THESIS

ALTERING WATER AND NITROGEN AVAILABILITY AFTER ROADSIDE DISTURBANCE TO FAVOR NATIVE PLANT SPECIES

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ABSTRACT

ALTERING WATER AND NITROGEN AVAILABILITY AFTER ROADSIDE DISTURBANCE TO FAVOR NATIVE PLANT SPECIES

This study evaluates the use of soil amendments in roadside restoration to promote native species and discourage invasive species establishment through manipulation of water and nitrogen (N) availability. Our goal was to decrease soil N availability and increase soil water content to foster growth of perennial native species on roadsides in Rocky Mountain National Park, Colorado. Roadside construction can increase soil bulk density, reduce aggregation, and cause an initial increase in resource availability, which encourages growth of early successional species. In addition, N deposition from the Front Range of Colorado is increasing nitrate and ammonium availability in this National Park. The study objective was to increase or decrease water and/or N availability with soil amendments to reduce weedy annual species establishment on roadsides. Treatments were hypothesized to 1) increase soil moisture and reduce plant-available N (synthetic polymer incorporation), 2) reduce soil surface temperatures, increase moisture and indirectly decrease N (wood mulch blanket), 3) decrease bulk density by changing soil structure and slowly increase N (yard-waste compost incorporation).

These amendments were applied alone and in pairwise combinations to six southeast facing roadsides slopes concurrent with seeding in fall of 2013. Ten perennial grass and forb species were hydro-seeded with tackifier to all roadsides. Plant density, cover, mineral nutrients, soil moisture, total C:N, soil temperature, and rainfall were measured during the growing season in 2014 and 2015. A paired greenhouse study was conducted in spring 2014 with analogue native and non-native grasses. Grass root and shoot biomass, plant height, seedling density, and soil moisture were measured after 9 weeks of growth.

On these roadsides, soil moisture, and density of native seeded species was significantly changed by soil treatments through time ($p=0.039$, $p=0.040$). Wood mulch alone and combined with compost or polymer increased soil moisture after rainfall in the field ($p=0.0007$) and after irrigation in the greenhouse ($p=0.0001$). In the field, seeded species density was highest in mulch/compost treatments in 2014 ($p=0.029$) and mulch/polymer treatments in 2015 ($p=0.003$).

After one year of decomposition, none of the treatments significantly changed carbon to nitrogen ratios ($p=0.27$) which averaged 18.7:1, although mulch/compost treatment had the lowest C:N ratio of 13.5:1. Mulch or mulch/polymer treatments combined had much less nitrate than yard-waste compost incorporation ($p=0.0002$). Mulch blanket immobilized N and decreased non-native density in summer 2015 to 2 plants/m^2. In contrast, compost/polymer treatments had 12 non-native plants/m^2 ($p=0.02$). In the greenhouse, nitrate was more limiting than water, and mulch blanket increased native grass growth relative to non-native grasses ($p=0.002$). Because of the immobilization of N, mulch also decreased germination rates ($p=0.001$) and biomass ($p=0.001$) across all species. With higher soil moisture availability in the greenhouse, non-native growth was lower than natives in control ($p=0.001$), polymer and mulch soil treatments. In contrast, compost incorporation, which increased N availability in the field, drastically increased growth of all species ($p<0.0001$) in the greenhouse.

These results begin to demonstrate how increased soil moisture and decreased soil N favors germination and seedling survival of desirable native perennial species, while simultaneously reducing non-native species establishment. By managing soil resource availability after disturbance, we can achieve resilient plant communities dominated by perennial native species.

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CHAPTER 1: INTRODUCTION

In the 21st century, environmental forcings are interacting to determine species composition in high-elevation biodiverse areas. Disturbance regimes, nitrogen (N) deposition, climate change, and species dispersal are increasing at an unprecedented rate (Hooper et al. 2005, Hooper et al. 2012, Stevens and Latimer 2015) in ecological history. In turn, change in species composition in ecosystems is changing fundamental process such as primary production and decomposition (Hooper et al. 2012), which impacts the functioning of habitats after disturbance. This research explores how plant establishment and competition are largely dependent on changes in resource availability (Toeroek et al. 2000), which can be a target for best management practices. We use a highly manipulated study system that is vulnerable to disturbance, climate change, and N deposition. By lowering the amount of plant-available N and increasing water availability, we can facilitate establishment and growth of late-successional plant species (Toeroek et al. 2000) and reduce the likelihood of invasion (He 2011, Rimer and Evans 2006, Vasquez et al. 2008).

Roadsides are a model ecosystem to examine how restoration methods that change nutrient availability direct species establishment and competitive outcomes. Road corridors make up only 1% of the surface of the United States (Forman and Alexander 1998), but their impacts extend beyond their limited surface area. They increase habitat fragmentation, pollution, erosion, and invasion while disrupting natural hydrology (Steinfeld et al. 2007, Leu et al. 2008). Conserved roadside habitat will simultaneously encourage native plant germination and discourage non-native plant germination (Harper-Lore and Wilson 2000). These roadsides can reduce the negative impacts on neighboring public and private lands (Leu et al. 2008). Conservation of roadside habitat is especially critical in biodiverse areas that host a high diversity of invasive species (Stohlgren et al. 1999). Establishment of native plant communities on roadsides will reduce invasion, erosion, and habitat fragmentation while improving infiltration and water-cycling (Steinfeld et al. 2007).

Road construction and associated disturbance increases the risk of invasion by providing a temporal and spatial niche for non-native species to establish. Invasive plants establish on roadsides because they have higher propagule pressure of seed (Tyser and Worley 1992), initial availability of N (McCrae and Wilson 1992, Lee et al. 2012), and bare soil. Non-native seed is transported to roadsides by wind, wildlife, gravity, cars, shoes, and construction equipment (Harper-Lore and Wilson 2000). Any disturbance, such as road construction, initially increases resource availability by removing the plant species which were actively using soil nutrients (Wilson and Tilman 1991). Car exhaust also increases N availability on roads by depositing nitrous oxides (McCrae and Wilson 1992, Adair 2008). Invasive plants are species from another region of the word that have escaped predation by establishing in a new place without native herbivore predators (Keane and Crawley 2002). This escape from predation increases the growth rate, reproduction, and competitive ability of non-native invasive species (Blumenthal 2006) relative to native plant species.

Other conditions for plant growth that are often altered on roadsides include loss of soil structure, increased bulk density, and reduced organic matter (Sanborn et al. 2004). The top 10-25 cm of a natural soil profile typically contains higher levels of organic matter and seed, which are critical to habitat function. Soil aggregation and structure are damaged as topsoil is moved during road re-surfacing. Construction equipment activity increases bulk density, or compaction, of the soil (Steinfeld et al. 2007). Soils cannot hold as much water after this disturbance because of compaction and homogenization of soil structure (Brady and Weil 2004). Reapplication of roadside topsoil increases soil fertility, microbial biomass, and plant growth (Claassen and Zasoski 1993).

Climate change is projected to cause increasing length of time between rainfall events, reducing the likelihood of seed germination (Bochet et al. 2007). This reduced water potential over time favors establishment of species with higher germination rates, which are often non-natives. The seedling stage is when plants are most susceptible to desiccation and death; this is a critical time for species in semiarid ecosystems where rainfall occurs in pulses followed by periods of drought (Snyder and Tartowski 2006). Higher soil temperatures associated with changing climates can also decrease the survival of both annual and perennial seedlings through greater water stress (Hovenden et al. 2008). The germination stage of plant species is when they are most sensitive to changes in temperature, light, moisture, and nutrient thresholds (Copeland 2001). Each plant species requires a specific length of time at a given range of temperature and soil moisture thresholds to germinate (Marschner 2012). Species-specific germination requirements and variation in seed production morphology within a species (Copeland 2001) ensure that plants can withstand future climate regimes by preventing germination in unfavorable years. But, in the face of climate change, dormancy requirements also increase selection towards annual species that have higher germination potential.

Roadside habitat is made up of plant populations of different species which are actively competing with each other for limiting resources (Gause 1934, Hardin 1960). If two non-breeding plant species populations occupy the same niche, the genetic and morphological traits of one population which increase its fitness relative to the other (Hardin 1960) will determine which species persists. Plant species can occupy complementary temporal and spatial habitats or niches, which reduces competition and ensures access to nutrients and water (Hooper and

Vitousek 1998). Species avoid direct competition by partitioning use of resources in time. For example, early season annuals use water and N available in the spring. In contrast, perennials survive on lower nutrients throughout the growing season, and late season annuals germinate at the end of the season. Alternatively, species may partition resources in space by having different spatial rooting patterns or canopy structures (Poorter et al. 2011). Increased functional species diversity also ensures complementary resource use (Santos et al. 2011). But habitats that support a high diversity of native species, also have higher diversity of non-native species (Stohlgren et al. 1999). This means that our biodiverse habitats, that are harboring species for the future, are also those most susceptible to the impacts of invasion.

