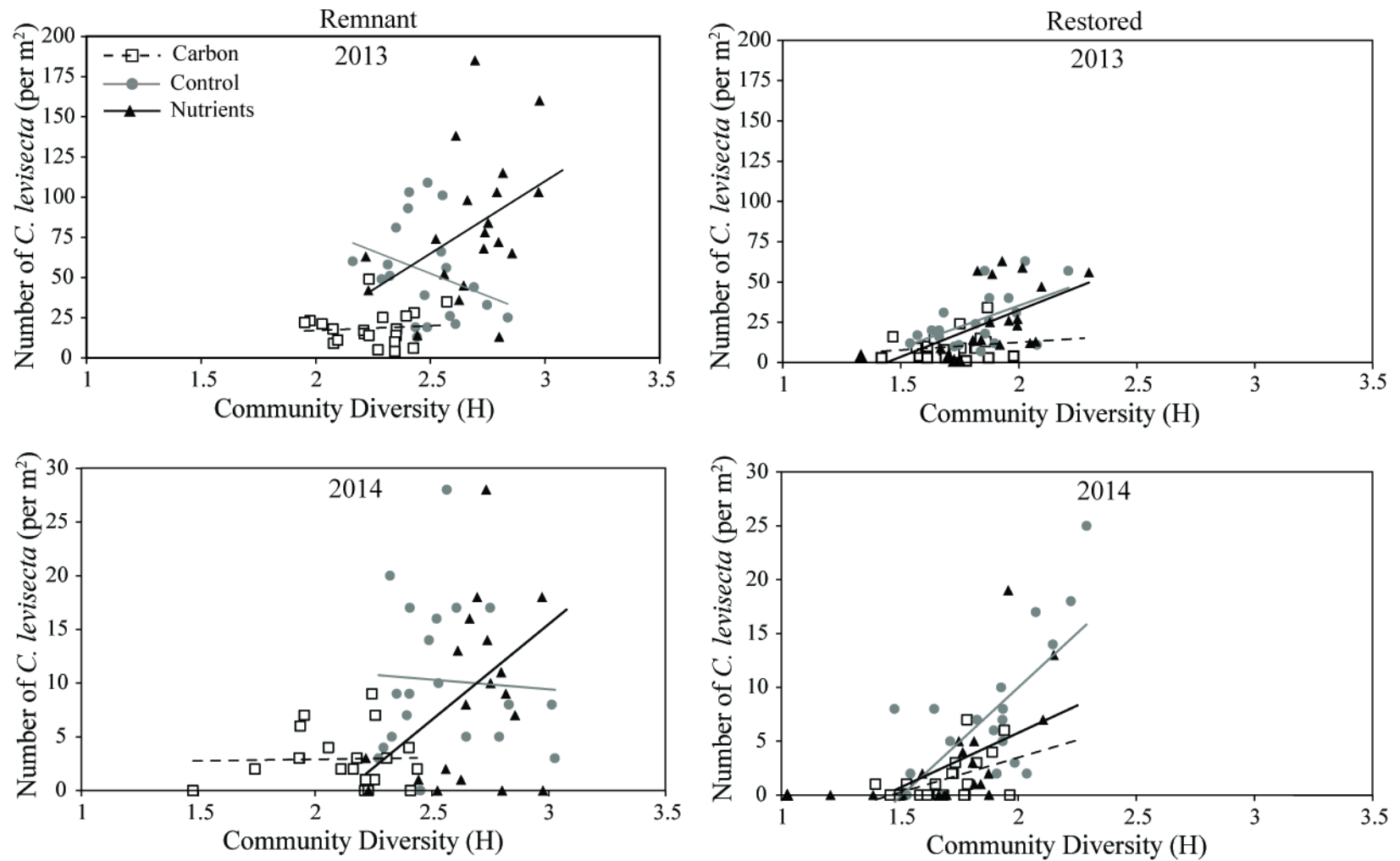
Figure 3.3 Relationship between counts of *C. levisecta* and community diversity (measured as Shannon-Weiner diversity). Trend lines are indicated for each of the nutrient levels separately. Scales differ on the y-axis between years.

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Chapter 4:
Conclusions

Soil Nutrient Availability

Control of Exotic Species

Our findings suggest that nutrient enrichment increases the abundance of exotic plants compared to native plants, primarily grasses, and that C addition can be an effective way to counter these effects. Carbon addition was effective at reducing vascular plant cover, specifically exotics, at two sites with very different site histories. Not all exotic species were affected by C addition, suggesting that the success of this control method may depend on the target species. Some exotic species do not have a stronger response to changes in available N than natives (Lowe et al. 2002, Blumenthal et al. 2003, Corbin and D'Antonio 2004), and site factors, including, species present, and the seed bank composition, likely influence the efficacy of C addition. These and other results (Mitchell and Bakker 2011), suggest that at least in PNW prairies, if the system is dominated by exotic annual grasses, or exotic forbs, C addition is more likely to be successful than if exotic perennial grasses are the problem. We would therefore recommend C addition as a restoration tool when these are the problem species groups; however, additional long term studies need to assess the community changes that occur following the cessation of treatments.

