Activated Carbon Decreases Invasive Plant Growth by Mediating Plant-Microbe Interactions

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ACTIVATED CARBON DECREASES INVASIVE PLANT GROWTH BY
MEDIATING PLANT-MICROBE INTERACTIONS

by

Nicole Nolan

A thesis submitted in partial fulfillment of the requirements for the degree
of
MASTER OF SCIENCE
in
Ecology

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UTAH STATE UNIVERSITY
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2014
ABSTRACT

Activated Carbon Decreases Invasive Plant Growth by Mediating Plant-Microbe Interactions

by

Nicole E. Nolan, Master of Science
Utah State University, 2014

Major Professor: Karen H. Beard
Program: Ecology

Abandoned agricultural lands in the Intermountain West are plagued by dense, persistent non-native vegetation. Targeted restoration tools are required to remove the competitive advantage of these non-natives while also removing the soil legacies they leave behind. Activated carbon (AC) is one such tool, with the ability to disrupt the mechanisms of allelopathy, positive plant-soil feedbacks, and altered nutrient cycling commonly used by non-native species. Previous studies have shown the success of high concentrations of AC in native plant community restoration on a small scale. Here, our goals are twofold: first, to test AC effectiveness in restoring desirable plant communities on a larger scale, and secondly, to identify the primary mechanism, allelopathy versus microbial changes, through which AC impacts native and non-native species. A large scale AC treatment in Methow Valley, Washington tested the effectiveness of AC restoration at a large scale and tested five concentrations and two types of AC to determine lowest effective application. Following treatment, sites were monitored for
vegetation cover for three years. The large-scale application produced similar results to the previous study at a 1000 g/m² application rate, with a 28% increase in the ratio of desirable:undesirable species cover and a decrease to 25% undesirable species cover. However, the effectiveness of AC concentrations below 1000 g/m² cannot yet be determined and may require a longer time scale and additional monitoring to assess restoration success. A greenhouse experiment was performed, which used native and non-native species common to the study site, grown in pairs in sterilized and live AC-treated soils to separate AC effects on allelopathy from that of microbial interactions. Both native and non-native species experienced a 25% decreased biomass in AC-treated live soils, with a minimal decrease in A-treated sterile soils for native species and no effect in AC-treated sterile soils for non-native species. Overall, AC live soils produced a positive effect on relative abundance; the ratio of native to non-native biomass was highest in AC-treated live soils. From these results, it is concluded that the primary pathway through which AC works is changes in the plant-microbial interactions of both native and non-native species.

(76 pages)
To restore lands invaded by dense, non-native vegetation, it may be necessary to develop targeted restoration tools than can remove the mechanism used by these non-native species as a competitive advantage. Activated carbon (AC) is one such tool, with the ability to disrupt the mechanisms of plant to plant communication (allelopathy) and positive plant to microbe communication commonly used by non-native species. Previous studies have shown the success of high concentrations, 1000 g/m$^2$, of AC in native plant community restoration on a small scale. Here, our goals are twofold: first, to test AC effectiveness in restoring desirable plant communities on a larger scale, and secondly, to identify the primary mechanism, allelopathy versus microbial interactions, through which AC impacts native and non-native species. AC treatments in Methow Valley, Washington tested the effectiveness of AC restoration at a large-scale and tested five concentrations and two type of AC to determine lowest effective concentration. Following three growing seasons, the large-scale application at a 1000 g/m$^2$ application rate decreased undesirable specie cover and positively impacted the relative abundance of desirable to undesirable species. However, the effectiveness of AC concentrations below 1000 g/m$^2$ cannot yet be
determined and may require a longer time scale and additional monitoring. A greenhouse experiment was performed to separate AC effects on allelopathy from that of microbial interactions, which used native and non-native species common to the study site, grown in pair in sterilized (i.e. all microbes removed) and live (i.e. containing microbial communities) AC-treated soils. Both native and non-native species experienced a decreased biomass in AC-treated live soils, with no effect in AC-treated sterile soils. Overall, AC live soils produced a positive effect on relative abundance; the ratio of native to non-native biomass was highest in AC-treated live soils. From these results, it is concluded that the primary pathway through which AC works is changes in the plant-microbial interactions of both native and non-native species.
ACKNOWLEDGMENTS

First, I would like to thank my major advisor, Dr. Karen Beard; without her support, patience, and encouragement, none of this would have been possible. I would also like to thank my committee members, Dr. Andrew Kulmatiski and Dr. Peter Adler, for their inputs and guidance. I am grateful to Susan Durham for her assistance with statistical analyses.

I would like to express thanks to everyone that helped with the research presented here and all the work I have done here at Utah State University. I would like to thank Brianne Loya, Andrew McNown, Anna Peschel, and Maureen Schweer for their assistance with the Washington field work. I greatly appreciate the insight, resources, and assistance for my field work in Tooele, UT provided by USU extension professor Linden Greenhalgh, Jerry Caldwell, and Bart Staples from Tooele Country Weed Control; and Darrell Johnson the Shambip Conservation District Board Chairman. I am indebted to my field technician Josiah Maughan and greenhouse technician Brittany Duncan, who were always enthusiastic and hard-working.

Additionally, I would like to thank my funding sources, the Utah Agricultural Experimental State, USDA NRI (award # 2010-85320-20402), and Utah State University Ecology Center. I am grateful to the Washington Department of Fish and Game for use of land and resources for our field experiment and the USDA Agricultural Research Station for use of greenhouse space.
Finally, I would like to acknowledge my friends for all their help and motivation, my parents, who have always been my biggest supporters, and my fiancé, Brian, who has been an endless source of encouragement.

Nicole Nolan
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Abandoned agricultural lands are growing worldwide and have long-term soil legacies that are highly susceptible to invasion by dense, persistent non-native species (Cramer et al. 2008; Kulmatiski & Beard 2008). These soil legacies can last decades and may encourage the growth of some plants (i.e., non-native) relative to other (i.e., native) plants. Non-native species utilize several mechanisms to invade these disturbed systems and decrease native growth, including faster dispersal rates (Rejmanek & Richardson 1996; Lloret et al. 2005), allelopathy (Callaway & Aschehoug 2000; Bais et al. 2003; Hierro & Callaway 2003; Callaway & Ridenour 2004), positive plant-soil feedbacks (PSFs) (Klironomos 2002; Levine et al. 2006; Reinhart & Callaway 2006), and altered nutrient cycling (Ehrenfeld 2003; Hawkes et al. 2005; Elgersma et al. 2011). Once present non-native species can create and persistent soil legacies that remain even after physical removal of the plants and hinder native plant community restoration (McKinley et al. 2005; Kulmatiski & Beard 2011). To recover such systems to native-dominated communities, it may be necessary to find control methods that not only decrease non-native species abundance but also overcome these legacies (Callaway & Aschehoug 2000; Elgersma et al. 2001; Kulmatiski et al. 2006). By identifying and targeting the mechanisms used by non-native species to change soils and decrease native plant growth, it may be possible to control non-native plants and restore native communities in these disturbed sites (Levine et al. 2003).

Activated carbon (AC) is one potential tool that could decrease the competitive advantage of non-native species through changes in the soil properties (Kulmatiski &
AC is a highly porous, non-toxic compound that indiscriminately binds organic molecules through Van der Waals forces (Cheremisinoff and Ellerbusch 1978). Therefore, AC can bind the carbohydrates and secondary metabolites that are released by plant roots and inhibit plant-plant (allelopathic) and plant–microbe (PSFs) communication (Compant et al. 2005; Khan et al. 2007; Pierson & Pierson 2007; Schaefer et al. 2008). AC is a commonly used tool in greenhouse experiments to test and manipulate allelopathic effects (Inderjit & Callaway 2003; Lau et al. 2008) and has also been shown to alter plant-microbial associations (Weisshuhn & Prati 2009; Wurst & van Beersum 2009; Aschehoug et al. 2012). AC may also alter nutrient levels, and has been shown in greenhouse experiments to alter nitrogen (N) concentrations (Berglund et al. 2004; Lau et al. 2008) and pH levels (Kabouw et al. 2010).

In a small-scale (1 m x 1 m) field experiment conducted in the Methow Valley, Washington, AC negatively affected non-native plant growth, positively affected native plant growth, decreased extractable organic C and N, and decreased microbial abundance (Kulmatiski & Beard 2006; Kulmatiski 2011). Though AC was successful at restoring small plots dominated by non-natives to native-dominated plots when a high concentration (1000 g/m²) of AC was applied, it is not known if these results are repeatable or achievable at larger spatial scales, nor was it known if lower, and more cost-effective concentrations, might have a similar effect. In addition, the primary mechanism through which AC elicits these plant responses in the field is still unclear. For example, AC has been shown to cause large, direct changes in the soil microbial
community that may change plant-plant competitive interactions (Kulmatiski 2011).

Alternatively, AC may change plant-plant interactions by binding allelopathic chemicals.

The goals of this study were two-fold: (1) to test AC effectiveness in restoring desirable plant communities on a larger scale, (2) determine the lowest effective AC concentration, and (3) to identify the primary mechanism, allelopathy versus microbial changes, through which AC impacts native and non-native plant growth. First, we conducted large-scale (15 m x 15 m plots) AC application in the same valley in Washington where the smaller scale application had previously been successful, using practical application techniques and machinery available for restoration. We tested multiple concentrations and two different types of AC to determine the lowest concentration to influence the plant community. Second, we conducted a greenhouse experiment to identify the mechanism through which AC affects native and non-native plant growth.