During early growth and establishment, plants are dependent on availability of water and plant-essential nutrients. Specifically, nitrogen, in the form of ammonium (NH_4^+) or nitrate (NO_3^-) and water (H_20), are co-limiting to plant establishment (Farrior et al. 2013, Figure 1). N is the most limiting plant nutrient because NH_4^+ and $N0_3^-$ make up less than 2% of the total N in soils (Reeder and Sabey 1987). Nitrogen is used in production of amino acids, proteins, enzymes, cell walls, and genetic material (Marschner 2012).The hydrogen and oxygen from water are used in photosynthesis and the compound also transports materials throughout the plant and helps maintain turgor pressure. Nitrogen stimulates root activity and growth and fosters uptake of other nutrients (Reeder and Sabey 1987). Other plant essential nutrients include aluminum, boron, cadmium, calcium, carbon, copper, chlorine, hydrogen, iron, magnesium, molybdenum, nickel, potasium, phospherous, sulfur, and zinc (Marchner 2012).

Soil properties and microsite variability determine availability of water and N in terrestrial ecosystems (Chapter 2, Figure 1) and can inform management of soil for restoration. Nutrient abundance, cycling and water storage and movement are determined by soil parent material, aggregation and texture (Brady and Weil 2004). The diversity of soil types is the reason for the diversity of plant species on this planet. The texture, organic matter content, soil structure, parent material, and landform characteristics can be used to guide any restoration practices to improve plant establishment. These abiotic factors will guide how much water and nutrients are available for plant growth and how they are cycled.

According to the resource-ratio hypothesis, there are tradeoffis in competition between multiple species, so species specialize in aquistion of a specific resource (Tilman 1977). Long-lived perennial plants can withstand poor nutrient environments, water-stress, and herbivory more than short-lived annuals (Puijalon et al. 2008). Because of adaptions associated with longevity, perennials have less responsive root systems (Campbell and Grime 1989 in response to increased soil N (Dupont et al. 2014, Roumet et al. 2006). Perennials are unable to photosynthesize and grow at rates as high as annuals, especially during early establishment (Marler and Claassen 1998). Perennials invest energy in production of anti-herbivory chemicals (Campbell and Grime 1989) and organs, like roots, that can survive until the next growing season (Diemer 1998).

Annuals put all of their resources, growth and reproduction into the current growing season.

Early-season annuals compete with perennials by pre-emptive use of soil N and water before perennial species are actively growing (Hooper and Vitousek 1998, Rejaniemi and Reynolds 2004). Successful non-native invaders are often species that respond to high resource availability (Blumenthal 2006) of N or water. The fast and strong growth response of annual species to increased N can result in dominance of annual invasive species in soils with high N availability (Chambers 2007, Paschke et al. 2000, Vasquez et al. 2008).

Organic matter is critical for plant growth because it increases the capacity of the mineral soil to retain rainfall and nutrients by reducing the rate of percolation (Franzluebbers 2002). The balance between carbon entering and leaving the belowground system determines organic matter content in soil (Davidson and Janssens 2006). The natural layer of organic litter on the soil surface returns carbon and N to the soil profile and causes aggregation of the soil profile (Curtis et al. 2009, Kowaljow 2007). Degrading plant material makes up two thirds of the terrestrial carbon pool (Krull et al. 2003). Most N in soil organic matter is stored in organic compounds, which decompose very slowly (Reeder and Sabey 1987) at rates dependent on their surface area and composition. Soil temperature regulates microbial activity and degradation and storage of organic matter (Krull et al. 2003).

To maintain desired roadside plant communities, we must address N availability, through management of the topsoil and effective revegetation (Rokich et al. 2000, Bowen et al. 2005, Claassen 2012). Slightly reduced soil N has been shown to increase rates of succession towards mid and late seral communities dominated by perennial species (Paschke et al. 2000). N availability often guides the successional direction of plant colonization after disturbance (Toeroek et al. 2010). Addition of organic matter can stimulate microbial species, which can break down DON into nitrate ($N0_3^-$) and ammonium (NH_4^+), to balance their C:N ratios (Sylvia et al. 2005, Marschner 2012). Nitrate is converted to ammonium through mineralization of dissolved organic N (Vitousek et al. 2011). Ammonium is rarely greater than 8% of the inorganic N that is available in wildland soils (Reeder and Sabey 1987) while nitrate usually dominates the composition of mineral N. Ammonium is oxidized by bacteria to nitrate and nitrite or volatized as ammonia (Chapin et al. 2011), which typically reduces its abundance in soils.

This mineral N is either released into the soil or quickly stored in microbial biomass and plants that are rapidly accessing it. As the soil wets or dries, fungal and bacterial microbes die, releasing the immobilized N, making it available to plant roots (Sylvia et al. 2005). There is large spatial heterogeneity in soil nutrient availability due to microsite variation in texture and organics, which increases the diversity of spatial niches plant roots can access. The amount of microsites where microbial growth is N limited affects which form of mineral N is most available (Vitousek et al. 2011).

Microbes have thresholds of activity based on the carbon to nitrogen (C:N) ratio of the soil (Qui et al. 2008), moisture availability (Garten et al. 2009), as well as beneficial relationships with plants. Because microbes use dissolved organic nitrogen as a carbon source and excrete excess mineral N, microbial species growth is carbon limited (Chapin, Matson, and Vitousek 2011). N cycling from plants to microbes to N pools begins with the decomposition of plant litter on the soil surface (Claassen and Hogan 2002). Respiration of microbes in soil, or release of CO_2, depends on moisture, rates of plant litterfall, metabolism, and photosynthesis (Ryan and Law 2005). Arbuscular mycorrhizal fungi exchange plant-fixed carbon within the colonized roots for other plant-essential nutrients, such as P and N (Marschner 2012). Microbial species abundance and diversity regulate cycling of nutrients, and therefore, plant growth (Cotrufo et al. 2013).

Nitrogen mineralization rates by microbial species in the soil respond to soil C:N and carbon quality because the absorption or release of N is dependent on their carbon status (Chapin et al 2011). The C:N ratio in microbial biomass is around 10:1 (Cleveland and Lipzin 2007), which regulates the percentage of C and N these species obtain from the environment, relative to the initial C:N of the soil. If microbial species are incorporating organic matter at 40% efficiency, they need substrates with a C:N of 25:1 (Chapin et al. 2011).

Carbon, N, and water cycling, as well as microbial activity, are tightly interwoven in terrestrial ecosystems (Chapin et al. 2011, Marschner 2012) and influence both plant growth and plant community composition (Stohlgren et al. 1999). The removal of plants during construction alters soil C:N ratios, due to an initial increase in N and decrease in carbon (Pineiro et al. 2006). There is the potential for organic amendments that are high in carbon to restore pre-disturbance soil C:N ratios (Brown et al. 2007), reduce compaction (Sanborn et al. 2014, Curtis and Claassen 2009), and ultimately increase native plant establishment.

The soil water holding capacity regulates the maximum amount of water it can retain. Soil water holding capacity is determined by soil texture, landform influences on texture and profile depth, aggregation, but also organic matter content (Haynes and Nadu 1998). Water availability greatly influences plant germination and growth. In addition, as soil moisture increases, soil N availability increases (Marschner 2012). Soils that are high in sand have higher water infiltration rates and are more likely to experience erosion with loss of organic matter or plant cover. As the percentage of clay in the soil increases, so does the number of micropores that can store water (Brady and Weil 2004). Landforms that are flat often have higher soil development, and tend to have higher percentage of clay in the soils. If plant growth and organic matter inputs to the soil are high, these soils can have good structure and infiltration, but if the soil is disturbed and the structure is degraded, these high clay soils can have very low infiltration (Troeh et al. 2004)). In contrast, soils on slopes have higher runoff, lower infiltration, less soil development, and less accumulation of nutrients and clays (Brady and Weil 2004).

Nutrient and water availability can be manipulated with soil amendments to achieve establishment of desired species establishment on roadsides. Changes in organic matter content, N availability, and water availability (Figure 1) will impact microbial activity and diversity and the composition of plant communities (Osanai et al. 2013, Zak et al. 2003). Through these pathways, certain amendments will promote rapid establishment of annual cover, while others will foster establishment of perennial species or exclude invasive species of concern (Curtis and Claassen 2005). Soil amendments that readily increase both water and nutrient availability will favor germination and establishment of annual species (Prober and Wiehl 2012). In contrast, soil amendments that decrease nutrient availability may increase establishment of long-lived perennial species (Toeroek et al. 2000) that withstand poorer nutrient environments.