Carbon Addition as a Restoration Tool

While C addition was effective at reducing exotic plant cover, we did not address the persistence of the effects on the community multiple years post-treatment. In many studies, C is applied in only one year (Averett et al. 2004; Kirkpatrick & Lubetkin 2011; Mitchell & Bakker 2011) or for a few years (Prober et al. 2005; Eschen et al. 2007), and even when applied long term, the soil and community effects do not persist for long (Burke et al. 2013). Our study

showed that with continued treatment, effects on the community persisted; however, further research is needed on the maintenance of these effects long-term. Other studies (Prober et al. 2005) have also found that by maintaining treatments, effects on the plant community can be maintained; sometimes, however, when treatments cease there can be a marked post-treatment recovery of the exotic species (Kirkpatrick & Lubetkin 2011). This suggests that, while C addition can initially be effective, when treatments cease there is a risk of the community being reinvaded by exotic species. In another study, after 8 years of continued sucrose treatments, a post-treatment soil N level of 250% the controls was observed in the year following the cessation of treatments (Burke et al. 2013). Soil N may not remain reduced long term and communities may not remain affected post-treatment. Seeding or planting of native species immediately after C additions cease may be a way to minimize the reinvasion of exotics. Carbon addition may also be useful in combination with other treatments, such as herbicides, in areas where exotic annual and perennial grasses dominate.

One additional issue with C addition treatments involves scaling them up to larger scale restorations (Perry et al. 2010). Our results, combined with other studies, suggest that high levels and frequent treatments may be necessary to achieve the desired results. At the rates we applied, 15 000 kg•ha^{-1}yr^{-1} would be required to achieve effects similar to those that we observed. If applied in well-timed increments, lower rates might be effective; further research should explore ways to scale these treatments up to large scales. While there are still some obstacles to using C addition in managing exotic species, it has shown great potential in many regions and should be studied further as a restoration tool.

Recovery of C. levisecta

Our soil nutrient manipulations did not provide evidence that either C addition or nutrient addition would have a long term positive effect on reintroduction of *C. levisecta*. C addition reduced *C. levisecta* numbers in both years and at both sites, and nutrient addition had mixed effects – positive, neutral and negative, on *C. levisecta* numbers. These results, along with the results from an additional C. *levisecta* fertilization study where no effect was seen, (Dunwiddie et al. 2013), suggest that fertilization is not a useful restoration tool for *C. levisecta,* and may actually be detrimental to restoration.

Effects of Plant Community Diversity

Our seeding treatments did not significantly increase community diversity or alter community composition. However, a positive correlation was observed between Shannon-Weiner diversity and counts of *C. levisecta* at the restored site. While further research is need to confirm whether this correlative relationship is causative, previous work has also found a positive relationship between functional diversity of the plant community and the success of hemiparasitic plants (Joshi et al 2000). Thus, we recommend seeding *C. levisecta* into highly diverse sites as a way increase reintroduction success for this species.

Effects of *C. levisecta*

Within two years, *C. levisecta* did not exert a measureable effect on the vascular plant community. We did not observe changes in total vascular plant cover, or diversity, as others have observed (Callaway & Pennings 1998; Joshi et al. 2000; Westbury & Dunnett 2000; Bardgett et al. 2006; Mudrák & Lepš 2010; Borowicz & Armstrong 2012). It is possible that we observed no

measurable effects as a result of low establishment of plants, lack of root connections with the host species, or the relatively short time period of observation after reintroduction. Future work should explore the effects of *C. levisecta* over longer time spans, and with a higher density of established plants.

Overall Conclusions

In sum, C addition was shown to be an effective tool for reducing exotic species cover to a greater degree than native cover. We therefore recommend C addition for the control of exotic species, especially annual grasses. However, further long-term work should assess post-treatment reinvasion of exotics, and explore ways to maintain the positive effects of C addition. C addition and nutrient addition, however, were not highly beneficial to the reintroduction success of *C. levisecta*. *C. levisecta* did appear to benefit from a highly diverse plant community; we therefore recommend seeding *C. levisecta* into diverse sites to increase the likelihood of establishing viable populations.

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Appendices

Table A1: Remnant Site Full Species List

Scientific Name	Common Name	Native Status	Growth Habit	Family	Duration
Achillea millefolium L.	Common yarrow	N	F	Asteraceae	P
Agrostis stolonifera L.	Creeping bentgrass	I	G	Poaceae	P
Aira caryophyllea L.	Silver hairgrass	I	G	Poaceae	A
Amelanchier alnifolia (Nutt.) Nutt. ex M. Roem.	Saskatoon serviceberry	N	F/T	Rosaceae	P
Aphanes arvensis L.	Field parsley piert	I	F	Rosaceae	A
Aquilegia formosa Fisch. ex DC.	Western columbine	N	F	Ranunculaceae	P
Arrhenatherum elatius (L.) P. Beauv. ex J. Presl & C. Presl	Tall oatgrass	I	G	Poaceae	P
Briza minor L.	Little quakinggrass	I	G	Poaceae	A
Bromus carinatus Hook. & Arn.	California brome	N	G	Poaceae	A/P
Bromus hordeaceus L.	Soft brome	I	G	Poaceae	A
Bromus sterilis L.	Poverty brome	I	G	Poaceae	A
Calochortus tolmiei Hook. & Arn.	Tolmie star-tulip	N	F	Liliaceae	P
Calystegia atriplicifolia Hallier f. ssp. atriplicifolia	Nightblooming false bindweed	N	F	Convolvulaceae	P
Carex tumulicola Mack.	Splitawn sedge	N	G	Cyperaceae	P
Castilleja levisecta Greenm.	Golden paintbrush	N	F	Scrophulariaceae	P
Centaurium erythraea Rafn	European centaury	I	F	Gentianaceae	A/B
Cerastium glomeratum Thuill.	Sticky chickweed	I	F	Caryophyllaceae	A
Cirsium arvense (L.) Scop.	Canada thistle	I	F	Asteraceae	P
Clarkia amoena (Lehm.) A. Nelson & J.F. Macbr.	Farewell to spring	N	F	Onagraceae	A
Clarkia purpurea (W. Curtis) A. Nelson & J.F. Macbr.	Winecup clarkia	N	F	Onagraceae	A
Crataegus douglasii Lindl.	Black hawthorn	I	S/T	Rosaceae	P
Crataegus monogyna Jacq.	Oneseed hawthorn	I	F	Rosaceae	P
Crepis capillaris (L.) Wallr.	Smooth hawksbeard	I	F	Asteraceae	A/B
Cynosurus echinatus L.	Bristly dogstail grass	I	G	Poaceae	A
Dactylis glomerata L.	Orchardgrass	I	G	Poaceae	P
Danthonia californica Bol.	California oatgrass	N	G	Poaceae	P
Daucus carota L.	Queen Anne's lace	I	F	Apiaceae	B
Delphinium menziesii DC.	Menzies' larkspur	N	F	Ranunculaceae	P
Elymus glaucus Buckley	Blue wildrye	N	G	Poaceae	P
Eriophyllum lanatum (Pursh) Forbes	Woolly sunflower	N	F/SS	Asteraceae	A/P