More specifically, the greenhouse experiment was designed to separate the effects of AC induced changes in plant growth via the microbial community from those of allelopathy. While the definition of allelopathy has recently been expanded by some to include allelopathic effects mediated by soil organisms (Inderjit 2005; Aschehoug et al. 2012), we test the more traditional definition of allelopathy, direct chemical effects of one plant on another without a role for microbial mediation. By using sterilized and live AC-treated soils, we could identify whether native and non-native plant growth was altered by a disruption in plant-plant communication or microbial communication pathways. Plant growth can be either negatively or positively affected by soil manipulation through AC, dependent upon the strength of communication interruption.
and its importance to plant growth (Fig. 1). However, in general, an allelopathic response will be equally present in both live and sterile treatments, and will differ from the control. In contrast, microbial-driven mechanisms will produce different plant responses in live and sterile soils.

Figure 1. Possible effects of AC addition on plant biomass for native and non-native species. 1) Allelopathy will be affected equally in both AC treatments and cause a similar increase in native biomass and decrease in non-native biomass in AC live and sterile soils, 2) Pathogens will decrease plant association in AC live soil causing increased biomass for native and non-native species. No pathogens will be present in AC sterile, causing increased biomass in native and non-native species, 3) Symbionts will decrease plant association in AC live soil causing decreased biomass in native and non-native species. All symbionts will be removed in sterilized soil decreasing biomass in native and non-native species. Multiple effects may be present and combined, altering plant response.
CHAPTER 2
LITERATURE REVIEW

Degraded and disturbed natural systems are increasing globally with human land-use practices (Foley et al. 2005; Safriel 2007). For example, in North American over the last century, there has been a large increase in the amount of abandoned agricultural lands (Ramankutty & Foley 1999). Agriculture creates long-term changes to nitrogen (N) and carbon (C) cycles as well as changes to the microbial community (Kulmatiski & Beard 2008), often requiring centuries to recover (Knops & Tilman 2000). More specifically, soils previously used for agriculture often demonstrate 75% less N, 80% less carbon, and less microbial abundance and diversity when compared to undisturbed soils (McKinley et al. 2005; Fraterrigo et al. 2006; Kulmatiski & Beard 2008). Lands formerly used for agriculture are also often highly susceptible to dense, persistent non-native vegetation that not only decrease land value but also serves as sources of seed pressure for other agricultural and range lands (Lake & Leishman 2004; Cramer et al. 2008). Non-native species found on abandoned agricultural fields are able to create areas resistant to re-invasion by native species (Kulmatiski 2006), which even following restoration efforts may not return to historical or native communities (Cramer et al. 2008). It is challenging for native plant species to overcome and erase previous agricultural and non-native plant growth legacies and create their own plant soil legacy, even following short-term plant growth success with restoration (McKinley et al. 2005; Fraterrigo et al. 2006; Kulmatiski & Beard 2011).

There are many reasons that non-natives are often undesirable in a system. Undesirable non-native species decrease forage value for native and commercial grazers,
disturb ecosystem services, and decrease habitat for native species (Sheley et al. 1998; Ehrenfeld 2003). Thus, they are often a high priority topic for management and restoration efforts. Numerous techniques are currently used to manage and attempt eradication of undesirable plant species, and restore native communities, including mechanical, cultural, biological, and chemical controls. However, some degraded systems are resilient to traditional restoration efforts due to changes in biotic and abiotic feedback systems (Suding et al. 2004). Additionally, management techniques often have high costs and variable success (Sheley et al. 1998), with possible negative impacts, including negative impacts on native species growth and their seed bank with herbicide applications (Davies & Sheley 2011), tillage decreasing microbial diversity (Lupwayi et al. 2001), and biological controls altering ecosystem food-webs (Pearson & Callawa, 2005).

There is a need to research restoration methods that will not only successfully decrease non-native species abundance, but also improve the degraded community and promote native plant communities. A better understanding of the mechanisms through which non-natives invade a system and the cascading changes caused by their establishment will lead to new, more effective control methods. Targeted techniques can inhibit the mechanisms used by non-native species to change soils and deter native plant growth; thus it may be possible to restore native communities in disturbed sites resistant to traditional restoration methods (Levine et al. 2003). Non-native species utilize several mechanisms to invade disturbed systems and deter native growth, including: positive plant-soil feedbacks (PSFs) (Klironomos 2002; Levine et al. 2006; Reinhart & Callaway 2006), allelopathy (Callaway & Ascheloung 2000; Bais et al. 2003; Hierro & Callaway 2003; Callaway & Ridenour 2004), and altered nutrient cycling (Ehrenfeld 2003; Hawkes...
et al. 2005; Elgersma et al. 2011). Thus, techniques that target these specific mechanisms might be successful.

Recent research has indicated that non-native plant success is related to changes in PSFs, and, more specifically, that non-natives often exhibit positive PSFs (Callaway & Aschehoug 2000; Ehrenfeld & Scott 2001; Kulmatiski et al. 2008). A potential reason for this relationship is that soils in introduced habitats are expected to be relatively enemy-free and symbiont-rich, because root herbivores and pathogens have not co-evolved to specialize on non-native plant species, while common symbionts are generalists (Callaway & Aschehoug 2000; Keane & Crawley 2002; Colautti et al. 2004). Plant-soil feedbacks describe the ability of a plant to change soil conditions, through the alteration of subsequent plant growth (Klironomos 2002; Bever 2003; Callaway et al. 2004; Kulmatiski et al. 2008). The plant-soil feedback hypothesis speculates that legacies of past plant growth can provide a selective advantaged to one plant species over another typically thorough specific microbial communities but also potentially through nutrient availability (Bever 2003; Reynolds et al. 2003). Positive PSFs are the production of soil condition by a species that improve the performance of conspecifics, while negative PSFs are the production of soil conditions that decrease performance of conspecifics (van der Putten et al. 2013). Most species exhibit negative PSFs (Kulmatiski et al. 2008). However, non-natives seem to utilize positive, or at least more positive, PSFs to gain a competitive advantage in native ecosystems (Kulmatiski et al. 2008). In support of this idea, microbial communities have been observed to differ from roots of non-native species to native species of a system (Kourtev et al. 2002).
Another potential mechanism used by non-native species is allelopathy, where successful non-native species release novel biochemical weapons used to deter native species growth (Callaway & Ridenour 2004). Allelopathy is the release of root exudates (allelochemicals) by one species that have an impact on the growth and survival of another species. The definition of allelopathy has recently been expanded by some to include allelopathic effects mediated by soil organisms, as microorganisms are influential in degradation and accumulation along chemical pathways (Inderjit 2005; Aschehoug et al. 2012). The contemporary expanded definition differs slightly from the more traditional definition of allelopathy, which has been understood as the direct chemical effects of one plant on another without any interaction through microbial mediation. Studies have also shown allelopathy to play a role in the competitive advantage of some non-native species (Mahall & Callaway 1991; Ridenour & Callaway 2001). Native species are naïve to the allelochemicals of introduced species, and can experience strong negative impacts (Callaway & Ridenour 2004). By using allelopathic weapons, some non-native species are able to eliminate native diversity and establish monocultures (Ridenour & Callaway 2001; Bais et al. 2003). Allelopathy has the potential to inhibit individual native species growth and germination (Bais et al. 2003), but effects of allelopathy can ultimately alter plant communities and ecosystem functions (Wardle et al. 1998). It is possible for native species to increase resistance to allelochemicals over prolonged exposure, possibly leading to an eventual evolutionary tolerance (Callaway et al. 2005).

In addition to causing biotic changes in introduced ecosystems, non-native species are able to alter the nutrients levels and cycling rates. Nutrient effects can be caused by
the primary effects of non-native species presence, through growth and nutrient use habits of the species, or through secondary channels, such as elicited changes in microbial communities or changes in fire regimes (Ehrenfeld 2003). Non-native species can cause changes in N availability and cycling, including decreased available N (Evans et al. 2001) and higher N mineralization and nitrification rates (Ehrenfeld 2003; Ehrenfeld 2004). Such drastic changes can have negative impacts on the native plant community. In addition, non-native annual species reduce C storage and increase C cycling upon invading systems historically composed of perennial species (Bradley et al. 2006).

Where PSF, allelopathy, or altered nutrient cycling allow plant invasions by changing plant-soil interactions, it may be necessary to manipulate soils to restore native plants and the ecosystem services they provide (Kulmatiski & Beard 2006; Cramer et al. 2008; Eviner & Hawkes 2008). This a growing field of research, with a variety of techniques available, to manipulate soil microbial, chemical, and nutrient pools. In crop systems, the concept and application of ‘changing plant-soil interactions’ or ‘domesticating microbial communities’ is growing (Compant et al. 2005; Hafeez et al. 2006). Bacterial applications have been used for plant and nematode suppression (Weissmann & Gerhardson 2001; Talavera et al. 2002) as well as plant growth enhancement (Canbolat et al. 2006). An example altering soil nutrient pools is the concept of reverse fertilization. The idea with reverse fertilization is to decrease nutrient pools used by early successional, fast growing non-native invaders. Carbon amendment, usually added as sugar or sawdust, targets nitrophilic invaders by stimulating microorganisms and causing the immobilization of inorganic nitrogen (Blumenthal et al. 2003;
Booth et al. 2003). It is primarily intended to increase N immobilization by heterotrophic soil microbes and, therefore, decrease N availability. This technique has mixed success in restoration of native plant communities, but it has shown the potential to alter plant community composition by changing microbial activity (Morghan & Seastedt 1999; Blumenthal et al. 2003; Corbin & D’Antonio 2004).