Motivation for this study was to identify which soil treatment best promotes the establishment of native species while minimizing abundance of invasive plants. Amendments encourage native plant establishment on roadsides by increasing capacity to retain soil moisture and reducing readily available N (Curtis and Claassen 2009). In this study, three amendments (mulch, compost and polymer) were selected because of their physical and chemical properties known to increase or decrease water and nutrient availability.

Wood mulch, which is high in decomposable carbon, can increase microbial activity and indirectly reduce the amount of readily available N, discouraging invasion (Claasssen 2012, Grice et al. 2013, Yao et al. 2005). The increase in carbon and reduction of soil temperatures alter microbial activity and composition, also changing which plant species germinate and establish (Zink and Allen 1998, Toeroek et al. 2010). Lower temperatures may act to extend microbial activity into dry periods by conserving soil moisture. The carbon in wood mulch causes microbes to immobilize N from the soil to balance their cellular C:N ratios (Marschner 2012, Zink and Allen 1998). Repeated sawdust and sucrose amendments on grasslands in Hungary significantly decreased plant-available N (Toeroek et al. 2000). At disturbed well pad sites, mulch incorporation prior to seeding decreased the ratio of non-native to native species by

50% (Eldridge et al. 2012). In addition, organic mulch application on top of the soil slows infiltration of water into the soil (Agassi et al. 1998), increases soil moisture, and decreases soil temperatures (Schonbeck and Evanylo 1998). This decrease in soil temperatures slows activity of microbes that decompose organic matter (Cadavid et al. 1998, Zogg et al. 1997) and can also alter seed germination rates.

Compost incorporated into soils changes N and water availability over time through its effects on soil properties and microbial processes (Zhang and Zak 1998). Yard-waste compost may be a beneficial alternative to commercial N fertilizers because its structure limits the rate of decomposition and extends N release (Cotrufo et al. 2012). Microbes can mineralize the organic matter in compost into nitrate and ammonium, releasing plant-available N over time (Sylvia et al. 2005). In addition, compost is known to improve water infiltration, water holding capacity, soil structure, cation-exchange capacity, and to reduce bulk density (Dexin et al. 2012). Curtis et al. (2009) found that roadside compost applied in four soil types significantly increased plant available water, biomass, and rooting density.

Super-absorbent polymers change the timing of water availability. These polymers absorb up to 400 times their mass in water and can increase soil moisture by up to 30% in sandy soils (Bai et al. 2010). Super-absorbent polymers not only absorb water, but have been shown to bind available cations such as ammonium, which is a form of mineralizable N (Orikiriza et al. 2009). Super-absorbent polymers are available commercially for nursery use and have been used in hydro-seeding to reduce soil erosion (Wang et al. 2011). Additionally, polymer amendments have decreased cheatgrass (*Bromus tectorum* L.) cover after restoration under some conditions (Johnston 2012). This observed reduction of cheatgrass, which is an annual species, may be explained by changes in the timing of soil water and N availability due to polymer amendments.

This concept has been tested on roadsides in California (Curtis and Claassen 2009), Arizona (Elseroad et al. 2003), British Columbia (Sanborn et al. 2004), and mine tailings in Colorado (Brown et al. 2007). Yard-waste compost was used with other types of organic matter to alter plant establishment on granitic roadsides (Curtis 2005, Curtis and Claassen 2009). In this roadside study, compost incorporation decreased bulk-density, increased hydraulic conductivity and caused a significant increase in plant biomass (Curtis and Claasssen 2009). Similarly, mulch incorporation on roadsides decreased bulk density and increased native pine establishment (Sanborn et al. 2014). Mulch application on a road obliteration increased establishment of native fescue (Elseroad et al. 2003).

Soil conditions, nutrient inputs, species diversity, and cycling of resources can determine ecosystem function. Because of existing water and nutrient cycling, organic amendments can alter nutrient and water availability in soils and therefore microbial activity and diversity. We evaluated possible post-disturbance soil restoration management strategies that promote the growth of native perennial species without promoting invasive annual plant growth on a high-elevation roadside in Rocky Mountain National Park.

CHAPTER 2: FIELD AND PRELIMINARY GREENHOUSE STUDY

Introduction

In the 21st century, mechanical disturbance, nitrogen deposition, and climate change are interacting to determine species composition in high-elevation biodiverse areas. These factors are causing species loss that is unprecedented in ecological history (Hooper et al. 2005, Hooper et al. 2012, Stevens and Latimer 2015). In turn, change in species composition in ecosystems is changing fundamental processes such as primary production and decomposition (Hooper et al. 2012), which impacts the functioning of habitats after disturbance. This research explores how resource availability influences plant establishment and competition (Toeroek et al. 2000) after roadside restoration. We use a highly manipulated study system vulnerable to disturbance, climate change, and N deposition. The management of soil resource availability, a restoration best management practice, can improve both restoration and invasion outcomes. Lowering the amount of plant-available nitrogen (N) and increasing soil water holding capacity has been shown elsewhere to facilitate establishment and growth of late-successional plant species (Toeroek et al. 2000) and reduce the likelihood of invasion (He et al. 2011, Perry et al. 2013, Rimer 2006, Vasquez et al. 2008).

Roadsides are a model ecosystem to see how changes in nutrient availability direct species establishment and competitive outcomes. Conserved roadside habitat will simultaneously encourage native plant germination and discourage non-native plant germination (Harper-Lore and Wilson 2000). Establishment of native plant communities on roadsides will reduce invasion, erosion, and habitat fragmentation while improving water cycling (Steinfeld et al. 2007). Road corridors make up only 1% of the surface of the United States (Forman and Alexander 1998), but their impacts extend beyond their limited surface area. They increase habitat fragmentation, pollution, erosion, and invasion while disrupting natural hydrology (Steinfeld et al. 2007, Leu et al. 2008). Conservation of roadside habitat is especially critical in biodiverse areas that host a high diversity of invasive species (Stohlgren et al. 1999). Ecosystems that support a high diversity of native species also have higher diversity of non-native species (Stohlgren et al. 1999). Our biodiverse habitats, which are harboring species for the future, are also those most susceptible to the impacts of invasion from road corridors.

Road construction increases the risk of invasion by providing a temporal and spatial niche for non-native species to establish. Invasive plants establish on roadsides because they have higher propagule pressure of seed (Tyser and Worley 1992), initial availability of N (McCrae and Wilson 1992, Lee et al. 2012), and bare soil. Non-native seed is transported to roadsides by wind, wildlife, gravity, cars, shoes, imported material, and construction equipment (Harper-Lore and Wilson 2000).

Any disturbance, such as road construction, initially increases resource availability by removing the plant species that actively use soil nutrients (Wilson and Tilman 1991). Car exhaust with nitrous oxides also increases N availability on roads (McCrae and Wilson 1992, Adair 2008). Invasive plants are species from another ecosystem that have evolved with high genetic diversity (Dlugosch et al. 2007) and escaped predation by establishing in a new place without native herbivore predators (Keane and Crawley 2002). This escape from predation increases the growth rate, reproduction, and competitive ability of non-native invasive species (Blumenthal 2006) relative to native species.

Roadside soils have typically lost soil structure and organic matter and have higher bulk density (Sanborn et al. 2004) due to the construction process. The top 10-25 cm of a natural soil profile typically contains higher levels of soil organic matter and seed, which are critical to habitat function. Soil aggregation is damaged as topsoil is moved during excavation and grading. Construction equipment activity increases bulk density, or compaction, of the soil (Steinfeld et al. 2007), reducing soil water holding capacity (Brady and Weil 2004). Harvest and reapplication of roadside topsoil increases soil fertility, microbial biomass, and plant growth (Claassen and Zasoski 1993).

Climate change is projected to increase variability and seasonality of rainfall events worldwide (Huntingford et al. 2016, Fowler and Kilsby 2003), which could affect which species germinate and survive on roadsides. Reduced soil water potential from construction impacts through time can increase the germination of some species and discourage the germination of others (Bochet et al. 2007). For example, generalist non-native species typically have higher germination potential (Morgan 1998) at lower soil moisture thresholds than native species (Hoyle et al. 2013). Early plant growth is a critical time in semiarid ecosystems where rainfall occurs in pulses followed by periods of drought (Snyder and Tartowski 2006). Higher soil temperatures, due to climate change, decrease the survival of both annual and perennial seedlings through greater water stress (Hovenden et al. 2008). In addition, non-native annual species, like *Bromus tectorum*, can be genetically adapted to drought (Kathiresan and Gualbert 2016). Increased soil temperatures and decreased water availability favors establishment of generalist non-native species on roadsides.