Table A1: continued

Festuca idahoensis Elmer ssp. roemeri (Pavlick) S. Aiken	Roemer's fescue	N	G	Poaceae	P
Fragaria virginiana Duchesne	Virginia strawberry	N	F	Rosaceae	P
Galium parisiense L.	Wall bedstraw	I	F	Rubiaceae	A
Geranium dissectum L.	Cutleaf geranium	I	F	Geraniaceae	A/B
Holcus lanatus L.	Common velvetgrass	I	G	Poaceae	P
Hypericum perforatum L.	common St. Johnswort	I	F	Clusiaceae	P
Hypochaeris radicata L.	Hairy cat's ear	I	F	Asteraceae	P
Koeleria macrantha (Ledeb.) Schult.	Prairie Junegrass	N	G	Poaceae	P
Lathyrus sphaericus Retz.	Grass pea	I	F/V	Fabaceae	A
Linum bienne Mill.	Pale flax	I	F	Linaceae	A/B/P
Lomatium macrocarpum (Nutt. ex Torr. & A. Gray) J.M. Coult. & Rose	Bigseed biscuitroot	N	F	Apiaceae	P
Lomatium nudicaule (Pursh) J.M. Coult. & Rose	Barestem biscuitroot	N	F	Apiaceae	P
Lotus micranthus Benth.	Desert deervetch	N	F	Fabaceae	A
Lupinus oreganus A. Heller var. kincaidii C.P. Sm.	Kincaid's lupine	N	F	Fabaceae	P
Luzula campestris (L.) DC.	Field woodrush	I	G	Juncaceae	P
Moenchia erecta (L.) G. Gaertn., B. Mey. & Scherb.	Upright chickweed	I	F	Caryophyllaceae	A
Myosotis discolor Pers.	Forget-me-not	I	F	Boraginaceae	A/P
Parentucellia viscosa (L.) Caruel	Yellow glandweed	I	F	Scrophulariaceae	A
Plantago lanceolata L.	Narrowleaf plantain	N	F	Plantaginaceae	A/B/P
Plectritis congesta (Lindl.) DC.	Shortspur seablush	N	F	Valerianaceae	A
Poa pratensis L.	Kentucky bluegrass	I/N	G	Poaceae	P
Potentilla gracilis Douglas ex Hook.	Slender cinquefoil	N	F/SS	Rosaceae	P
Prunella vulgaris L.	Common selfheal	N	F	Lamiaceae	P
Ranunculus occidentalis Nutt.	Western buttercup	N	F	Ranunculaceae	P
Rosa rubiginosa L.	Sweetbriar rose	I	SS	Rosaceae	P
Rumex acetosella L.	Common sheep sorrel	I	F	Polygonaceae	P
Sanicula bipinnatifida Douglas ex Hook.	Purple sanicle	N	F	Apiaceae	P
Schedonorus arundinaceus (Schreb.) Dumort., nom. cons.	Tall fescue	I	G	Poaceae	P
Senecio jacobaea L.	Stinking willie	I	F	Asteraceae	P

Table A1: continued

Sherardia arvensis L.	Blue fieldmadder	I	F	Rubiaceae	A
Sisyrinchium idahoense E.P. Bicknell	Idaho blue-eyed grass	N	F	Iridaceae	P
Symphyotrichum hallii (A. Gray) G.L. Nesom	Hall's aster	N	F	Asteraceae	P
Taeniatherum caput-medusae (L.) Nevski	Medusahead	I	G	Poaceae	A
Toxicodendron diversilobum (Torr. & A. Gray) Greene	Pacific poision oak	N	S/V	Anacardiaceae	P
Trifolium dubium Sibth.	Suckling clover	I	F	Fabaceae	A
Trifolum sp.	Clover	I	F	Fabaceae	A
Triphysaria pusilla (Benth.) T.I. Chuang & Heckard	Dwarf owl's-clover	N	F	Scrophulariaceae	A
Valerianella locusta (L.) Lat.	Lewiston cornsalad	I	F	Valerianaceae	A
Veronica arvensis L.	Corn speedwell	I	F	Scrophulariaceae	A
Vicia sativa L.	Garden vetch	I	F/V	Fabaceae	A
Vicia hirsuta (L.) Gray	Tiny vetch	I	F/V	Fabaceae	A
Vicia tetrasperma (L.) Schreb.	lentil vetch	I	F/V	Fabaceae	A
Vicia villosa Roth	Winter vetch	I	F/V	Fabaceae	A/B/P
Vulpia bromoides (L.) Gray	Brome fescue	I	G	Poaceae	A
Zigadenus glaberrimus Michx.	Sandbog deathcamas	N	F	Liliaceae	P