Additionally, biochar amendments have been attempted to improve soil qualities and promote plant growth. Biochar, a processed carbon charcoal, is used to increase crop yields and soil quality, reduce the leaching of nutrients, improve soil structure and the retention of soil moisture, and stimulate soil microbial activity (Brodowski et al. 2006; Kolb et al. 2009; Quilliam et al. 2012). Research has shown that biochar has the greatest impacts in low quality soils, such as those used in agriculture (Quilliam et al. 2012; Jones et al. 2012). Its addition has been shown to increased soil respiration and microbial growth rates (Jones et al. 2012). It has been indicated that biochar may be as useful tool in restoration, through an increase in native plant growth (Adams et al. 2013).

An emerging field is the use of AC to inhibit plant-plant and plant-soil interactions (Callaway & Aschehoug 2000; Lau et al. 2008). AC is a highly porous carbon substance with high surface areas. It is processed from several carbon based sources: coal, nutshells, wood, or peat. AC is able to efficiently bind organic compounds through Van der Waals forces; however, it is poor at adsorbing alcohols, glycols, ammonia, and non-organic compounds (Cheremisinoff & Ellerbusch 1978). AC differs from biochar in the compound processing. AC is ‘activated’ through exposure to CO₂, steam, or acids at high temperatures to increase surface area, this allows AC to have an increased ability to bind organic compounds compared to biochar (Azargohar & Dalai
Depending on the carbon source and processing method, AC will have macro (> 5 nm), meso (2-5 nm) or micropore (<2 nm) structure. Macropores adsorb large organic molecules (e.g., tannins, fulvins), while micropores adsorb small organic molecules (e.g., auxin, or the allelochemicals catechin and 8-hydroxyquinoline). AC can bind the carbohydrates and secondary metabolites that are released by plant roots thereby inhibiting plant-plant and plant–microbe communication (Compant et al. 2005; Khan et al. 2007; Pierson & Pierson 2007; Schaefer et al. 2008). Similarly, AC is able to adsorb phytotoxic root exudates, which may give non-native species a competitive advantage (Callaway & Aschehoug 2000; Inderjit & Callaway 2003).

It is possible AC can be a useful tool in the restoration of native plant communities. The ability of AC to disrupt plant-soil communications may decrease non-native plant growth through several mechanisms. AC can bind allelochemicals, which will benefit native plants that are naïve to these (Callaway & Aschehoug 2000; Lau et al. 2008), decrease plant-microbe communication, which will decrease the positive PSFs on which many non-natives rely (Kulmatiski et al. 2008; Wurst & van Beersum 2009; Kulmatiski & Beard 2011), and decrease nutrient availability, which will decrease the advantage of fast-growing, non-native species (Kulmatiski 2006; Lau et al. 2008; Weisshuhn & Prati 2009).

AC is a commonly used tool in greenhouse experiments to test and manipulate allelopathic effects (Inderjit & Callaway 2003; Lau et al. 2008). AC decreases allelopathic effects, with positive effects on native aboveground biomass (Lau et al. 2008; Murrell et al. 2011) and root growth (Ridenour & Callaway 2001). However, AC soil and
plant interactions may produce undesirable effects when studying pure plant-plant allelopathy (Lau et al. 2008; Weisshuhn & Prati 2009). AC has also been used experimentally to alter plants microbial associations (Weisshuhn & Prati 2009; Wurst & van Beersum 2009; Aschehoug et al. 2012). AC addition to soil decreased plant infection with arbuscular mycorrhizal fungi (AMF; Weisshuhn & Prati 2009). Additionally, AC has been shown to negatively impact N-fixing species that depend on symbiont bacteria; the disrupted communication with the microbial community decreases bacterial associations (Wurst & van Beersum 2009). In agricultural applications, AC is used to protect seeds from pre-emergence herbicide applications (Toth et al. 1987). AC is able to strongly absorb some herbicides (Coffey & Warren 1969), though effectiveness is dependent upon herbicide and application rate, soil depths, and soil types (Burr et al. 1972). Recently, research has been done testing the use of AC in restoration efforts as a protective coating for native seed in herbicide-treated sites (Madsen et al. 2014).

AC’s impact on soil microbial communities may lead to altered nutrient levels. In greenhouse experiments, AC soil additions increased available N concentrations (Berglund et al. 2004; Lau et al. 2008), available phosphate levels (Weisshuhn & Prati 2009), and, dependent upon AC source, can increase or decrease pH levels (Kabouw et al. 2010). In small scale field experiments, AC soil treatments decreased extractable C and N in topsoil (Kulmatiski & Beard 2006). While AC has a strong affinity and bonding capability for organic compounds, it has a weak affinity to inorganic nutrients, such as \( \text{NH}_4^+ \) and \( \text{NO}_3^- \) (Chermisinoff & Ellderbusch 1978). However, nutrient changes likely occur due to AC effects on microbial communities rather than nutrient inputs from AC addition or binding of nutrients to AC. AC has been shown to change C:N ratios in
greenhouse studies, suggesting increased microbial activity (Weisshuhn & Prati 2009). AC can negatively impact N-fixing species’ bacterial symbiont associations (Wurst & van Beersum 2009), which could lead to decreased available N.

A small-scale assessment of AC effects in field conditions to restore native plant communities was performed in 2002 in the Methow Valley, Washington (Kulmatiski & Beard 2006; Kulmatiski 2011). AC was applied to 60, 1-m² plots in three fallowed fields at a concentration of 1000 g/m². AC additions reduced concentrations of extractable organic C and N and induced consistent changes in plant community composition. Six years following this single field application, native species were dominant in plots that had been dominated by non-natives for up to 47 years. Dominant non-native species decreased on AC treated plots compared to controls, while native perennial grasses increases on AC plots compared to controls. In an attempt to identify the mechanism through which AC changes plant growth, it was found that AC decreased the microbial lipids associated with non-native plant success. Specifically, AC treatments caused a large decrease in AMF abundance. This suggests that AC decreased non-native plant growth by decreasing the soil microbes upon which non-natives rely for vigorous growth (Kulmatiski & Beard 2006; Kulmatiski 2011). Pyrosequencing of bacteria and archaea communities was performed in the large-scale application of AC in the field experiment described in this thesis. Results from this analysis may identify specific organisms, or groups, which are significantly altered by the presence of AC. This information used with plant growth response could be a useful key to future research in targeted restoration.

The primary mechanism through which AC elicits plant community response is still unknown. Distinguishing whether a plant responds to AC because it binds
allelochemicals or inhibits plant-microbe interactions will help identify the mechanisms explaining non-native plant success and help direct the development of future, more efficient restoration techniques (Kulmatiski & Beard 2006; Lau et al. 2008; Weibhuhn & Prati 2009; Kulmatiski 2011). Both modes of operation have the potential to increase the growth of natives and decrease the growth of non-natives.
CHAPTER 3
METHODS

Field Experiment

To test the effectiveness of AC in the field at a large (15 m x 15 m) spatial scale, a field experiment was conducted in nine former agricultural fields located in the Methow Valley, Washington, USA (48°37’N, 107°10’W, 580–880 m a.s.l.). Fields were on Newbon-Conconully soil series association (coarse-loamy, mixed mesic Typic Haploxerolls). This semi-arid shrub-steppe ecotype has a mean annual precipitation of 380 mm, primarily occurring as snow outside of the growing season (October-March).

The vegetation and management history of the study area is representative of a significant portion of the northern Intermountain West; abandoned agricultural fields are dominated by non-native plants (Sheley 1998). Dominant non-native species include grasses: *Bromus inermis* Leyss. DC, *Bromus tectorum* L., and *Poa bulbosa* L. and forbs: *Cardaria draba* L. Desv., *Centaurea diffusa* Lam., *Lactuca serriola* L., *Medicago sativa* L., and *Sisymbrium spp.* (*S. altissimum* L. and *S. loeselli* L.). Native vegetation areas that have never been used for agriculture surround these abandoned agricultural fields and include grasses: *Festuca idahoensis* Elmer., *Koeleria macrantha* (Lede.) Schult., and *Pseudoroegneria spicata* Pursh., forbs: *Balsamorhiza sagittata* Pursh. and *Lupinus spp* (*L. arbusus* Dougl., *L. aridis* Dougl., and *L. sericeus* Pursh.), and shrubs: *Artemisia tridentata* Nutt and *Purshia tridentata* Pursh. (Kulmatiski 2006). Species in this area can be categorized as undesirable or desirable. Desirable species are native plant species commonly found in undisturbed native-dominated fields. Undesirable species include non-native species and some native annual forbs, for example *Amsinckia menziesii,*
which are common in weed dominated abandoned agricultural fields and rarely found in undisturbed native-dominated plant communities. For species considered undesirable, there are active campaigns in the study area to remove the species in this category.

At each of the nine sites, seven 15 m x 15 m plots were established with a 5 m buffer between plots and the following treatments were applied randomly to these plots: four concentrations of coal-based AC, 100 g/m² (100), 400 g/m² (400), 700 g/m² (700), 1000 g/m² (1000), one wood-based carbon treatment at 1000 g/m² concentration (1000w), and a control which received the same physical disturbance and seeding as the treatments but no AC additions, 0 g/m² (0). Each site also had one “complete” control (CC) with the same physical disturbance but no AC or seed addition. AC used was a commercial grade, coal-based carbon powder with a 300 mesh size and iodine number (measure of pore content) > 500 mg/m² and wood-based carbon with a 330 mesh size and iodine number > 500 mg/m² (Carbon Activated Corporation, Compton, California, USA). Sites were treated in October 2010.