Plant species occupy complementary temporal and spatial niches, reducing competition for nutrients and water (Hooper and Vitousek 1998). If two non-breeding plant species populations occupy the same niche, the genetic and morphological traits of one population which increase its fitness relative to the other (Hardin 1960) will determine which species persists. During early growth and establishment, plant growth is co-limited by availability of water and nitrogen (Farrior et al. 2013). Nitrogen is often the most limiting plant nutrient because inorganic N (NH_4^+ and $N0_3^-$) makes up less than 2% of the total N in soils (Reeder and Sabey 1987) and N is the primary nutrient needed for plant growth. Species may partition resources in space by having different spatial rooting patterns or canopy structures (Poorter et al. 2011). Early season annuals use resources in the spring and perennials have lower nutrient requirements throughout the growing season (Santos et al. 2011).

According to the resource-ratio hypothesis, there are tradeoffis in competition between multiple species, so species specialize in aquistion of a specific resource (Tilman 1977). Non-native species that have become successful invaders are often those that respond to higher soil resource availability (Blumenthal 2006). Annual and perennial invasive species tend to specialize in acquisition of inorganic N (Chambers 2007, Paschke et al. 2000), which favors their establishment with increased N availability (Vasquez et al. 2008). In general, both non-native and native annuals put all of their resources, growth and reproduction into the current growing season in order to produce seed. In contrast, perennial seedlings are generally less competitive than annual seedlings with increased N availability (Belsky 1992, Chalmers et al. 2005, Claassen and Marler 1998, Hulbert 1955) and spend extra energy on root production. Long-lived perennial plants can withstand poor nutrient environments, water-stress, and herbivory more than short-lived annuals (Puijalon et al. 2008). Because of adaptions associated with longevity (Diemer 1998, Dupont et al. 2014), perennials have less responsive root systems to changes in nutrient availability (Campbell and Grime 1989, Roumet et al. 2006).

To maintain desired roadside plant communities, we must address initial increased N availability, through management of the topsoil and effective revegetation (Rokich et al. 2000, Bowen et al.

2005, Claassen 2012). Nitrogen availability often guides the successional direction of plant colonization after disturbance (Toeroek et al. 2010). Slightly reduced soil N has been shown to increase rates of succession towards mid and late seral communities dominated by perennial species (Paschke et al. 2000) while highly decreased soil N can discourage all plant growth (Rhoades et al. 2015). Addition of organic matter can stimulate microbial species growth. These microbial species mineralize organic molecules into nitrate ($N0_3^-$) and ammonium (NH_4^+) (Sylvia et al. 2005, Marschner 2012). Ammonium is rarely greater than 8% of the inorganic N that is available in wildland soils (Reeder and Sabey 1987) while nitrate usually dominates the composition of inorganic N. Under aerobic conditions, ammonium is lost through rapid oxidization by bacteria to nitrate and nitrite or volatization as ammonia (Chapin et al. 2011).

Inorganic N is either present in the soil solution or quickly assimilated into microbial or plant biomass. There is large spatial heterogeneity in soil nutrient availability due to microsite variation in texture and organic matter. The amount of microsites where microbial growth is N limited affects the form of inorganic N that is most available (Vitousek et al. 2011). Microbes have thresholds of activity based on the carbon to nitrogen (C:N) ratio of the soil (Qui et al. 2008), moisture availability (Garten et al. 2009), as well as symbiotic relationships with plants. Respiration rates of microbes respond to moisture, rates of plant litterfall, metabolism, and photosynthesis (Ryan and Law 2005, Sylvia et al. 2005). Microbes use organic nitrogen compounds as a carbon source and excrete excess inorganic N and their growth is often carbon limited (Chapin et al. 2011). Nitrogen mineralization rates by microbial species are influenced by soil C:N and carbon biochemistry (Chapin et al 2011). The C:N ratio in microbial biomass is around 10:1 (Cleveland and Lipzin 2007), which regulates the percentage of C and N these species obtain relative to the initial C:N of the soil. Net nitrogen mineralization occurs at lower C:N ratios, (Chapin et al. 2011), increasing the mineral N available to plant species.

Carbon, N, and water cycling, as well as microbial activity, are tightly interwoven in terrestrial ecosystems (Chapin et al. 2011, Marschner 2012) and influence both plant growth and community composition (Stohlgren et al. 1999). The removal of plants during construction alters soil C:N ratios by increasing N availability and decreasing organic carbon due to topsoil disturbance (Pineiro et al. 2006). There is the potential for organic amendments that are high in carbon to restore pre-disturbance soil carbon, reduce soil N availability (Brown et al. 2007), reduce compaction (Sanborn et al. 2014, Curtis and Claassen 2009), and ultimately increase native plant establishment.

The soil water holding capacity regulates the maximum amount of water it can retain. Water availability greatly influences plant germination and growth. Soil water holding capacity is determined by soil texture, landform influence on texture and profile depth, aggregation, but also organic matter content (Haynes and Nadu 1998). Soils with higher percentage of fine clay or loam particles are able to store more water than those with coarse sands. Organic matter increases water holding capacity of soils (Brady and Weil 2004). In addition, as soil moisture increases, soil N availability increases (Marschner 2012).

Nutrient and water availability can be manipulated with soil amendments to achieve establishment of desired species establishment on roadsides. Changes in organic matter content, N availability, and water availability (Figure 1) will impact microbial activity and diversity and the composition of plant communities (Osanai et al. 2013, Zak et al. 2003). Native perennial species may be limited by water availability in competition with non-native annuals (Hamilton et al. 1999). Through these pathways, certain amendments will promote rapid establishment of annual cover, while others will foster establishment of perennial species or exclude invasive species of concern (Curtis and Claassen 2005).

Soil amendments that readily increase both water and nutrient availability will favor germination and establishment of annual species (Prober and Wiehl 2012). In contrast, soil amendments that decrease nutrient availability may increase establishment of long-lived perennial species (Toeroek et al. 2000) that withstand poorer nutrient environments. Amendments can encourage native plant establishment on roadsides by increasing capacity to retain soil moisture and reducing readily available N (Curtis and Claassen 2009). In this study, three amendments (compost, mulch, or polymer) were selected because of their physical and chemical properties known to increase or decrease water and nutrient availability.

Compost incorporated into soils changes N and water availability over time through its effects on soil properties and microbial processes (Zhang and Zak 1998). Yard-waste compost may be a beneficial alternative to commercial N fertilizers because its complex structure limits the rate of decomposition and extends N release (Cotrufo et al. 2012, Sylvia et al. 2005). In addition, compost is known to improve water infiltration, water holding capacity, soil structure, cation-exchange capacity, and to reduce bulk density (Dexin et al. 2012). Curtis et al. (2009) found that compost applied in four roadside soil types significantly increased plant available water, biomass, and rooting density.

Wood mulch, which is high in decomposable carbon, can increase microbial activity and indirectly reduce the amount of readily available N, indirectly discouraging invasion (Claasssen 2012, Perry et al. 2013, Yao et al. 2005). The mulch causes an increase in carbon and reduction of soil temperatures, which alters microbial activity and composition, changing which plant species establish (Zink and Allen 1998, Toeroek et al. 2010). The carbon in wood mulch causes microbes to immobilize N from the soil (Marschner 2012, Zink and Allen 1998). In addition, organic mulch application on top of the soil can slow infiltration of water into the soil (Agassi et al. 1998), increases soil moisture, and decreases soil temperatures (Schonbeck and Evanylo 1998). This decrease in soil temperatures and extension of soil moisture availability changes the rate of microbial activity (Cadavid et al. 1998, Zogg et al. 1997) and can also alter seed germination rates.

Super-absorbent polymers change the timing of water availability. These polymers absorb up to 400 times their mass in water and can increase soil moisture by up to 30% in sandy soils (Bai et al. 2010). Super-absorbent polymers not only absorb water, but can bind available cations such as ammonium (Orikiriza et al. 2009). Super-absorbent polymers are available commercially for nursery use and have been used in hydro-seeding to reduce soil erosion (Wang et al. 2011). Additionally, polymer amendments have decreased *Bromus tectorum* cover after restoration under some conditions (Johnston 2012). This observed reduction of cheatgrass, which is an annual species, may be explained by changes in the timing of soil water and N availability due to polymer amendments.