*Native Status: I=Introduced, N=Native, Growth Form: G=Graminoid, F=Forb, S=Shrub, SS=Subshrub, T=Tree, V=Vine, Duration: A=Annual, B=Biennial, P=Perennial

Table A2: Restored Site Full Species List

Scientific Name	Common Name	Native Status	Growth Habit	Family	Duration
Achillea millefolium L.	Common yarrow	N	F	Asteraceae	P
Agrostis stolonifera L.	Creeping bentgrass	I	G	Poaceae	P
Aira caryophyllea L.	Silver hairgrass	I	G	Poaceae	A
Anthoxanthum odoratum L.	Sweet vernalgrass	I	G	Poaceae	P
Briza minor L.	Little quakinggrass	I	G	Poaceae	A
Bromus hordeaceus L.	Soft brome	I	G	Poaceae	A
Castilleja levisecta Greenm.	Golden Indian paintbrush	N	F	Scrophulariaceae	P
Centaurium erythraea Rafn	European centaury	I	F	Gentianaceae	A/B
Cerastium glomeratum Thuill.	Sticky chickweed	I	F	Caryophyllaceae	A
Clarkia amoena (Lehm.) A. Nelson & J.F. Macbr.	Farewell to spring	N	F	Onagraceae	A
Crepis capillaris (L.) Wallr.	Smooth hawksbeard	I	F	Asteraceae	A/B
Danthonia californica Bol.	California oatgrass	N	G	Poaceae	P
Daucus carota L.	Queen Anne's lace	I	F	Apiaceae	B
Elymus glaucus Buckley	Blue wildrye	N	G	Poaceae	P
Eriophyllum lanatum (Pursh) Forbes	Woolly sunflower	N	F/SS	Asteraceae	A/P
Festuca idahoensis Elmer ssp. roemeri (Pavlick) S. Aiken	Roemer's fescue	N	G	Poaceae	P
Galium parisiense L.	Wall bedstraw	I	F	Rubiaceae	A
Geranium dissectum L.	Cutleaf geranium	I	F	Geraniaceae	A/B
Holcus lanatus L.	Common velvetgrass	I	G	Poaceae	P
Hypericum perforatum L.	Common St. Johnswort	I	F	Clusiaceae	P
Hypochaeris radicata L.	Hairy cat's ear	I	F	Asteraceae	P
Koeleria macrantha (Ledeb.) Schult.	Prairie Junegrass	N	G	Poaceae	P
Lathyrus angulatus L.	Angled pea	I	F/V	Fabaceae	A
Lomatium nudicaule (Pursh) J.M. Coult. & Rose	Barestem biscuitroot	N	F	Apiaceae	P
Lotus micranthus Benth.	Desert deervetch	N	F	Fabaceae	A
Lotus unifoliolatus (Hook.) Benth.	American bird's-foot trefoil	N	F	Fabaceae	A
Lupinus albicaulis Douglas	Sicklekeel lupine	N	F	Fabaceae	P
Luzula campestris (L.) DC.	Field woodrush	I	G	Juncaceae	P
Myosotis discolor Pers.	Changing forget-me-not	I	F	Boraginaceae	A/P
Parentucellia viscosa (L.) Caruel	Yellow glandweed	I	F	Scrophulariaceae	A
Plantago lanceolata L.	Narrowleaf plantain	N	F	Plantaginaceae	A/B/P
Plectritis congesta (Lindl.) DC.	Shortspur seablush	N	F	Valerianaceae	A

Table A2: continued

Poa pratensis L.	Kentucky bluegrass	I/N	G	Poaceae	P
Potentilla gracilis Douglas ex Hook.	Slender cinquefoil	N	F/SS	Rosaceae	P
Prunella vulgaris L.	Common selfheal	N	F	Lamiaceae	P
Ranunculus occidentalis Nutt.	Western buttercup	N	F	Ranunculaceae	P
Rubus armeniacus Focke	Himalayan blackberry	I	SS	Rosaceae	P
Rubus laciniatus Willd.	Cutleaf blackberry	I	SS/V	Rosaceae	P
Rumex acetosella L.	Common sheep sorrel	I	F	Polygonaceae	P
Senecio jacobaea L.	Stinking willie	I	F	Asteraceae	P
Senecio jacobaea L.	Stinking willie	I	F	Asteraceae	P
Trifolium dubium Sibth.	Suckling clover	I	F	Fabaceae	A
Vicia sativa L.	Garden vetch	I	F/V	Fabaceae	A
Vulpia bromoides (L.) Gray	Brome fescue	I	G	Poaceae	A

*Native Status: I=Introduced, N=Native, Growth Form: G=Graminoid, F=Forb, S=Shrub, SS=Subshrub, T=Tree, V=Vine, Duration: A=Annual, B=Biennial, P=Perennial

Table A3: Remnant Site Pearson and Kendall Correlations with Ordination Axes 2014