AC was manually applied in powdered form using a push seed spreader and mixed into the top soil to 15 cm depth using two passes with a disc harrow pulled by a tractor. This soil mixing also removed standing vegetation. A mix of native seeds was broadcast by a hand seed spreader at a rate of 5.6 g/m². The seed mix included by weight: 7.5 % B. sagittata, 12.6% Collomia grandiflora Douglas ex Lindl., 17.9% F. idahoensis, 17.4% K. macrantha, 9.0% L. sericeus, 7.9% Lomatium dissectum Nutt., and 27.7% P. spicata, using local varieties when available (BFI Native Seeds, Moses Lake, WA, USA).

Plant growth was monitored for three growing seasons following treatment. In the first year, germination and emergence was measured in May 2011 using vegetation
counts in twenty-seven 10-cm² sampling grids in each treatment plot. Vegetation cover was measured in June 2011, 2012, and 2013 using the point-intercept method in nine 1-m² sampling grids within each treatment plot.

In 2011, to determine the effect of AC on N cycling rates, we determined N mineralization rates in the g/m², 100 g/m², 1000 g/m², and CC plots for all nine sites using the buried bag technique (Robertson et al. 1999). Briefly, in early June, 10, 4-cm diameter core samples were collected to a depth 15 cm in each plot. Five samples were processed immediately and five were placed individually into polyethylene bags and reburied for one month. From each core sample, 10 g of soil was extracted in 100 mL 2.0 M KCl. Inorganic N was determined from colorimetric analysis of KCl extracts using a Lachat autoanalyzer (Lachat Instruments, Loveland, Colorado, USA). Rates of microbial mineralization were calculated using the change in NH₄⁺ and NO₃⁻ [incubated (NH₄⁺ + NO₃⁻) - initial field (NH₄⁺ + NO₃⁻)] and nitrification rates were calculated from the change in NO₃⁻ (incubated NO₃⁻ - initial field NO₃⁻).

**Greenhouse Experiment**

To isolate the mechanism through which AC changes plant growth, a greenhouse experiment was conducted in an Agricultural Research Station greenhouse facility at Utah State University, Logan, Utah, USA from August to November 2011. A total of 740 1-L polyethylene pots (7.6 cm width and 20.3 cm height; Stuewe & Sons, Tangent, Oregon, USA, model MT38) were randomly assigned to one of three soil treatments: live soil with AC, sterile soil with AC, and a control live soil. All pots were filled with a steam sterilized 6:1 ratio of sand and peat. The two treatments that received live soils (i.e., both AC live and control live) were inoculated with 5% by volume field soil.
collected from the Methow Valley. The two AC treatments received 1% AC by mass (~10 g AC Kg\(^{-1}\) soil), which was mixed throughout the pot. A coal-based, 300 mesh, commercial grade AC was used. Soils in ‘AC sterile’ treatments were inoculated with 5% by volume of gamma-irradiated field soil, 25 kGy dose prior to AC addition (JS8900 Batch Gamma Irradiator, Steris Isomedix, Temecula, California, USA).

One native and one non-native plant species was grown in each pot. For the plant species listed below, all possible native-non-native pairing combinations were replicated for each treatment. Each pairing was replicated 15 times in AC-live and control-live treatments, and 12 times in AC-sterile treatments. Species included the most common species found at the field study site: three native grass species *F. idahoensis*, *K. macrantha*, and *P. spicata*, and two native forbs, *L. sericeus* and *B. sagittata*, and five common non-native species: the grass *B. tectorum*, and four non-native forbs: *C. diffusa*, *L. serriola*, *Tragopogon dubius* Scop., and *S. altissimum*. *B. sagittata* experienced successful growth in fewer than 10% of pots and was removed from the primary analysis. Non-native plant growth in the pots in which *B. sagittata* never grew was used as a measure of the direct effects of AC and sterilization on non-native plants (i.e., in the absence of competition from native plants). Native seeds were purchased and local Methow Valley varieties were used when possible (BFI Native Seeds, Moses Lake, WA, USA). Non-native seeds were collected from the Methow Valley study sites in summer 2011.

Seeds were kept moist and germinated at a mean temperature of 22°C and 14 hours of light daily then transplanted into each pot. Three successful germinants of each species were planted per pot. After 3 weeks, the tallest individual of each species was
allowed to remain in each pot while the shorter two were removed. Greenhouse conditions were at a mean temperature of 22°C and sodium lamps were used to maintain 14 hours of light daily. All pots were watered daily. Pots were rotated within the greenhouse weekly.

Three months after the initiation of the experiment, aboveground biomass was clipped for each species. Belowground biomass was separated from the soil for each species. Above- and belowground biomass for each species in each pot was dried at 70°C until constant weight and weighed.

Statistical Analyses

Two sets of test were conducted on the field data. First, we tested the effect of the high concentration of AC on plant growth responses to determine if the results were similar to those from the previous small scale experiment (Kulmatiski & Beard 2006). Second, we tested the effects of multiple AC concentrations to determine at which concentration AC had measurable effects on plant growth response.

To test the effect of the high concentration treatment, treatment effects on the 0, 1000, and CC plots were tested on the percent cover of desirable species primarily found in the native fields (hereafter, desirable species), the percent cover of undesirable species primarily found in non-native fields (hereafter, non-native species), the ratio of desirable species to undesirable species (desirable:undesirable ratio) using a two-way randomized block design with repeated measures and subsamples analysis of variance (ANOVA). Fixed effect were treatment and year, random effects were field, field*treatment. To look at individual species responses, we also tested the effectiveness of AC on the ten most abundant species in 0 and 1000 plots using a one-way randomized block design with
repeated measures and subsamples; fixed effects were treatment and random effects were field and field\*treatment.

To compare the effects of different AC concentrations, treatment effects in the 100, 400, 700, 1000, and 1000w plots were tested on the percent cover of desirable species, undesirable species, the ratio of desirable:undesirable species, and N mineralization and nitrification rates using a one-way randomized block design with repeated measures and subsamples ANOVA. Fixed effect were treatment, random effects were field, field\*treatment. Post-hoc Tukey-Kramer method was used to adjust for Type I error and determine pairwise differences among least square means.

Species were categorized into the following groupings for analysis. Species were considered undesirable that are commonly found in non-native dominated abandoned agricultural fields. This category included all non-native species, many species considered “weedy” by the Western Society of Weed Science, and five native annual forbs. These native forbs, for example *Amsinckia menziesii*, are all common in weedy fields and rarely, if ever, found in undisturbed native-dominated plant communities. They are all typically considered undesirable (DiTomaso 2000), and there are active campaigns in the study area to remove all the species in this category. Species were considered desirable if they were commonly found in undisturbed native-dominated field. This category only included native species. We used this classification system instead of native and non-native because there are some species, like native annual forbs species that did not equate with categories of species found in abandoned agricultural field and native-dominated undisturbed areas, and in this study we are trying to identify ways to restore the communities found in the abandoned agricultural fields. Prior to analysis, raw
percent cover values were square-root transformed and ratio data was log-transformed to better meet assumptions of normality and homogeneity of variance.

For the greenhouse experiment, effects of treatment were tested on ratio of native:non-native aboveground biomass, native aboveground biomass, non-native aboveground biomass, native belowground biomass, and non-native belowground biomass using a three-way factorial in a completely randomized design ANOVA. Fixed effects were treatment, native species, and non-native species, pots were considered replicates and included in residual effects. Additionally, monoculture non-native biomass was tested in a one-way factorial with treatment and non-native species as fixed effects. Post-hoc Tukey-Kramer method was used to adjust for Type I error and determine pairwise differences among least square means. Prior to analysis, pots with no total growth were removed from the dataset for data to better meet assumptions of normality and homogeneity, leaving 284 pots for AC live, 194 pots for AC sterile, and 239 for control live,. Above- and belowground biomass raw values were square-root transformed and native:non-native aboveground ratio data was log-transformed to better meet assumptions of normality and homogeneity of variance. All tests were considered significant at the $\alpha = 0.05$ level. Analyses were conducted using the GLIMMIX procedure in SAS for Windows v. 9.3 (SAS Institute Inc., Cary, North Carolina, USA).
CHAPTER 4
RESULTS

Field Experiment
Large-Scale Application Test

For all years, complete control (CC) plots differed from seed treated plots (AC applied at both 0 g/m² and 1000 g/m²) for desirable percent cover, undesirable percent cover, and desirable:undesirable ratio ($F_{2,712} = 121.63$, $p = 0.0001$, $F_{2,712} = 125.84$, $p = 0.0001$, $F_{2,712} = 125.84$, $p = 0.0001$, respectively). Desirable species percent cover was lower in CC plots than seed addition plots with 0 g/m² and 1000 g/m² AC treatments, while 0 g/m² and 1000 g/m² AC did not significantly differ (Fig. 2b). Undesirable percent cover was higher in CC plots than 0 g/m² and 1000 g/m² treatments and lower in 1000 g/m² than 0 g/m² treatment (Fig. 2c). The desirable:undesirable species ratio was lower in the CC plots than 0 g/m² and 1000 g/m² treatments and lower in 0 g/m² than 1000 g/m² (Fig. 2a).