This concept has been tested on roadsides in California (Curtis and Claassen 2009), Arizona (Elseroad et al. 2003), British Columbia (Sanborn et al. 2004), and mine tailings in Colorado (Brown et al. 2007). Yard-waste compost was used with other types of organic matter to alter plant establishment on granitic roadsides (Curtis 2005, Curtis and Claassen 2009). Compost incorporation decreased bulk-density, increased hydraulic conductivity and caused a significant increase in plant biomass (Curtis and Claassen 2009). Similarly, wood chip mulch incorporation on roadsides decreased bulk density and increased native pine establishment (Sanborn et al. 2014). Mulch application on a road obliteration increased establishment of native fescue

(Elseroad et al. 2003). The dynamic thresholds of readily available N (Lowe et al. 2003) and water (Jankju 2008) determine relative plant establishment in terrestrial ecosystems. Soil conditions, nutrient inputs, species diversity, and cycling of resources are interacting to direct ecosystem function. Because of existing water and nutrient cycling, organic amendments can alter nutrient and water availability in soils and therefore microbial activity and diversity. We evaluated possible post-disturbance soil restoration management strategies that promote the growth of native perennial species without promoting invasive annual plant growth on a high-elevation roadside in Rocky Mountain National Park.

Study location and rationale

We evaluated plant growth in response to soil amendments on disturbed roadsides in Rocky Mountain National Park and in complementary greenhouse studies. Bear Lake Road in Rocky Mountain National Park, located in Estes Park, CO, has a history of nearly 10 years of roadway regrading and construction. Phase II of this project was completed in summer of 2013 and 2014. This low-elevation roadway project ranged from 2486 to 2614 meters in a montane ponderosa pine forest community. Native species revegetation was a high priority because of the low water-holding capacity of the granitic soils (Claassen 2012) and adjacent invasive populations. Non-native cheatgrass (*Bromus tectorum*) occurrence in this park can be predicted by distance to roads, elevation, and plant community (Bromberg et al. 2011). *B. tectorum* is a winter annual invasive brome that was introduced in the American West in the 1850's and is now present in every state. *B. tectorum* invasion is of management concern because it changes water and nitrogen cycling (Blank et al. 2008), increases fire return intervals (Germino, Chambers and Brown 2016), and moves up in elevation with climate change (West et al. 2015). Because of these factors, Bear Lake Road was selected for a study to determine how soil treatments direct plant establishment after road resurfacing and realignment.

Objectives and Hypotheses

Soil treatment strategies can promote the growth of native species and discourage invasive plant growth. This research examines the impact of soil amendments on soil moisture, N availability, and early establishment of species. Ultimately, the objective is to identify effective methods that enhance habitat for slow-growing native species rather than fast-growing, weedy species.

Three soil treatments were chosen to achieve specific effects in soil characteristics that impact water and nutrient availability. Super-absorbent polymers increase soil moisture (Yao et al. 2005) and reduce plant-available N (Agaba et al. 2010). Compost improves soil structure and slowly increases soil moisture and N availability (Cotrufo et al. 2013). Wood mulch reduces soil surface temperatures, which increases soil moisture retention and indirectly decreases N availability (Fornwalt and Rhoades 2011).

Wood mulch application can increase microbial activity and indirectly reduce the amount of readily available N, discouraging invasion (Classsen 2012, Perry et al. 2013, Yao et al. 2005). Application of wood chips as an organic mulch on top of the soil slows percolation of water through the soil (Agassi et al. 1998), increases soil moisture, and decreases soil temperatures (Schonbeck and Evanylo 1998) and evaporation of water. Decreased soil temperatures slows germination and activity of microbes that decompose organic matter (Cadavid et al. 1998, Zogg et al. 1997). Increased carbon and reduction of soil temperatures alter microbial activity and composition, changing which plant species establish (Zink and Allen 1998, Toeroek et al. 2010). Mulch increases dominance of soil fungal species (Silva et al. 2014). The carbon in wood mulch causes microbes to pull N from the soil to balance their carbon: nitrogen ratios (Marschner 2012, Zink and Allen 1998). Repeated sawdust and sucrose amendments on grasslands significantly decreased plant-available N (Toeroek et al. 2000). At disturbed sites, straw mulch incorporation prior to seeding decreased the ratio of non-native to native species by 50% (Eldridge et al. 2012). Mulch application rates of 4-6 cm in the Front Range of Colorado have decreased ammonium and nitrate and increased seeded grass cover (Fornwalt and Rhoades 2011).

Compost incorporated into soils changes N and water availability through its effects on soil properties and microbial processes (Zhang and Zak 1998). Yard-waste compost is a great alternative to commercial N fertilizers because its structure can block access of decomposers to substrate and limits the rate of decomposition (Cotrufo et al. 2012). Microbes can mineralize the organic matter in compost into nitrate and ammonium, releasing plant-available N over time (Sylvia et al. 2005). In addition, compost is known to improve water infiltration, water holding capacity, soil structure, cation-exchange capacity, and to reduce bulk density (Dexin et al. 2012). Curtis et al. (2009) found that roadside compost applied in four soil types significantly increased plant available water, biomass, and rooting density.

Super-absorbent polymers were used in this study to change the timing of water availability. These polymers absorb up to 400 times their mass in water and can increase soil moisture by up to 30% in sandy soils (Bai et al. 2010). Super-absorbent polymers not only absorb water, but have been shown to bind available cations such as ammonium (NH_4^+), which is a plant-available form of N (Orikiriza et al. 2009). Super-absorbent polymers are available commercially for nursery use and have been used in hydro-seeding to reduce soil erosion (Wang et al. 2011). Additionally, polymer amendments have decreased *Bromus tectorum* L. cover after restoration under some conditions (Johnston 2012). *B. tectorum* is especially dependent on soil moisture in the early spring of the growing season. This observed reduction of annual *B. tectorum* cover, may be explained by changes in the timing of soil water and N availability due to polymer amendments. The ability of the polymers to retain water decreases over time with each wetting event and as the gel degrades (Wang et al. 2011).

Hypotheses are:

1) Polymer and/or compost application will increase soil water retention and improve germination and establishment of native species;

2) Mulch application will reduce water loss and regulate changes in temperature and thus improve establishment of native species;

3) Mulch application will reduce readily available N, decreasing invasive species establishment;

4) Compost will extend N availability and increase native seedling establishment.

Comparisons used to test each of these field hypotheses and trends observed are in Appendix 1, Table 2.

Methods: Field Study

Site Selection

The study was along a roadside cutting through forested montane habitats in Rocky Mountain National Park, Larimer County, Colorado. Site selection was prioritized based on the aspect of the location, so that evaporation was higher and plant growth was limited by water availability. The semi-arid climate averages 330 mm of rain and 720 mm of snow per year. Field sites were south, southeast or east facing slopes with a 10-30 degree grade (Appendix 1, Table 3). There are two roadside sites at three different elevations: Moraine Park (2500 m), Hollowell Park (2550 m), and Glacier Basin (2600 m) (Appendix 1, Figure 14). The two fill slopes were at the lower road obliteration (site 3) and in Moraine Park (site 5). The remaining cut slope sites were at the upper road obliteration, realignment, in Hollowell Park, and in Moraine Park. Initial rooting depth averaged 16.3 cm across all sites. Low elevation sites 5 and 6 had the lowest rooting depth of 12.5 cm and higher elevation sites tended to have rooting depth ranging from 14-22 cm.

Bear Lake Road soils have a sandy loam soil texture, which developed from granitic, glacial till, parent material. These sandy loam soils are well drained, low in nutrients, and prone to erosion (Claassen 2012). Soil texture across the six sites averaged 75% sand, 14% silt, and 11% clay in the top 7.5 cm and 72% sand, 17% silt, and 11% clay from 7.5-15 cm (A & L Western Laboratories, Appendix 1, Table 5). The proportion of clay particle class ranged from 9-13% between sites. Soil pH averaged 6 across all sites, with slightly higher pH at 7.5-15 cm depth. The organic matter content in the top 7.5 cm varied between sites from 44 to 73 lbs/acre (Appendix 1, Figure 15). Cation exchange capacity averaged 5.7 meq/100 g in the top 7.5 cm and 4.8 meq/100 g from 7.5-15 cm depth.

Amendment Description

The pine-duff wood mulch used in this study is a mixture of small and large particles of approximately 35% pine needles and cones and 65% larger wood particles (A1 Organics, Eaton, CO). The particles sizes ranges from 1.5x5 cm wood chips to .1 x 1cm pine needles. The C:N ratio of the pine-duff mulch was approximately 57:1 (Appendix 1, Table 3). The yard-waste compost used in this study is approximately 1% N, 0.4% P, 0.5% K, 1% Ca, and 39% organic matter by dry weight (Yost 2012, Appendix 1, Figure 12). Compost carbon to nitrogen ratio averaged 10:1 (Appendix 1, Table 4). Polymers used in this study are made of a potassium polyacrylamide-acrylate copolymer gel that biodegrades in 5 years or less (Terra-sorb, Plant Health Care, Inc., Lebanon, PA), depending on the exposure to temperatures, sunlight and water. A fine-grade polymer granule size was used. Polymer C:N ratio was approximately 4:1.