Axis:	1			2			3		
	r	r-sq	tau	r	r-sq	tau	r	r-sq	tau
Carbon	-0.73	**0.54**	-0.63	-0.03	0.00	-0.03	0.21	0.04	0.19
Nutrients	0.82	**0.67**	0.65	-0.02	0.00	-0.05	0.13	0.02	0.10
Soil moisture	-0.52	**0.28**	-0.38	-0.09	0.01	-0.11	0.12	0.01	0.12
Bare ground	-0.58	**0.34**	-0.41	-0.48	**0.23**	-0.32	-0.03	0.00	-0.04
Litter	0.59	**0.35**	0.40	0.31	0.10	0.20	0.02	0.00	0.02
Moss/lichen	0.20	0.04	0.22	0.48	**0.23**	0.41	0.02	0.00	-0.02
Disturbance level	-0.21	0.04	-0.13	-0.34	**0.12**	-0.28	-0.12	0.01	-0.08
Natives	0.14	0.02	0.10	0.27	0.07	0.22	0.43	**0.19**	0.29
Exotics	0.86	**0.74**	0.72	0.28	0.08	0.16	-0.14	0.02	-0.15
Perennials	0.53	**0.28**	0.40	0.62	**0.38**	0.40	0.25	0.06	0.14
Annuals	0.74	**0.55**	0.62	-0.21	0.04	-0.07	-0.26	0.07	-0.23
Grasses	0.84	**0.70**	0.65	0.22	0.05	0.12	0.20	0.04	0.10
Forbs	0.23	0.05	0.22	0.40	**0.16**	0.29	-0.33	**0.11**	-0.25
Legumes	0.57	**0.33**	0.44	0.10	0.01	0.12	0.16	0.03	0.08
Total cover	0.83	**0.69**	0.67	0.36	**0.13**	0.23	0.04	0.00	0.00
Richness	0.48	**0.23**	0.34	0.27	0.07	0.21	0.01	0.00	0.01
Diversity	0.50	**0.25**	0.37	0.16	0.03	0.15	-0.10	0.01	-0.10

Table A3: continued

Aira caryophyllea	0.44	**0.19**	0.37	-0.33	**0.11**	-0.20	-0.03	0.00	-0.10
Arrhenatherum elatius	0.39	**0.15**	0.31	0.69	**0.48**	0.56	-0.16	0.02	-0.10
Aquilegia formosa	0.07	0.01	0.12	0.42	**0.17**	0.27	-0.12	0.02	-0.14
Bromus hordeaceus	0.46	**0.21**	0.39	0.04	0.00	0.00	-0.17	0.03	-0.23
Carex tumulicola	0.34	**0.12**	0.27	0.43	**0.19**	0.39	-0.35	**0.12**	-0.29
Calochortus tolmiei	-0.06	0.00	-0.09	-0.11	0.01	-0.04	0.41	**0.17**	0.39
Cerastium glomeratum	0.46	**0.21**	0.54	-0.29	0.08	-0.16	-0.04	0.00	-0.03
Crepis capillaris	0.26	0.07	0.09	0.47	**0.22**	0.38	-0.23	0.05	-0.13
Cynosurus echinatus	0.62	**0.39**	0.57	-0.38	**0.14**	-0.18	-0.13	0.02	-0.16
Dactylis glomerata	0.32	**0.10**	0.22	0.33	**0.11**	0.34	0.27	0.08	0.21
Daucus carota	0.03	0.00	0.06	-0.12	0.01	-0.04	-0.48	**0.23**	-0.40
Elymus glaucus	0.07	0.01	0.07	0.24	0.06	0.23	-0.40	**0.16**	-0.16
Festuca idahoensis ssp. *roemeri*	0.07	0.00	-0.04	-0.24	0.06	-0.06	0.39	**0.16**	0.39
Fragaria virginiana	-0.12	0.01	-0.07	0.31	0.10	0.19	0.49	**0.24**	0.40
Galium parisiense	-0.03	0.00	0.01	0.14	0.02	0.33	-0.32	**0.10**	-0.17
Geranium dissectum	0.39	**0.16**	0.23	0.26	0.07	0.08	-0.36	**0.13**	-0.36
Holcus lanatus	0.46	**0.21**	0.41	0.24	0.06	0.14	0.25	0.06	0.20
Koeleria macrantha	0.12	0.01	0.02	-0.58	**0.34**	-0.53	0.31	0.10	0.25
Lathyrus sphaericus	0.37	**0.14**	0.21	-0.31	0.10	-0.38	0.02	0.00	0.05
Linum bienne	-0.07	0.01	-0.12	-0.10	0.01	-0.03	-0.39	**0.16**	-0.31
Lupinus oreganus var. *kincaidii*	-0.05	0.00	-0.09	0.14	0.02	0.11	0.38	**0.15**	0.38
Luzula campestris	0.44	**0.19**	0.44	-0.09	0.01	0.05	0.08	0.01	-0.01
Myosotis discolor	0.35	**0.12**	0.48	-0.07	0.00	-0.17	-0.17	0.03	-0.15
Poa pratensis	0.40	**0.16**	0.30	0.43	**0.19**	0.47	0.20	0.04	0.00
Rosa rubiginosa	0.28	0.08	0.12	0.61	**0.37**	0.37	-0.23	0.05	-0.19
Schedonorus arundinaceus	0.07	0.01	0.17	0.37	**0.14**	0.24	-0.05	0.00	-0.06
Sherardia arvensis	0.03	0.00	0.04	-0.08	0.01	-0.06	-0.42	**0.18**	-0.31
Toxicodendron diversilobum	0.24	0.06	0.14	0.61	**0.37**	0.39	-0.21	0.05	-0.21
Trifolium dubium	0.40	**0.16**	0.34	0.02	0.00	0.08	-0.13	0.02	-0.09
Vicia hirsuta	0.38	**0.14**	0.28	0.40	**0.16**	0.19	0.19	0.04	-0.02
Vicia sativa	0.51	**0.26**	0.39	0.09	0.01	0.06	0.02	0.00	0.03
Vicia villosa	0.33	**0.11**	0.18	0.22	0.05	0.27	-0.05	0.00	-0.15
Vulpia bromoides	0.41	**0.17**	0.32	0.07	0.00	0.03	0.02	0.00	-0.01

*Environmental variables and species with an r^2 correlation of 0.1 or greater with one of the ordination axes.
Correlations of 0.1 or more are bolded in the table.