Different Concentrations of AC

In May 2011, there was no effect of AC treatment on desirable species germination ($F_{5,40} = 0.91$, $p = 0.4839$, Fig. 3b), but there was an effect of treatment on undesirable species germination ($F_{5,40} = 2.57$, $p = 0.0417$, Fig. 3c); 0 g/m² had the greatest germination and 1000 g/m² had the least germination. There was also a treatment effect on desirable:undesirable germination ratio with 1000 g/m² having the highest ratio ($F_{5,40} = 1.72$, $p = 0.1520$, Fig. 3a).
Figure 2. Treatments complete control (CC), seeded control (0), and AC treated (1000) for each year for: A) Desirable:undesirable ratio, B) Desirable species percent cover, C) Undesirable percent cover (mean ± SE). Letters denote differences from combined years results. Year was significant for desirable:undesirable ratio, desirable species cover, and undesirable species cover ($F_{2,712} = 25.98$, $p = 0.0001$, $F_{2,712} = 52.11$, $p = 0.0001$, $F_{2,712} = 52.11$, $p = 0.0001$, respectively).
Figure 3. Treatments 0 g/m$^2$, 400 g/m$^2$, 700 g/m$^2$, 1000 g/m$^2$, and 1000w g/m$^2$ for May 2011, June 2011, June 2012, and June 2013 for A) desirable:undesirable species cover ratio, B) desirable species percent cover, and C) undesirable species percent cover. (mean ± SE) Letters denote significant differences between treatments.

In June 2011, there was no AC treatment effect on desirable species (F$_{5,40} = 0.84$, p = 0.5295, Fig. 3b), undesirable species (F$_{5,40} = 0.79$, p = 0.5629, Fig. 3c), or the desirable:undesirable ratio (F$_{5,40} = 2.00$, p = 0.1018, Fig. 3a). In May and June, $B. officinalis$ had greater percent cover in 0 g/m$^2$ than 1000 g/m$^2$ treatments (F$_{1,8} = 0.41$, p = 0.0914; F$_{1,8} = 4.81$, p = 0.0595). $B. tectorum$ had greater percent cover in 0 g/m$^2$ than 1000 g/m$^2$ treatments in June 2011 (F$_{1,8} = 4.81$, p = 0.0595; Table 8, Fig. 3).

In June 2012, there was no AC treatment effect for desirable species cover (F$_{5,40} = 0.70$, p = 0.6248, Fig. 3b), undesirable species cover (F$_{5,40} = 0.56$, p = 0.7288, Fig. 3c), or the desirable:undesirable ratio (F$_{5,40} = 0.73$, p = 0.6075, Fig. 3a).
In June 2013, there was no AC treatment effect for desirable species \((F_{5,40} = 0.53, p = 0.7554, \text{Fig. 3b})\). But, undesirable species differed among treatments \((F_{5,40} = 2.99, p = 0.0222, \text{Fig. 3c})\), 0 g/m\(^2\) had greater undesirable species cover than all other treatments. Furthermore, the desirable:undesirable ratio was the lowest in the 0 g/m\(^2\), lower than 400 g/m\(^2\), 700 g/m\(^2\), and 1000 g/m\(^2\) AC treatments \((F_{5,40} = 1.97, p = 0.1050, \text{Fig. 3a})\).

There was no treatment effect for nitrogen mineralization or nitrification rates \((F_{3,21} = 0.37, p = 0.7753, F_{3,21} = 0.67, p = 0.5781)\). Mean final nitrogen levels were 0.35 mg NH\(_4\)+/kg soil and 1.23 mg NO\(_3\)-/kg soil, with a nitrogen mineralization mean rate of 0.83 mg/kg and nitrification mean rate of 0.85 mg/kg for the incubation period.

**Greenhouse Experiment**

The highest ratio of native:non-native aboveground biomass was found in the AC live soils; the native:non-native aboveground biomass was greater in AC live soil than AC sterile and control soils \((F_{2,657} = 3.29, p = 0.0380; \text{Fig. 4a})\).

Across all native species, native aboveground biomass in AC live soil was lower than native aboveground biomass in the control soil, while native aboveground biomass in the AC sterile treatment was not significantly different from biomass in AC live and control soil \((F_{2,657} = 5.41, p = 0.0047; \text{Fig. 4b})\). Across all non-native species, non-native aboveground biomass in AC live soil was lower than non-native aboveground biomass in AC sterile and control soils \((F_{2,657} = 6.06, p = 0.0025; \text{Fig. 4b})\). This was also true for non-native species grown in monoculture (i.e., unsuccessful *B. sagittata* pairings) \((F_{2,182} = 5.18, p = 0.0065; \text{Fig. 5})\).

Individual native species responded similarly to treatment for the ratio of native:non-native aboveground biomass \((F_{6, 657} = 0.85, p = 0.5352)\). Individual non-native
species responded differently to treatment for the ratio of native:non-native aboveground biomass ($F_{8,657} = 3.93$, $p = 0.0001$). *L. serriola* showed a significant native:non-native aboveground biomass response to treatment, whereas the other non-native did not respond differently to treatment. *L. serriola* followed the general trend of all pots, the ratio was higher for *L. serriola* in AC live than AC sterile and control soils.

![Figure 4](image)

Figure 4. A. Native:non-native species above ground biomass ratio by treatment. B. Total aboveground biomass for all native species and non-native by treatment. (Mean ±SE)
As would be expected, species exhibited different total growth which affected their native:non-native ratio values. There were differences among species for native:non-native aboveground biomass, native aboveground biomass, and non-native aboveground biomass ($F_{4,657} = 121.45$, $p = 0.0001$; $F_{3,657} = 24.22$, $p = 0.0001$; $F_{3,657} = 54.33$, $p = 0.0001$; $F_{8,657} = 4.13$, $p = 0.0001$).

![Figure 5](https://via.placeholder.com/150)

Figure 5. Non-native biomass (g) for individuals grown in monoculture (mean ± SE).

Individual species growth was strongly influenced by the composition of their paired species. Non-native species affected the growth of native species ($F_{4,657} = 10.95$, $p = 0.0001$). Natives had the greatest biomass with *S. altissimum* and the least with *B. tectorum*. Native species in turn affected the growth of non-native species ($F_{3,657} = 4.48$, $p = 0.0040$). Non-natives had the greatest aboveground biomass with *L. sericeus* and the least with *K. macrantha* compared to the other native species.
Belowground native biomass and non-native biomass showed no response to treatment ($F_{2,657} = 1.83, p = 0.1605$; $F_{2} = 1.51, p = 0.2208$, respectively).
CHAPTER 5
DISCUSSION

Across the field and greenhouse experiments, AC decreased undesirable plant species growth and improved the relative abundance of desirable to undesirable plant species. These AC effects appear to be driven by the ability of AC to limit positive interactions between non-native plants and soil microbes. Unfortunately, these effects were only observed at the highest AC concentrations of 1000 g/m$^2$. As a result, this technique may have applications in high-value sites such as abandoned oil exploration sites but is not likely to see wide use. Results do, however, suggest that future efforts that focus on manipulating plant-microbe interactions may produce novel, potent and cost-effective approaches to native plant restoration.

We found that at a large-scale application the addition of 1000 g/m$^2$ of coal-based AC increased the ratio of desirable to undesirable plant species. These results are similar to previous research conducted on a smaller spatial scale (1 m x 1 m plots), and indicates that AC is an effective restoration tool at large scale (Kulmatiski & Beard 2006; Kulmatiski 2011). This result was not detectable in the first year of the study, but was measurable by the third year of the study and across years. Across the three years, we found an increase in the ratio of desirable:undesirable plant species of 28% in 1000 g/m$^2$ plots compared to control plots that received similar disturbance and seeding but no AC (seeded controls). Undesirable species were the drivers of this community composition change. The addition of 1000 g/m$^2$ of AC decreased undesirable growth to 25% cover from 29% in seeded controls and 42% in unseeded controls. While this is not a large effect, previous results suggest that AC effects on native growth will increase with time.
as native perennials continue to expand and outcompete weedy species in AC treated plots.

In the greenhouse experiment, similar to the field experiment, AC addition to live soil decreased non-native growth, and increased the ratio of native to non-native plants. More specifically, with the addition of 1000 g/m² of AC to live soils, non-native species had 25% less biomass in pairs with native species, and a 37% less biomass when grown in monoculture compared to control soils. However, unlike the field experiment, native species also had 25% less biomass in AC live soil treatments compared to controls. Even though both non-native and native species had lower biomass in AC live soil, because non-natives had more biomass than natives, the reduction in biomass of non-native species created an overall increase in the native:non-native ratio by 3% in AC live soil compared to control soil.

A goal of our experiment was to determine whether AC changes plant growth by changing plant-microbial interactions or by reducing allelopathic chemicals. We accomplished this by measuring changes in the plant biomass in live and sterilized AC-treated soils. If AC benefits native plants by binding allelochemicals, then native plants competing with allelopathic non-native species should grow better in both AC-live and AC-sterile soils (Fig. 1), but this is not what we found. Because we did not measure significant changes in biomass in sterilized AC soils compared to control soil, native and non-native species appear to be unaffected by loss of allelopathy. We found little evidence to suggest that AC affects plant growth in sterile soils. Rather all plants grew poorly in AC-live soils suggesting that AC limits interactions between plants and beneficial soil organisms (e.g., growth-promoting bacteria). This negative effect of AC in
live soils was stronger for non-native resulting in a net benefit for native plants. Because we found greater differences in both native and non-native plant responses in AC live soils than in AC sterile soils compared to control soils, our results support the hypothesis that the primary pathway through which AC changes plant growth is through the microbial community for both native and non-native species. Many experiments have been conducted testing allelopathic effects using AC (Lau et al. 2008; Wurst et al. 2010; Murrell et al. 2011); however, the results of this experiment suggest that the effects of AC on plant-microbial interactions are more influential than allelopathic effects.