Amendment Application Method

All sites had been restored with salvaged topsoil and seed in fall 2013. Topsoil was applied at 10 cm depth to cut and fill slopes (Claassen 2012). Afterwards, seven soil amendment treatments were randomly applied to 17.5 m long strips parallel to Bear Lake Rd. and extending perpendicular from the roadside. Each individual treatment area within this strip was 2.5 m wide and extended for the distance of the disturbance, which varied from 1.5-3 m in length. We installed two-way combinations of mulch, compost, and polymer at six sites along Bear Lake Road.

Compost and polymer amendments were incorporated by raking to a depth of 10 cm within the mineral soil prior to seeding. We broadcast super-absorbent polymer to the soil surface at the rate of 49 g/m^2 and raked it to 10 cm depth. Yard-waste compost was applied with buckets at 1.3 cm thickness and raked to 10 cm depth. Mulch was applied two weeks after seeding on the surface as a 2.5 cm blanket.

Restoration Seeding

In late September 2013, a mixture of ten native perennial grasses and forbs were hydro-seeded at the rate of 2,173 seeds/m^2 (200 pure live seed (PLS)/ft^2). Seed was applied to the soil surface and then bonded wood-fiber matrix was applied evenly across all treatment, including the control (Figure 5). Fiber matrix and tackifier were applied using a hydraulic pump at the rate of 3,368 kg/ha (3,000 lbs/acre) (Wildlands Inc. 2013). A green dye was added to the fiber matrix and seed to improve application efficiency.

The seed mix was 80% grasses and 20% forbs, with 10 native species total (Appendix 1, Table 7). Native rye bunch grasses, *Elymus elymoides* and *Elymus canadensis* each composed 22.5% of the seed mix. *E. elymoides* is known for its competitive ability with *B. tectorum*. Once established, it can effectively compete despite increased and decreased water and N availability (McGlone et al. 2012).

Other perennial, fibrous-rooted grasses in the seed mix were *Bouteloua gracillis* (6.2%), *Koeleria macrantha,* (14%), and *Muhlenbergia montana*, (13%). Both *B. gracillis* and *M. montana* are warm-season grasses, while *K. macrantha* is a cool-season grass. The five perennial forbs in the seed mix were *Heterotheca villosa*, (1.5%), *Antennaria parviflora* (1%), *Artemisia frigida* (6%), *Oxytropis lambertii* (11%), *Thermopsis divaricarpa* (0.08%). Both *O. lambertii* and *T. divaricarpa* are early-season, N-fixing species. Fringed sagebrush, the only woody subshrub in the mix, readily responds to disturbance and grows asexually by adventitious roots.

Field Sampling

To determine plant growth response to soil amendments, we monitored plant density and cover in early August 2014 and 2015. One linear transect was installed perpendicular to the road 0.75 m from the edge of the amendment treatment in each of the seven soil treatments at each site. Four 1 x 0.5 m quadrats were evenly spaced along these linear transects. Species were observed in late July to determine plant species composition at peak biomass. Plant density was recorded by counting the number of seedlings per species in each 0.5 m^2 quadrat. After recording the number of each plant species, we estimated total native and non-native percentage aerial cover using Daubemire cover class ranges (Davis and Brown 2014). We also recorded percent cover of litter and bare ground.

Soil temperature, ambient temperature, rainfall, and soil N were monitored at the study sites during the growing season. In June and July 2014 and midsummer 2015, we installed soil plant root simulator (PRS) probes that absorb plant available nutrients and demonstrate N availability during a saturation period of 30 days (Western Ag Innovations Inc., Saskatoon, Canada). These probes contain an ion exchange resin membrane that is saturated with ions of either negative or positive charge. When in contact with the soil, soil ions displace these charged ions at a rate that depends on their natural diffusion. Probes are 10 cm in length and are installed at a 45 degree angle to the soil surface. PRS probes were placed in the control, mulch, mulch/compost, compost, polymer, and mulch/polymer treatment at all sites. There were four subsamples in each sampling location. Soil temperature sensors with data-loggers were buried at 7.5-10 cm depth in the compost, mulch, and control treatments. One additional sensor measured ambient temperature. Rain gauges at the center of each study site were monitored weekly during the summer. Weather stations in Moraine Park serve as a reference for rainfall data (40.3625, -105.60194).

Soil samples from 0-7.5 cm and 7.5-15 cm depth were collected for texture prior to amendment application in fall 2013. We pooled soil samples from 5-10 cm depth in four locations in each treatment plot in mid July 2014 and measured total soil carbon and N (Costech 4130 CNHSO flash combustion gas chromatograph, Valencia, CA, USA). Lastly, we collected soils to determine gravimetric water content by pooling from 5-10 cm depth at four locations in each treatment at each site twice per growing season (7/23/14, 9/8/14, 7/13/15 and 9/8/15). In late July of each year, soil moisture was measured 3-4 days after significant rainfall of 80-120 mm. There were no rainfall events of this size in late August or early September of 2014 and 2015. These field soils were double bagged or collected in metal tins and bagged and put on ice. Then they were pooled, weighed, dried at 100˚C for 24 hours, and weighed again to determine the amount of water they contained by subtraction.

Statistical Analysis

The experiment was a complete randomized block design with six study sites as the blocking factor. One replication of seven amendment treatments was at each study site. Mixed model ANOVA was performed on each response variable with site as a random effect and soil amendment treatment as a fixed effect. Repeated measures ANOVA was used to evaluate the effects of soil treatments over time. Soil amendment treatment, soil nitrate in July 2014, and their interaction were predictor variables of plant growth response in multiple regression. These predictors were chosen because they were in our hypothesis and explained the majority of the variation in the response variables. These models had the highest R^2 and lowest AIC values with the least number of predictors.

Plant growth response (native cover, native seeded density, non-native cover, or non-native density) or soil response (moisture, N) in 2014 and 2015 were the response variables. For each sampling method, we calculated means of the subsamples collected in each treatment plot, and this value was used as the block response variable. We assessed equality of variance by observing equal scatter in plots of residual vs. predicted values. For the repeated measures ANOVA, the assumption of sphericity must be met by the data.

We assessed this using Pillai's trace multivariate test for repeated measures with more than two time points. Tukey's Honestly Significant Difference was used to identify which means differed when ANOVA indicated a significant effect of treatment or soil nitrate. Non-native density, non-native cover, native density, and native cover were square root transformed prior to analysis to meet the assumptions of the statistical models used. To calculate the ratio of non-native to native species, we divided non-native cover by native and multiplied by 100. This ratio was square root transformed to meet assumptions of normality and equality of variance.

The alpha level for our analyses was set at 0.05, but we include results with p-values between 0.05 and 0.15 because they suggest a non-significant trend. Analyses were conducted in JMP®, Version 11 **(**SAS Institute Inc., Cary, NC).

Methods: Greenhouse Competition Study

Objectives and Hypotheses

The greenhouse study was designed to evaluate how soil amendments alter initial competition between native and non-native species. The preliminary experiment reported here evaluated grass species growth response to soil amendments during germination and early establishment, a period of 8 weeks. The objectives of this study were to 1) evaluate the effect of soil amendments on the competitive relationship between invasive *Bromus spp.* and native *Elymus spp.* during early establishment, 2) compare which combination of soil treatments promotes the establishment of native species while discouraging invasive species establishment, 3) compare the growth response of short-lived and long-lived analogue native: non-native species to each of the soil amendments and 4) examine how the amendments may be changing loss of soil moisture over time.

We tested the hypothesis below with comparisons that are in Table 8 of the Appendix.

H1: Polymer incorporation will slightly decrease nutrient availability and increase soil water, favoring native over non-native establishment.

H2: Compost incorporation will increase nutrient availability and soil water, favoring non-native growth over native.

H3: Mulch application will increase soil water and decrease nutrient availability, favoring native over non-native establishment.

Experimental design

Competition can be evaluated by growing analogue species together (McGlone et al. 2012, West et al. 2010). We paired native: non-native species with similar functional roles in this experiment. Bottlebrush squireltail (*Elymus elymoides* (Raf.) Swezey) was paired with cheatgrass (*Bromus tectorum* L.) which are both shallow-rooting, short-lived species (Brown and Rice 2010, Tiley et al. 2006). Canada wild-rye (*Elymus canadensis* L.) was paired with smooth brome (*Bromus inermis Leyss.),* which are deep-rooting, long-lived perennials (East and Felker 1993, Chantigny et al. 1997).