Table A4: Restored Site Pearson and Kendall Correlations with Ordination Axes 2014

Axis:	1			2			3		
	r	r-sq	tau	r	r-sq	tau	r	r-sq	tau
Carbon	-0.49	**0.24**	-0.40	0.00	0.00	-0.01	-0.01	0.00	-0.02
Nutrients	0.82	**0.68**	0.64	-0.01	0.00	0.01	-0.08	0.01	-0.08
Soil moisture	-0.34	**0.11**	-0.23	-0.37	**0.14**	-0.28	-0.25	0.06	-0.18
Bare ground	-0.32	**0.10**	-0.37	0.21	0.05	0.18	0.03	0.00	0.09
Moss/lichen	-0.26	0.07	-0.19	0.30	0.09	0.27	0.36	**0.13**	0.22
Natives	-0.03	0.00	0.00	0.63	**0.40**	0.45	-0.39	**0.15**	-0.26
Exotics	0.71	**0.50**	0.52	-0.20	0.04	-0.15	0.28	0.08	0.18
Perennials	0.43	**0.19**	0.33	0.02	0.00	-0.01	-0.32	**0.10**	-0.23
Annuals	0.56	**0.31**	0.30	0.48	**0.23**	0.37	0.32	**0.10**	0.22
Grasses	0.72	**0.51**	0.57	0.08	0.01	0.03	-0.21	0.04	-0.18
Forbs	-0.28	0.08	-0.24	0.37	**0.13**	0.24	0.27	0.07	0.20
Total cover	0.63	**0.40**	0.46	0.30	0.09	0.20	-0.03	0.00	0.38
Richness	-0.01	0.00	-0.02	0.42	**0.18**	0.29	0.18	0.03	-0.05
Diversity	0.09	0.01	0.06	0.51	**0.26**	0.31	0.37	**0.14**	0.14
Agrostis stolonifera	0.12	0.01	0.13	-0.82	**0.67**	-0.61	0.27	0.07	0.18
Aira caryophyllea	-0.02	0.00	-0.03	0.38	**0.15**	0.28	0.30	0.09	0.21
Anthoxanthum odoratum	0.78	**0.61**	0.55	-0.06	0.00	-0.10	-0.33	0.11	-0.27
Danthonia californica	-0.07	0.01	0.00	-0.02	0.00	-0.01	-0.89	**0.78**	-0.73
Festuca idahoensis ssp. *roemeri*	-0.07	0.01	-0.08	0.81	**0.65**	0.64	0.23	0.05	0.15
Hypochaeris radicata	-0.48	**0.23**	-0.37	0.37	**0.13**	0.24	0.32	**0.10**	-0.06
Lotus micranthus	-0.32	**0.11**	-0.32	0.33	**0.11**	0.34	0.25	0.06	0.23
Myosotis discolor	0.37	**0.14**	0.29	-0.02	0.00	-0.12	-0.07	0.00	0.33
Rumex acetosella	0.36	**0.13**	0.30	0.07	0.01	0.14	-0.10	0.01	-0.14
Vicia sativa	-0.15	0.02	-0.07	0.30	0.09	0.32	0.45	**0.20**	0.00
Vulpia bromoides	0.62	**0.39**	0.41	0.38	**0.14**	0.27	0.20	0.04	0.18

*Environmental variables and species with an r^2 correlation of 0.1 or greater with one of the ordination axes. Correlations of 0.1 or more are bolded in the table.

Table A5:
Estimates, Bonferroni-corrected confidence intervals, t and p-values for the cover, and richness/diversity related linear mixed effects models at the remnant site

		2013					2014				
		Estimate	**Lower**	**Upper**	**t**	**p**	**Estimate**	**Lower**	**Upper**	**t**	**p**
Total Cover	Control v. Carbon	35.48	25.64	45.64	9.24	<0.001	66.46	52.78	80.15	12.43	<0.001
	Nutrients v. Control	68.95	52.93	84.96	11.02	<0.001	48.39	30.73	66.05	7.01	<0.001
	Nutrients v. Carbon	104.43	88.70	120.16	16.99	<0.001	114.85	99.13	130.57	18.69	<0.001
Native Grasses	Control v. Carbon	--	--	--	--	--	--	--	--	--	--
	Nutrients v. Control	--	--	--	--	--	--	--	--	--	--
	Nutrients v. Carbon	--	--	--	--	--	--	--	--	--	--
Exotic Grasses	Control v. Carbon	14.79	9.50	20.08	7.16	<0.001	19.77	12.45	27.08	6.92	<0.001
	Nutrients v. Control	44.20	35.62	52.78	13.19	<0.001	66.92	52.59	81.26	11.95	<0.001
	Nutrients v. Carbon	58.99	50.96	67.01	18.81	<0.001	86.69	72.91	100.46	16.10	<0.001
Native Forbs	Control v. Carbon	2.89	-3.31	9.08	1.19	0.245	--	--	--	--	--
	Nutrients v. Control	4.86	-1.96	11.67	1.82	0.080	--	--	--	--	--
	Nutrients v. Carbon	7.74	1.75	13.73	3.30	0.003	--	--	--	--	--
Exotic Forbs	Control v. Carbon	18.49	9.73	27.25	5.40	<0.001	34.56	26.37	42.74	10.81	<0.001
	Nutrients v. Control	23.13	14.37	31.88	6.76	<0.001	-13.60	-21.79	-5.42	-4.25	<0.001
	Nutrients v. Carbon	41.62	32.86	50.37	12.16	<0.001	20.95	12.77	29.14	6.55	<0.001
Richness	Control v. Carbon	2.08	0.42	3.73	3.21	0.004	5.20	3.13	7.27	6.44	<0.001
	Nutrients v. Control	3.88	2.22	5.53	5.99	<0.001	0.25	-1.82	2.32	0.31	0.759
	Nutrients v. Carbon	5.95	4.29	7.61	9.19	<0.001	5.45	3.38	7.52	6.75	<0.001
Native Richness	Control v. Carbon	--	--	--	--	--	1.18	-0.08	2.43	2.40	0.024
	Nutrients v. Control	--	--	--	--	--	-1.38	-2.63	-0.12	-2.80	0.010
	Nutrients v. Carbon	--	--	--	--	--	-0.20	-1.45	1.05	-0.41	0.687
Exotic Richness	Control v. Carbon	2.00	0.69	3.31	3.92	<0.001	4.03	2.57	5.48	7.06	<0.001
	Nutrients v. Control	3.53	2.22	4.83	6.91	<0.001	1.60	0.14	3.06	2.81	0.010
	Nutrients v. Carbon	5.53	4.22	6.83	10.82	<0.001	5.63	4.17	7.08	9.87	<0.001
Diversity (H')	Control v. Carbon	0.22	0.11	0.33	5.23	<0.001	0.37	0.24	0.50	7.47	<0.001
	Nutrients v. Control	0.16	0.05	0.27	3.78	<0.001	-0.01	-0.14	0.12	-0.20	0.844
	Nutrients v. Carbon	0.38	0.27	0.48	9.01	<0.001	0.36	0.23	0.49	7.27	<0.001