The specific mechanism through which AC may be creating this response is through a loss of positive plant-soil feedbacks. Previous research suggests that non-native species create positive relationships with the microbial community (Callaway & Aschehoug 2000; Mangla et al. 2008), but AC addition could interrupt this communication by sequestering organic compounds, and thus this positive effect could be lost (Weisshuhn & Prati 2009; Wurst & van Beersum 2009). For example, non-native species increase growth by using root exudes against pathogens (Van Der Putten 2003; Bais et al. 2004; Orians & Ward 2010; Doornbos et al. 2012). AC can decrease this defense by binding root exudes, which would lead to lower non-native biomass in AC live soils, while plants in sterile soils would not experience these effects due to total pathogen removal. Finally, AC may suppress symbiont associations with non-native species (Fig. 1, Non-native Symbiont), which would decrease growth in the live soil. In support of this, in the small-scale field experiment there was 44% less arbuscular mycorrhizal fungi in AC soils compared to control soils (Kulmatiski 2011). Symbiont loss would also be present in sterilized soils, but soil sterilization can cause positive plant
response because gamma irradiation also removes all potential pathogens (Callaway et al. 2004; De Deyn et al. 2004) and can release nutrients (Powlson & Jenkinson 1976; McNamara et al. 2003). It is more likely result is due to pathogen loss because nutrient release from gamma irradiated soil is unlikely to have a large impact in this case due to the small amount of live soil added (5% of total soil volume). Native species also experienced decreased growth in AC live soil treatments, which may also be caused by symbiont suppression (Fig. 1, Native Symbiont). Further research into the specific microbial changes occurring may be able to produce techniques that can target non-native positive microbial associations.

Neither the field nor greenhouse results suggest that AC decreases plant growth or increases the relative abundance of native plants by decreasing nutrient cycling rates. First, we did not find that AC affected N cycling or concentrations between our 1000 g/m² and control plots in our field experiment. Second, in the greenhouse experiment, AC addition does not appear to elicit plant growth changes through altered nutrient levels or cycling because a change caused by AC addition would be present equally in both live and sterile soils for native and non-native species, while we found significantly decreased biomass in only AC live soils.

Two other goals of this experiment were to determine: 1) whether different types of AC may be equally effective, and 2) the effectiveness of different concentrations of AC. In the previous field (Kulmatiski & Beard 2006) and greenhouse experiments (Lau et al. 2008; Weissahuhn & Prati 2009; Kabouw et al. 2010), coal-based AC has been typically used. We wanted to test the effectiveness of wood-based carbon because wood-based AC treatments would hold the benefit of fixing carbon into soils and may allow the
development of on-site production. On-site carbon production could have the benefit of both removing woody materials that present a fire hazard (e.g., ponderosa pine thickets) while simultaneously fixing carbon in the soil. Between the two AC types tested, coal and wood-based AC, there were no differences at a 1000 g/m² concentration for desirable species cover or undesirable species cover for any individual year. The ratio of desirable:undesirable species was higher for wood-based AC in June 2011, but there are no differences in 2012 or 2013.

There is some suggestion in the data that concentrations below 1000 g/m² may be effective in restoring desirable plant species. For example, by 2013, undesirable species were significantly lower in 100 g/m² and 1000 g/m² AC concentrations, a 31% and 23% decrease, respectively, and the desirable:undesirable ratio was significantly higher for 400 g/m², 700 g/m², and 1000 g/m² AC concentrations compared to seeded control treatments. Further monitoring of these experimental sites may show long term success of lower AC concentration treatments.

There are several potential reasons that the response may not have been as strong in the current study as in the previous study. The difference may be due to more realistic larger scale applications in more fields, 9 fields compared to 3 fields. In the small-scale experiment, AC was thoroughly mixed into plots by hand, ensuring even application in the top 10 cm of soil. This experiment used techniques and equipment commonly used in restoration efforts, including tractors, disc harrows, and broadcast seed spreaders, which do not provide as thorough distribution and even mixture into top soil. The small-scale experiment also received a much higher rate of seeding, 13.2 g/m² compared to 5.6 g/m² in this experiment, as well as herbicide treatment prior to AC addition and seeding.
(Kulmatiski & Beard 2006). This more intensive site preparation possibly led to higher desirable species growth from seeding and lower undesirable growth from remaining seed or vegetation. Furthermore, due to favorable, wet conditions the spring following treatment in this study, seeding had a large positive effect on desirable species growth in both the AC and control plots, making it difficult for any treatments to increase desirable success beyond seeding success. More specifically, spring rainfall in the recent experiment greatly exceeded the area average of 7.4 cm. Between March and May 2011, the sites received 21.5 cm of precipitation, in contrast with 9.3 cm in 2003, the spring following treatment in the previous small-scale study. In this study, plots receiving no AC but receiving native seed mix experienced 7.3% higher desirable vegetation cover and 12.8% lower undesirable vegetation cover than treatments with no seeding across sampling periods. This high seeding success likely reduced the effect of AC addition, with no differences in desirable species cover between AC-treated plots and seeded control for any year and no difference in desirable:undesirable ratio between AC treatments and seeded control in 2011 and 2012. However, this seeding effect began to decrease by the third growing season. It is possible these trends will continue over time. Our targeted desirable natives are long-lived perennials and can be expected to persist, increase cover, and provide a native seed source.

It is necessary to consider the cost of AC application before considering broad uses. Costs for tested AC concentrations are approximately: 100 g/m² - $454/ ha, 400 g/m² - $1817/ ha, 700 g/m² - $3180/ ha, 1000 g/m² - $4,543/ ha, and 1000w g/m² - $5618/ ha. Due to its high costs, AC may not be a practical large-scale restoration tool. It may be useful for targeted treatments, in small highly concentrated sites, such as restoring
abandoned oil pad sites. Because AC is eliciting driving community changes by decreasing positive microbial interactions with non-native species, it may be more beneficial to further research and explore techniques in this field.

Figure 6. Percent cover in seeded control, 0 g/m², and AC-treated, 1000 g/m², plots of A. non-native grass species *B. tectorum* and B. native grass species *P. spicata*. Significant differences between treatments in a year noted with *.
CHAPTER 6

CONCLUSIONS

Our research demonstrated that AC is effective at restoring desirable plant communities at a larger scale. At concentrations of 1000 g/m\(^2\), across three years AC treatments were able to increase the ratio of desirable to undesirable species cover by 28% compared to seeded controls, primarily through a decrease in undesirable vegetation. However, this high application rate and the methods used in this experiment are not practical for typical restoration projects. AC costs alone are $4,500/ha. With additional expenses towards equipment, labor, and seed, this is not a cost-effective option for most restoration projects (Holl & Howarth 2000; Dorrough et al. 2008). With the strong seeding success in this study, non-native removal, either physical (e.g. tillage) or chemical (e.g. herbicides), followed by native species seeding may be a more desirable management option. AC may be useful in small, highly disturbed areas at a great risk of invasion by non-native plant species. An example is the growing disturbed lands from oil pad sites as oil and natural gas development expanded across the Intermountain West within sagebrush-dominated landscapes (Gilbert & Chalfoun 2011). At these oil pad sites, vegetation is removed and soil is greatly disturbed, typically experiencing compaction and loss of soil structure, which leaves a site that requires restoration of the plant community and soil.

Lower AC concentration treatments are not at this time effective in restoration. But, if treatments continue growth trends seen in June 2013, it may be possible to use greatly reduced concentrations of AC in plant community restoration. In June 2013, 400 g/m\(^2\) and 700 g/m\(^2\) AC treatments produced an increase in the desirable to undesirable
species cover ratio, but did not have significant decreases in undesirable species cover or increases in desirable species cover. The 100 g/m$^2$ did have a 31% decrease cover of undesirable species compared to seeded controls in June 2013. If this treatment concentration is able to maintain low undesirable species cover while desirable cover increases over time, it may be possible to have positive impacts on the ratio of desirable to undesirable species over time. The cost at an AC application of 100 g/m$^2$ is $450/ha, a more feasible cost for large scale applications. The high seeding success experienced may have delayed the positive impacts on community composition caused by AC additions. The target desirable species are long-lived perennial bunchgrasses, which are expected to persist, increase cover and abundance.

Though this research used equipment commonly available and used in restoration projects, the methods used are not practical for sites larger than the experimental scale. Tractors and disc harrows were used to mix topsoil and remove vegetation. But, AC was spread across plots through hand raking and seed was broadcast by hand, which is far too slow and labor intensive for broad restoration uses. Research is underway to improve AC application techniques. In an AC restoration experiment in Tooele, UT, AC was applied in solution. Spraying an AC slurry onto the treatment areas provided an even and time-efficient application method. At these sites in Tooele, UT, seeds were broadcast at a constant rate from a seed spreader attached to a tractor, but it is also possible to use rangeland drills for seeding. Monitoring of this experiment is still required to assess the success of these application techniques on community restoration; however, the techniques themselves were successful in applying AC. AC has the potential to be a useful tool in many types of systems and among diverse plant species. As AC
indiscriminately binds all organic molecules, AC will inhibit plant-soil communication and interrupt plant microbial interactions in all systems. However, in sites where other biotic or abiotic factors are more influential to plant success, such as systems with limited nutrient or water availability, the loss of microbial communication due to AC treatment will not affect plant growth patterns or community composition.