We used a replacement design with constant species density similar to other grass competition greenhouse studies (Leger et al. 2014, Lowe et al. 2003, McGlone et al. 2012). There were monocultures of both the native and non-native grass and a biculture of both. The planting ratios for the paired grasses were 0:100, 50:50, and 100:0; (Figure 2).

In the 50:50 multi-species containers we used a checkerboard pattern of the native and non-native species, alternating their locations so that the neighbors of each individual plant were the other species (Figure 2). Grass seeds were planted at a density of 30 seeds per 6 inch diameter container, which is a higher density than was observed in the field in 2014. This density was chosen to ensure that the plant species were in close competition with each other during early establishment. *E. elymoides* and *E. canadensis* seed was from Bear Lake Road Phase II restoration stock and *B. tectorum* and *B. inermis* seed were collected from adjacent to Bear Lake Road. The experiment was set up with three blocks, to account for differences in sunlight and water pressure in the irrigation system along the greenhouse bench. Container location was randomized within each of these blocks every 2 weeks. We watered plants with a raised mist irrigation system for 2 minutes twice daily during the first two weeks and once daily for the remainder of the study. Plants were grown with a 16 hour day and 8 hour night photoperiod. Average day temperatures were 18˚C and night temperatures were 15˚C. During the day, half the amount of natural sunlight was supplied by 430 Watt HID lights.

We combined soils from different containers for each batch mix to reduce heterogeneity among containers. Soils and amendments were mixed using a mechanized soil mixer. Amendment application rates and depth for polymer, compost, and mulch were exactly the same as in the field study. We combined 1 gram of polymer or 250 ml of compost with 1000 ml of soil to fill the top 10 cm of each container. Containers were marked with tape to determine amendment depth. Lastly, we added 500 ml of wood mulch to the soil surface to create a 2.5 cm mulch blanket.

Soil Water Loss Study

To test amendment impact on soil moisture through time, we watered containers with 1 month old grasses to field capacity or saturation, then let them dry naturally in a week-long dry-down study. Using a HydroSense probe (Campbell Scientific, Inc., Logan, UT), we measured volumetric water content in each container twice daily at 10 am and 4 pm for 7 days. The hydro-sense probe is 20 cm long and measures dielectric permittivity around the metal probes to determine the proportion of water relative to soil and air within the volume of a sphere within a 2 cm radius around each metal rod.

Statistical Analysis

We ran separate analysis of variance (ANOVA) models to compare total biomass in monoculture treatments. The predictor variables were species, treatment and their interaction. Block was not significant to plant growth response, so it was removed from the model. The response variables were mean biomass per plant or mean biomass per species in each container. Total biomass was square root transformed to meet the assumptions of ANOVA. Levene's test indicated equal variance for the transformed data. Analyses were conducted using JMP Pro 11 (JMP$^{®}$, Version **11**. SAS Institute Inc., Cary, NC, 1989-2007). The selected alpha value of 0.05 was compared to p values to detect significance.

To analyze the dry down study, we used repeated measures ANOVA, with time as the repeated measure. The response variable was volumetric water content at the 11 times it was measured during the week-long study. The data did not meet assumptions of sphericity, so we used the Greenhouse-Geisser adjusted p-value. To determine differences between soil treatments at a given time point, we ran an ANOVA with species and treatment as the predictor variables and water content as the response.

Results: Field Study

Soil Nitrogen

We did not detect a significant change in plant-available N over time due to the amendments (p=0.31, Figure 3), although it was significantly different between treatments at two sampling periods. Across time, there was a marginally non-significant trend that ammonium (NH_4^+) availability was higher in control treatments than all others ($F_{5,18}$ =2.4, p=0.07). Soil nitrate (NO_3^-) comprised the majority of available N (Figure 3). In both years, yard-waste compost incorporation treatment increased the amount of plant-available N during the middle of the growing season. There were significant differences in soil N availability among amendment treatments in June through July in both 2014 ($F_{5,5}$ =4.7, p=0.003) and 2015 ($F_{5,5}$ =4.7, p=0.017). In 2015, compost had greater N than mulch and mulch/polymer. In 2014, plant-available N was highest in compost at 44 ug/10 cm^2/burial length compared with averages in control, mulch, and polymer treatments. Two years after installation, compost had nearly five times more nitrate than all other treatments except for polymer.

Carbon: Nitrogen

Total C:N ratios (Figure 4) were significantly influenced by site (p=0.006) and amendment treatment (p=0.01) one year after restoration. Initial roadside f soils in 2013 had a C:N ratio of 19:1 across the six sites. The initial C:N ratios of the amendments were 57:1 for mulch, 10:1 for compost and 4:1 for super-absorbent polymer (Figure 4). One year after decomposition, mulch/compost amended soils had the lowest C:N ratio relative to control and mulch/polymer ($F_{1,30}$=15.3, p=0.0005). Control soils changed the least, with bonded wood-fiber matrix application in the hydro-seed slightly increasing C:N.

Rainfall and Soil Moisture

The soil treatments significantly changed soil moisture over time ($p=0.039$, $F_{6,24}=2.6$) due to the mulch/compost and mulch/polymer treatments. The differences in soil moisture among treatments were most apparent in the first year of sampling in mid-July. Rainfall was high in spring of 2014 and 2015, but limited in the summer months. In 2014, there was 0.9 cm of rainfall in the month of April, 5.9 cm in May, and 0.3 cm in June. In 2015, there was 5.5 cm of rainfall in April, 4.8 cm in May and 3.3 cm in June.

Mulch amended soils did not lose moisture as quickly as other treatments after rainfall events (Figure 5a). In late July 2014, one week after 14-21 mm of rainfall, there was a significant difference in soil moisture among treatments ($p = 0.0007$). The soil moisture in mulch/compost treatment (27%) was higher than compost (12.5%), compost/polymer (13 %), control (11%) and polymer (8%) (Figure 5a). Similarly in late July of 2015, soil moisture in the mulch/polymer treatment was significantly higher than control ($p=0.001$) after 35 mm of rainfall. In contrast, when there was little or no rainfall (≤ 2.5 mm), there were no differences in soil moisture between treatments (Figure 5c-d). In early September 2014, no treatments were different from each other and they averaged 5-7% soil moisture (Figure 5c). Similarly in mid-September 2015, soil moisture averaged 2-3% for all treatments (Figure 5d).

Native Species Composition

Restoration soil treatments significantly increased native density over time ($F_{12,56} =2.6$, $p=0.007$, Figure 6) with the highest number of seeded plants in mulch alone or combined with compost or polymer. In summer 2014, seeded native species density was higher in the mulch (97 plants/m^2) and mulch/compost (90 plants/m^2) compared with control (34 plants/m^2). Seeded native density was lowest in compost (27 plants/m^2). In summer 2015, seeded plant density was higher in mulch combined with compost ($F_{1,30}$=10.9, p=0.002) or polymer ($F_{1,30}$=16.3, p=0.0003) than in soils amended with compost and polymer (Figure 5). All other soil treatments did not have different densities of seeded species, although there were trends similar to those of 2014 (Figure 5). One year after seeding in 2014, composition of mulch/compost treatments was 34 *E. canadensis*, 7 *E. elymoides*, 1.3 *H. villosa* and 0.6 *A. frigida* per m^2 (Appendix 1, Figure 15). In contrast, control treatments averaged 6 *E. canadensis,* 4.4 *E. elymoides*, 1 *H. villosa* and 0.7 *A. frigida* plants per m^2 (Appendix 1, Figure 15).

In both 2014 and 2015, native species cover was not significantly different between soil treatments ($F_{6,30}$=0.39, p=0.87 ; $F_{6,30}$=0.75, p=0.61, Table 1). Both soil nitrate level ($F_{1,24}$ =4.7, p=0.04) and site location ($F_{5,24}$ =7.3, p=0.0003) influenced native cover through time.

Yet, native cover increased by nearly 50% in all soil treatments from 2014 to 2015. In summer 2015, native cover was highest in mulch with compost (22 ± 6%), mulch alone (18 ± 6%), compost with polymer (18 ± 5%) and compost alone (17 ± 3%). Control soils averaged 15% native species cover. Polymer amendment and mulch with polymer had the lowest native species cover of 12% and 13%, respectively. Since site 5 was an outlier with high non-native density due to *B. tectorum*, we also analyzed native cover data with all field sites except site 5. In this revised analysis, mulch with compost treatment had higher native cover (21%) than control (11.5%) and polymer (11%) amended soils ($F_{1,24}$=7, p=0.014).