Table A6:
Estimates, Bonferroni-corrected confidence intervals, t and p-values for the cover, and richness/diversity related linear mixed effects models at the restored site

		2013					2014				
		Estimate	**Lower**	**Upper**	**t**	**p**	**Estimate**	**Lower**	**Upper**	**t**	**p**
Total Cover	Control v. Carbon	30.06	21.18	38.94	8.67	<0.001	34.43	24.54	44.32	8.90	<0.001
	Nutrients v. Control	66.40	55.57	77.22	15.69	<0.001	29.72	16.41	43.03	5.71	<0.001
	Nutrients v. Carbon	96.46	84.64	108.28	20.87	<0.001	64.15	50.31	77.98	11.86	<0.001
Native Grasses	Control v. Carbon	--	--	--	--	--	--	--	--	--	--
	Nutrients v. Control	--	--	--	--	--	--	--	--	--	--
	Nutrients v. Carbon	--	--	--	--	--	--	--	--	--	--
Exotic Grasses	Control v. Carbon	15.82	5.38	26.26	3.88	<0.001	11.38	0.96	21.80	2.79	0.008
	Nutrients v. Control	57.68	44.75	70.60	11.42	<0.001	50.71	39.17	62.24	11.25	<0.001
	Nutrients v. Carbon	73.50	62.11	84.88	16.52	<0.001	62.09	51.89	72.28	15.58	<0.001
Native Forbs	Control v. Carbon	2.22	0.78	3.74	3.91	<0.001	4.26	1.02	7.49	3.37	0.005
	Nutrients v. Control	1.27	-1.33	3.59	1.34	0.192	-2.19	-5.57	1.19	-1.66	0.137
	Nutrients v. Carbon	3.49	1.44	5.53	4.37	<0.001	2.07	-1.30	5.44	1.57	0.130
Exotic Forbs	Control v. Carbon	9.41	3.46	15.36	4.05	<0.001	13.46	6.71	20.21	5.10	<0.001
	Nutrients v. Control	2.97	-2.98	8.92	1.28	0.214	-18.11	-24.86	-11.36	-6.87	<0.001
	Nutrients v. Carbon	12.38	6.43	18.33	5.32	<0.001	-4.65	-11.40	2.10	-1.76	0.090
Richness	Control v. Carbon	1.23	0.16	2.29	2.95	0.007	3.08	1.63	4.52	5.44	<0.001
	Nutrients v. Control	0.08	-0.99	1.14	0.18	0.858	-2.00	-3.45	-0.55	-3.54	0.002
	Nutrients v. Carbon	1.30	0.24	2.36	3.13	0.004	1.08	-0.37	2.52	1.90	0.069
Native Richness	Control v. Carbon	--	--	--	--	--	1.35	0.50	2.20	4.06	<0.001
	Nutrients v. Control	--	--	--	--	--	-1.23	-2.08	-0.37	-3.68	0.001
	Nutrients v. Carbon	--	--	--	--	--	0.13	-0.73	0.98	0.38	0.710
Exotic Richness	Control v. Carbon	0.75	-0.09	1.59	2.27	0.032	1.73	0.76	2.69	4.57	<0.001
	Nutrients v. Control	0.33	-0.52	1.17	0.98	0.334	-0.78	-1.74	0.19	-2.05	0.051
	Nutrients v. Carbon	1.08	0.23	1.92	3.26	0.003	0.95	-0.02	1.92	2.82	0.019
Diversity (H')	Control v. Carbon	0.08	-0.01	0.17	2.39	0.025	0.15	0.05	0.25	3.81	<0.001
	Nutrients v. Control	0.12	0.03	0.21	3.49	0.002	-0.06	-0.16	0.04	-1.53	0.139
	Nutrients v. Carbon	0.20	0.11	0.29	5.88	<0.001	0.09	-0.01	0.19	2.28	0.031

Table A7:

Post-hoc pairwise comparison tests including back-transformed estimates, back-transformed Bonferroni-corrected confidence intervals, t and p-values for the species-specific linear mixed effects models at the remnant site