A goal of this research was to determine the mechanism through which AC affects plant growth. Our results support the hypothesis that AC is eliciting plant response through disruption in plant-microbe communication. Native and non-native species experienced a 25% decreased growth in AC-treated live soils, with no significant decreases in AC-treated sterile soils. Despite equal percent decreases in native and non-native species biomass, AC additions to live soils positively impact community composition through the greater biomass reduction of non-native species. AC live soil treatments had the greatest native to non-native biomass ratio. Our results determine that the role of allelopathy and its inhibition from AC presence are not important competitive factors for our study species. The strong microbial response suggests that AC is not an effective tool in testing or manipulating plant-plant allelopathy as results can be strongly influenced by microbial interactions.

From the results of this study, we are unable to determine the specific changes in microbial associations. However, from these results and results of other AC research studies, it is suggested that AC is causing a decrease in symbiont associations for both native and non-native species. A loss in symbionts due to AC disruption of plant-soil communication would lead to decreased biomass, which occurred in our results. In previous AC field experiments, AC caused a decrease in AMF soil abundance
(Kulmatiski 2011). In greenhouse conditions, AC additions to soil decreased plant infection with AMF (Weisshuhn & Prati 2009). Some successful non-native species form strong symbiont relationships with introduced soil AMF communities (Greipsson and DiTommaso. 2006), and create soil legacies of altered AMF diversity and abundance (Jordan et al. 2012).

Future research should aim to investigate the hypothesis that AC decreases symbiont associations, particularly with non-native species. If it determined that plant biomass responses are a direct cause of symbiont loss, it may be possible to isolate specific organisms groups, such as AMF, or species that are influential to growth success of specific, problematic non-native species. The next step would be to pursue research to create a targeted method to decrease non-native species symbionts. A targeted restoration approach could remove important competitive advantages of non-native species.
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APPENDICES
APPENDIX A
Supplemental figures and table
Figure A-1. Root biomass (g) by treatment for A. May 2011, B. June 2011, and C. July 2011. (mean ±SE)
Figure A-2. Treatments 0 g/m², 100 g/m², 1000 g/m², and CC net nitrogen mineralization and net nitrification rates (mean ±SE)
Figure A-3. Relationship between mean percent cover between control plots, 0 g/m$^2$, and AC treated plots, 1000 g/m$^2$. Amal, A. albus; Boof, B. officinalis; Cedi, C. diffusa; Chal, C. album; Cogr, C. grandiflora; Copa, C. parviflora; Koer, K. Cristata; Lase, L. serriola; Mesa, M. sativa; Pobu, P. bulbosa; Podo, P. douglasii; Pssp, P. spicata; Sial, S. altissimum.
Figure A-4. Native species above ground biomass by treatment (Mean ±SE). Significant differences from control denoted by *.
Figure A-5. Non-native species above ground biomass by treatment (Mean ±SE). Significant differences from control denoted by *.
Table A-1. Species grouping and classification for field experiment results

| Species                              | Origin     | Growth Habit | Duration | Categorization |
|--------------------------------------|------------|--------------|----------|----------------|
| Acroptilon repens (L.) DC.           | non-native | forb         | perennial| undesirable    |
| Agoseris glauca (Pursh) Raf.         | native     | forb         | perennial| desirable      |
| Agropyron cristatum (L.) Gaertn.     | non-native | grass        | perennial| undesirable    |
| Amaranthus albus L.                  | non-native | forb         | annual   | undesirable    |
| Amsinckia menziesii (Lehm.) A. Nelson & J.F. Macbr. | native | forb         | annual   | undesirable    |
| Artemisia tridentata Nutt.           | native     | shrub        | perennial| desirable      |
| Balsamorhiza sagittata (Pursh) Nutt. | native     | forb         | annual   | desirable      |
| Borago officinalis L.                | non-native | forb         | annual   | undesirable    |
| Bromus inermis Leyss.                | non-native | grass        | perennial| undesirable    |
| Bromus tectorum L.                   | non-native | grass        | annual   | undesirable    |
| Buglossoides arvensis (L.) I.M. Johnst. | non-native | forb         | annual   | undesirable    |
| Cardaria draba (L.) Desv.            | non-native | forb         | perennial| undesirable    |
| Centaurea diffusa Lam.               | non-native | forb         | perennial| undesirable    |
| Chenopodium album L.                 | native     | forb         | annual   | undesirable    |
| Chorispora tenella (Pall.) DC.       | non-native | forb         | annual   | undesirable    |
| Collinsia parviflora Lindl.          | native     | forb         | annual   | undesirable    |
| Collomia grandiflora Douglas ex Lindl. | native | forb         | annual   | desirable      |
| Convolvulus arvensis L.              | non-native | forb         | perennial| undesirable    |
| Festuca idahoensis Elmer             | native     | grass        | perennial| desirable      |
| Hesperostipa comata (Trin. & Rupr.) Barkworth | native | grass        | perennial| desirable      |
| Koeleria cristata Schult.            | native     | grass        | perennial| desirable      |
| Lactuca serriola L.                  | non-native | grass        | annual   | undesirable    |
| Leymus cinereus (Scribn. & Merr.) Á. Löve | native | grass        | perennial| desirable      |
| Lomatium spp.                        | native     | forb         | perennial| desirable      |
| Lomatium dissectum (Nutt.) Mathias & Constance | native | forb         | perennial| desirable      |
| Lupinus sericeus Pursh               | native     | forb         | perennial| desirable      |
| Madia citriodora Greene              | native     | forb         | annual   | undesirable    |
| Medicago sativa L.                   | non-native | forb         | perennial| undesirable    |
| Microsteris gracilis (Hook.) Greene var. humilior (Hook.) Cronquist | native | forb         | annual   | undesirable    |
| Species                                      | Origin | Type  | Life Cycle   | Desirability |
|----------------------------------------------|--------|-------|--------------|--------------|
| *Phacelia linearis* (Pursh) Holz.            | native | forb  | annual       | undesirable  |
| *Poa bulbosa* L.                             | non-native | grass | perennial    | undesirable  |
| *Polygonum douglasii* Greene                 | native | forb  | annual       | undesirable  |
| *Pseudoroegneria spicata* (Pursh) Á. Löve    | native | grass | perennial    | desirable    |
| *Purshia tridentata* (Pursh) DC.             | native | shrub | perennial    | desirable    |
| *Sisymbrium alitissimum* L.                  | non-native | forb  | annual       | undesirable  |
| *Taraxacum F.H. Wigg.*                      | non-native | forb  | perennial    | undesirable  |
| *Thlaspi arvense* L.                         | non-native | forb  | perennial    | undesirable  |
| *Tragopogon dubius* Scop.                   | non-native | forb  | annual       | undesirable  |
APPENDIX B
Field experiment ANOVA tables
Table B-1. ANOVA results to estimate the effects of treatment and year on desirable:undesirable species percent cover ratio; CC, 0 g/m$^2$, and 1000g/m$^2$ treatments for June 2011, June 2012, and June 2013.

| Effect          | Num DF | Den DF | F Value | Pr > F |
|-----------------|--------|--------|---------|--------|
| Treatment       | 2      | 712    | 121.63  | <.0001 |
| Year            | 2      | 712    | 25.98   | <.0001 |
| Treatment*Year  | 4      | 712    | 1.68    | 0.1529 |

Table B-2. ANOVA results to estimate the effects of treatment on desirable species percent cover; CC, 0 g/m$^2$, and 1000g/m$^2$ treatments for June 2011, June 2012, and June 2013.

| Effect          | Num DF | Den DF | F Value | Pr > F |
|-----------------|--------|--------|---------|--------|
| Treatment       | 2      | 712    | 125.84  | <.0001 |
| Year            | 2      | 712    | 52.11   | <.0001 |
| Treatment*Year  | 4      | 712    | 7.44    | <.0001 |

Table B-3. ANOVA results to estimate the effects of treatment and year undesirable species percent cover; CC, 0 g/m$^2$, and 1000g/m$^2$ treatments for June 2011, June 2012, and June 2013.

| Effect          | Num DF | Den DF | F Value | Pr > F |
|-----------------|--------|--------|---------|--------|
| Treatment       | 2      | 712    | 125.84  | <.0001 |
| Year            | 2      | 712    | 52.11   | 0.0268 |
| Treatment*Year  | 4      | 712    | 7.44    | 0.9964 |

Table B-4. ANOVA results to estimate the effects of treatment on desirable species percent cover: treatments 0 g/m$^2$, 100 g/m$^2$, 400 g/m$^2$, 700 g/m$^2$, 1000 g/m$^2$, and 1000w g/m$^2$.

| Effect          | Num DF | Den DF | F Value | Pr > F |
|-----------------|--------|--------|---------|--------|
| May 2011- Treatment | 5      | 40     | 0.91    | 0.4839 |
| June 2011- Treatment | 5      | 40     | 0.84    | 0.5295 |
| June 2012- Treatment | 5      | 40     | 0.70    | 0.6248 |
| June 2013- Treatment | 5      | 40     | 0.53    | 0.7554 |
Table B-5. ANOVA results to estimate the effect of treatment on undesirable species percent cover: treatments 0 g/m², 100 g/m², 400 g/m², 700 g/m², 1000 g/m², and 1000w g/m².

| Effect                  | Num DF | Den DF | F Value | Pr > F |
|-------------------------|--------|--------|---------|--------|
| May 2011- treatment     | 5      | 40     | 2.57    | 0.0417 |
| June 2011- treatment    | 5      | 40     | 0.79    | 0.5629 |
| June 2012- treatment    | 5      | 40     | 0.56    | 0.7288 |
| June 2013- treatment    | 5      | 40     | 2.99    | 0.0222 |

Table B-6. ANOVA results to estimate the effect of treatment on desirable:undesirable percent cover ratio: treatments 0 g/m², 100 g/m², 400 g/m², 700 g/m², 1000 g/m², and 1000w g/m².