		2013					2014				
		Estimate	**Lower**	**Upper**	**t**	**p**	**Estimate**	**Lower**	**Upper**	**t**	**p**
Aira caryophyllea	Control v. Carbon	2.23	1.16	4.30	3.13	0.004	7.57	3.45	16.63	6.59	<0.001
	Nutrients v. Control	3.47	1.80	6.68	4.85	<0.001	1.44	0.66	3.17	1.19	0.244
	Nutrients v. Carbon	7.75	4.03	14.92	7.99	<0.001	10.93	4.98	23.98	7.79	<0.001
Cynosurus echinatus	Control v. Carbon	3.22	1.76	5.90	4.93	<0.001	9.10	4.47	18.53	7.95	<0.001
	Nutrients v. Control	3.01	1.64	5.52	4.65	<0.001	4.09	2.01	8.32	5.07	<0.001
	Nutrients v. Carbon	9.69	5.28	17.76	9.58	<0.001	37.21	18.28	75.74	13.02	<0.001
Daucus carota	Control v. Carbon	1.78	1.31	2.43	4.76	<0.001	2.25	1.60	3.16	6.09	<0.001
	Nutrients v. Control	1.15	0.84	1.56	1.13	0.271	0.54	0.39	0.76	-4.59	<0.001
	Nutrients v. Carbon	2.04	1.50	2.78	5.89	<0.001	1.22	0.87	1.71	1.50	0.146
Hypochaeris radicata	Control v. Carbon	1.76	1.12	2.79	3.18	0.004	2.74	1.49	5.04	4.22	<0.001
	Nutrients v. Control	1.17	0.74	1.85	0.89	0.378	0.25	0.14	0.47	-5.73	<0.001
	Nutrients v. Carbon	2.07	1.31	3.27	4.08	<0.001	0.70	0.38	1.28	-1.51	0.143
Koeleria macrantha	Control v. Carbon	0.83	0.41	1.67	-0.67	0.510	0.82	0.37	1.81	-0.65	0.523
	Nutrients v. Control	1.99	0.98	4.06	2.50	0.019	2.20	1.00	4.87	2.55	0.017
	Nutrients v. Carbon	1.66	0.82	3.37	1.83	0.079	1.80	0.82	3.98	1.91	0.068

Table A8:

Post-hoc pairwise comparison tests including back-transformed estimates, back-transformed Bonferroni-corrected confidence intervals, t and p-values for the species-specific linear mixed effects models at the restored site

		2013					2014				
		Estimate	Lower	Upper	t	p	Estimate	Lower	Upper	t	p
Aira caryophyllea	Control v. Carbon	1.89	1.18	3.01	3.47	0.002	1.96	1.06	3.62	2.81	0.009
	Nutrients v. Control	1.50	0.94	2.40	2.23	0.035	0.63	0.34	1.17	-1.90	0.069
	Nutrients v. Carbon	2.83	1.78	4.53	5.71	<0.001	1.24	0.67	2.29	0.91	0.370
Anthoxanthum odoratum	Control v. Carbon	1.64	1.04	2.58	2.78	0.010	1.16	0.79	1.69	0.96	0.345
	Nutrients v. Control	1.91	1.21	3.00	3.64	0.001	2.40	1.64	3.53	5.86	<0.001
	Nutrients v. Carbon	3.12	1.98	4.91	6.42	<0.001	2.78	1.89	4.07	6.82	<0.001
Hypochaeris radicata	Control v. Carbon	1.39	1.09	1.77	3.49	0.002	1.46	0.97	2.21	2.37	0.026
	Nutrients v. Control	1.05	0.83	1.34	0.55	0.590	0.25	0.16	0.37	-8.69	<0.001
	Nutrients v. Carbon	1.46	1.15	1.86	4.06	<0.001	0.36	0.24	0.55	-6.32	<0.001
Vulpia bromoides	Control v. Carbon	2.45	1.22	4.89	3.30	0.003	2.74	1.47	5.12	4.15	<0.001
	Nutrients v. Control	12.90	6.45	25.80	9.44	<0.001	9.84	5.28	18.35	9.39	<0.001
	Nutrients v. Carbon	31.58	15.79	63.15	12.75	<0.001	27.01	14.48	50.37	13.54	<0.001

Table A9:
Post-hoc pairwise comparison tests including back-transformed estimates, back-transformed Bonferroni-corrected confidence
intervals, t and p-values for the paintbrush count generalized linear model at the remnant site

		2013					2014				
		Estimate	Lower	Upper	t	p	Estimate	Lower	Upper	t	p
	Control v. Carbon	2.79	1.89	4.13	6.46	<0.001	3.56	1.67	7.58	4.13	<0.001
Paintbrush count	Nutrients v. Control	1.53	1.05	2.22	2.80	0.007	0.95	0.47	1.91	-0.19	0.849
	Nutrients v. Carbon	4.27	2.90	6.30	9.20	<0.001	3.37	1.58	7.19	3.95	<0.001

Table A10:
Post-hoc pairwise comparison tests including back-transformed estimates, back-transformed Bonferroni-corrected confidence
intervals, t and p-values for the paintbrush count generalized linear model at the restored site

		2013					2014				
		Estimate	Lower	Upper	t	p	Estimate	Lower	Upper	t	p
	Control v. Carbon	2.84	1.68	4.79	4.91	<0.001	5.72	2.32	14.11	4.76	<0.001
Paintbrush count	Nutrients v. Control	0.94	0.57	1.55	-0.30	0.770	0.38	0.17	0.85	-2.95	0.005
	Nutrients v. Carbon	2.67	1.58	4.51	4.62	<0.001	2.17	0.84	5.58	2.01	0.050