| Effect                  | Num DF | Den DF | F Value | Pr > F |
|-------------------------|--------|--------|---------|--------|
| May 2011- Treatment     | 5      | 40     | 01.72   | 0.1520 |
| June 2011- Treatment    | 5      | 40     | 2.00    | 0.1018 |
| June 2012- Treatment    | 5      | 40     | 0.73    | 0.6075 |
| July 2013- Treatment    | 5      | 40     | 1.97    | 0.1050 |

Table B-7. ANOVA results to estimate the effect of treatment on nitrogen mineralization and nitrification rates: treatments 0 g/m², 100 g/m², 1000 g/m², and CC.

| Effect                  | Num DF | Den DF | F Value | Pr > F |
|-------------------------|--------|--------|---------|--------|
| Mineralization- Treatment| 3      | 21     | 0.37    | 0.7753 |
| Nitrification- Treatment | 3      | 21     | 0.67    | 0.5781 |

Table B-8. ANOVA results to estimate the effect of treatment on most abundant species in May 2011, treatments 0 g/m² and 1000 g/m².

| Effect        | Num DF | Den DF | F Value | Pr > F |
|---------------|--------|--------|---------|--------|
| P. spicata    | 1      | 8      | 0.18    | 0.6809 |
| B. officinalis| 1      | 8      | 0.41    | 0.0914 |
| A. albus      | 1      | 8      | 0.50    | 0.5006 |
| S. altissimum | 1      | 8      | 0.21    | 0.6607 |
| K. Cristata   | 1      | 8      | 0.33    | 0.5810 |
| L. serriola   | 1      | 8      | 1.40    | 0.2704 |
| P. bulbosa    | 1      | 8      | 0.29    | 0.6064 |
| C. album      | 1      | 8      | 0.04    | 0.8490 |
| C. parviflora | 1      | 8      | 1.07    | 0.3303 |
Table B-9. ANOVA results to estimate the effect of treatment on most abundant species in June 2011, treatments 0 g/m² and 1000 g/m²

| Effect       | Num DF | Den DF | F Value | Pr > F |
|--------------|--------|--------|---------|--------|
| *S. altissimum* | 1      | 8      | 0.02    | 0.9036 |
| *L. serriola*  | 1      | 8      | 0.43    | 0.5327 |
| *P. spicata*   | 1      | 8      | 0.11    | 0.7433 |
| *B. officinalis* | 1    | 8      | 0.21    | 0.5870 |
| *A. albus*     | 1      | 8      | 0.07    | 0.7996 |
| *L. sericeus*  | 1      | 8      | 0.01    | 0.9343 |
| *P. douglasii* | 1      | 8      | 2.65    | 0.1425 |
| *M. sativa*    | 1      | 8      | 0.74    | 0.4151 |
| *B. tectorum*  | 1      | 8      | 4.81    | 0.0595 |
| *C. diffusa*   | 1      | 8      | 0.72    | 0.4193 |

Table B-10. ANOVA results to estimate the effect of treatment on most abundant species in June 2012, treatments 0 g/m² and 1000 g/m²

| Effect       | Num DF | Den DF | F Value | Pr > F |
|--------------|--------|--------|---------|--------|
| *P. spicata* | 1      | 8      | 0.45    | 0.5217 |
| *C. diffusa* | 1      | 8      | 0.05    | 0.8307 |
| *K. cristata* | 1    | 8      | 0.01    | 0.9462 |
| *S. altissimum* | 1  | 8      | 0.01    | 0.9118 |
| *B. tectorum* | 1      | 8      | 1.83    | 0.2126 |
| *L. serriola* | 1      | 8      | 1.56    | 0.2467 |
| *M. citriodora* | 1 | 8      | 0.93    | 0.3621 |
| *C. grandiflora* | 1  | 8      | 0.35    | 0.5712 |
| *A. albus*    | 1      | 8      | 0.95    | 0.3584 |
| *M. sativa*   | 1      | 8      | 1.10    | 0.3255 |

Table 11. ANOVA results to estimate the effect of treatment on most abundant species in June 2013, treatments 0 g/m² and 1000 g/m²

| Effect       | Num DF | Den DF | F Value | Pr > F |
|--------------|--------|--------|---------|--------|
| *P. spicata* | 1      | 8      | 0.31    | 0.5921 |
| *C. diffusa* | 1      | 8      | 0.01    | 0.9821 |
| *K. cristata* | 1    | 8      | 0.53    | 0.4885 |
| *M. sativa*  | 1      | 8      | 2.92    | 0.1259 |
| *S. altissimum* | 1  | 8      | 0.10    | 0.7597 |
| *B. tectorum* | 1      | 8      | 0.04    | 0.8381 |
| *B. officinalis* | 1  | 8      | 0.87    | 0.3794 |
| *L. serriola* | 1      | 8      | 0.86    | 0.3797 |
| *A. albus*   | 1      | 8      | 0.83    | 0.3894 |
| *C. grandiflora* | 1  | 8      | 0.06    | 0.8161 |
APPENDIX C
Greenhouse experiment ANOVA tables
Table C-1. ANOVA results to estimate the effects of treatment, native species, and non-native species on the native: non-native species aboveground biomass ratio

| Effect                                 | Num DF | Den DF | F Value | Pr > F |
|----------------------------------------|--------|--------|---------|--------|
| Treatment                              | 2      | 657    | 3.29    | **0.0380** |
| Native species                         | 3      | 657    | 24.22   | **<.0001** |
| Treatment*Native species               | 6      | 657    | 0.85    | 0.5352 |
| Non-native species                     | 4      | 657    | 121.45  | **<.0001** |
| Treatment*Non-native species           | 8      | 657    | 3.93    | **0.0001** |
| Native species*Non-native species      | 12     | 657    | 2.42    | **0.0045** |
| Treatment*Native*Non-native           | 24     | 657    | 1.00    | 0.4647 |

Table C-2. ANOVA results to estimate the effects of treatment, native species, and non-native species on the native species aboveground biomass

| Effect                                 | Num DF | Den DF | F Value | Pr > F |
|----------------------------------------|--------|--------|---------|--------|
| Treatment                              | 2      | 657    | 5.41    | **0.0047** |
| Native Species                         | 3      | 657    | 54.33   | **<.0001** |
| Treatment*Native Species               | 6      | 657    | 1.34    | 0.2363 |
| Non-native Species                     | 4      | 657    | 10.95   | **<.0001** |
| Treatment*Non-native Species           | 8      | 657    | 2.26    | **0.0217** |
| Native Species*Non-native Species      | 12     | 657    | 2.75    | **0.0012** |
| Treatment*Native Species*Non-native   | 24     | 657    | 0.79    | 0.7465 |

Table C-3. ANOVA results to estimate the effects of treatment, native species, and non-native species on the non-native species aboveground biomass

| Effect                                 | Num DF | Den DF | F Value | Pr > F |
|----------------------------------------|--------|--------|---------|--------|
| Treatment                              | 2      | 657    | 6.06    | **0.0025** |
| Native Species                         | 3      | 657    | 4.48    | **0.0040** |
| Treatment*Native Species               | 6      | 657    | 1.67    | 0.1248 |
| Non-native Species                     | 4      | 657    | 118.56  | **<.0001** |
| Treatment*Non-native Species           | 8      | 657    | 4.13    | **<.0001** |
| Native Species*Non-native Species      | 12     | 657    | 1.79    | **0.0457** |
| Treatment*Native Species*Non-native   | 24     | 657    | 0.84    | 0.6835 |

Table C-4. ANOVA results to estimate the effects of treatment and non-native species on the non-native species biomass grown in monoculture

| Effect                                 | Num DF | Den DF | F Value | Pr > F |
|----------------------------------------|--------|--------|---------|--------|
| Treatment                              | 2      | 182    | 5.18    | **0.0065** |
| Non                                    | 4      | 182    | 17.43   | **<.0001** |
| Treatment*Non                           | 8      | 182    | 0.57    | 0.8055 |
Table C-5. ANOVA results to estimate the effects of treatment, native species, and non-native species on the native species belowground biomass

| Effect                                | Num DF | Den DF | F Value | Pr > F |
|---------------------------------------|--------|--------|---------|--------|
| Treatment                             | 2      | 657    | 1.83    | 0.1605 |
| Native species                        | 3      | 657    | 18.85   | <.0001 |
| Treatment*Native species              | 6      | 657    | 0.15    | 0.9886 |
| Non-native species                    | 4      | 657    | 26.43   | <.0001 |
| Treatment*Non-native species          | 8      | 657    | 1.71    | 0.0923 |
| Native species*Non-native species     | 12     | 657    | 5.47    | <.0001 |
| Treatment*Native species*Non-native species | 24    | 657    | 0.42    | 0.9942 |

Table C-6. ANOVA results to estimate the effects of treatment, native species, and non-native species on the non-native species belowground biomass

| Effect                                | Num DF | Den DF | F Value | Pr > F |
|---------------------------------------|--------|--------|---------|--------|
| Treatment                             | 2      | 657    | 1.51    | 0.2208 |
| Native species                        | 3      | 657    | 22.07   | <.0001 |
| Treatment*Native species              | 6      | 657    | 0.77    | 0.5931 |
| Non-native species                    | 4      | 657    | 25.43   | <.0001 |
| Treatment*Non-native species          | 8      | 657    | 1.99    | 0.0455 |
| Native species*Non-native species     | 12     | 657    | 3.60    | <.0001 |
| Treatment*Native species*Non-native species | 24    | 657    | 0.79    | 0.7461 |