Non-native Species Composition

Over time, treatment and soil N influenced non-native density, although this was a marginally non-significant trend (p=0.12, $F_{5,24}$=1.9). In 2014, we detected no difference in non-native species density among treatments (p=0.27, $F_{6,18}$=1.4, Figure 8). In control treatments in 2014, density of non-native species averaged 2.4 *R. acetosella* L., 2.6 *B. tectorum*, and 0.8 *Poa spp.* per m^2. In comparison, non-native density was 1 *R. acetosella* L., 1.7 *B. tectorum*, and 0 *Poa spp.* per m^2 in mulch/polymer (Appendix 1, Figure 16). In 2015, non-native density was highest in the compost with polymer treatment (12 plants/m^2) and lowest in the mulch (2 plants/m^2) or mulch with polymer (1 plant/m^2) (p=0.02, $F_{6,30}$=3). In the same year, site 5 had 83 *B. tectorum* plants/m^2 in compost treatments.

Non-native cover was not significantly different between treatments in 2014 ($F_{6,30}$=1.9, p=0.12) or 2015 ($F_{6,30}$=1.6, p=0.19). Although, non-native species cover was higher across all treatments in the first year after road resurfacing and realignment (Table 3). In summer 2015, non-native cover varied from 0.5% (mulch/polymer) to 1.9% (compost), although there were no significant differences in cover among treatments. In 2014, non-native cover was highest in compost (5.6%) and compost/polymer (5.2%), but this was not different from control (3.3%).

The ratio of non-native to native cover demonstrates the future potential for non-native species to dominate the roadside plant community, since these species typically have higher seed production and dispersal capabilities (Steinfeld et al. 2007). Soil treatments with lower non-native/native cover will likely be the most resistant to invasive species. Compost/polymer amended soils had a higher proportion of non-native species and the lowest non-native: native cover was in mulch/polymer and mulch/compost (Figure 7). Control soils had 9% proportion of non-native: native species cover.

Results: Greenhouse Competition Study

Plant Germination, Establishment and Competition

Plant growth over 8 weeks was dependent on the soil treatment ($p<0.001$, df=3, F=213), species ($p<0.001$, df=3, F=16), and the interaction between them ($p=0.001$, df=9, F=4.2). Total biomass per experimental unit varied from 0.13 g (*B. tectorum*, mulch, rep 2) to 3.05 g (*E. canadensis*, compost, rep 3). All grass species grew much more in compost amended soils than control ($F_{1,31} = 557$, $p<0.0001$, Figure 9-10). Plant growth was significantly lower in mulch treatments than in compost, polymer or control ($F_{1,31} = 123$, $p<0.001$; Figure 9, 10). Total plant biomass across all species averaged 0.25 g in mulch, 0.43 g in control, 0.53 g in polymer, and 1.9 g in compost. *Bromus tectorum* grew less than the other three species in the polymer, control and mulch treatments (Figure 10, $p=0.001$). *B. tectorum* biomass per plant across all soil treatments averaged 0.028 g which was significantly lower than *E. elymoides* biomass per plant of 0.045 g ($F_{1,31} =37$, $p<0.0001$). In particular, *B. tectorum* grew significantly less than *E. elymoides* in mulch ($p=0.002$, Figure 10d), polymer ($p=0.03$, Figure 10a), and control ($p=0.001$, Figure 14). Soil treatments changed the germination and establishment of the grass species in this study by altering water and nutrient availability, similar to the field study. Although 30 seeds were planted in each pot, the number of plants in each pot at 8 weeks varied between amendment treatments ($p<0.001$). There were significantly lower number of plants in mulch blanket application compared to control ($p=0.01$, $F_{1,31}= 7.3$). Containers with mulch averaged 14 grass seedlings, while all other treatments had 19-26 seedlings. There were not significant differences in germination among species ($p=0.16$), but there were differences in biomass per plant by species ($p=0.006$).

The native *Elymus spp*. had higher biomass per plant than non-native *Bromus spp.* across all treatments ($F_{1,31}$ =45, p<0.001). *Bromus spp*. grew less than *Elymus spp.* in control soils ($F_{1,31}$ =22, p<0.0001, Figure 9,10). In particular, *E. elymoides* especially grew more than *B. tectorum* in control field soils ($F_{1,31}$=25, p<0.0001, Figure 9). *B. inermis* grew less than *E. canadensis* in compost amended soil ($F_{1,31}$=20, p<.0001, Figure 9).

Soil Water Loss Study

Soil treatments lost water at significantly different rates over time during the dry-down study ($F_{30,165}$ =1.9, p=0.005, Figure 11). Initial soil moisture was 27.5% in compost, 24.4% in control, 24.0% in polymer and 22.5% in mulch. These differences were marginally non-significant ($F_{3,3}$ =2.8, p=0.09). Mulch treatment dried out at the slowest rate, reaching an average of 17% soil moisture after five days without water, which was 3.5% higher than the control and significantly higher than all other treatments ($F_{1,55}$ =22, p<0.0001, Figure 11). There was a trend that yard-waste compost initially retained more water than control ($F_{1,55}$=3, p=0.09). Yet, compost amended soils had the lowest moisture after five days without watering ($F_{1,55}$=4, p=0.046). Water content at the end of the study averaged 17% in mulch, 14% in polymer, 13% in control and 12.5% in compost.

Discussion: Field and Greenhouse Study

Soil amendments affect soil nutrient and water availability, which in turn influence seed germination, plant establishment and competition among species on roadsides. Seedling establishment increased in response to higher soil water content. The soil treatments significantly changed soil moisture over time (p = 0.039). As a result of higher moisture in mulch combined with compost or polymer, perennial seeded density increased. Yet, mulch blanket decreased germination rates of all species in the greenhouse (p=0.01) due to higher soil moisture and N immobilization. Similarly, yard-waste compost incorporation in the field decreased plant establishment in comparison with mulch, but increased germination (p=0.01) and growth ($p < 0.0001$) in the greenhouse due to higher water content.

These opposing trends can be explained by the interaction between water and nutrient availability. Plants in the greenhouse were watered daily, while plants in the field study were water limited (Appendix 1, Figure 17-18). In the field, gravimetric water content with mulch blanket ranged between 2 to 20% and control soils ranged from 2 to 11% (Figure 4). Increased soil moisture through time increased native seeded establishment in mulch/compost and mulch/polymer treatments. Constant soil moisture of 25-28% in compost amended soil in the greenhouse could have increased microbial decomposition rates of organic matter (Figure 10) and release of mineral N. This rapid release of mineral N increased plant growth (Figure 9-10). Similarly, mulch treatments maintained soil moisture between 21 and 18.5% when they were watered daily. In the greenhouse, germination and growth in mulch treatments was discouraged through rapid immobilization of N by microbial species.

Mulch composition and application depth are critical considerations in restoration. A 10 cm mulch blanket can discourage native-species growth (Rhoades et al. 2015), but thicker (15 cm) blanket can reduce mineral N (Rhoades et al. 2012). Rainfall patterns, landform, and soil texture of the field site will drastically change how mulch impacts soil nutrients and plant growth over time, so application rate should be adjusted to these factors. Increased rainfall and higher proportion of clay in the soil texture will speed the degradation of the mulch and immobilization of N. The mixture of particle sizes in this mulch allows for slow infiltration of snowmelt and rainfall to the soil surface. In addition, the pine needles have higher surface area and are a more labile form of carbon than traditional wood mulch. The tanins and resins in the pine needles reduce the rate of decomposition, but the high surface area to mass ratio increase it. In contrast, thicker wood mulch has lower tanins, but also lower surface area that reduces access of microbes to the carbon.

Both higher soil moisture (Hamilton et al. 1999) and decreased mineral N (Lowe et al. 2003, Toeroek et al. 2000) through time increased native perennial establishment relative to non-native annuals. In the field, mulch blanket treatment combined with compost or polymer raised water content and density of seeded native species while decreasing non-native density (Figure 6-8). The majority of observed seeded species were perennial rye grasses. All mulch blanket treatments maintained higher water content after rainfall (Figure 4a) and likely reduced seedling plant stress on south facing slopes. This trend is supported by the greenhouse water loss study, which had the greatest long-term moisture retention in soils with a 2.5 cm thick mulch blanket (Figure 11). Two years after restoration, there was a trend that native cover was highest in mulch with compost incorporated below (Table 1).

Mulch and polymer may additively reduce soil N (Figure 3), decreasing germination and establishment of fast-growing annual non-natives (Figure 8). In the field, soil N and non-native density did not decrease in mulch blanket treatment in comparison to control. Mulch + polymer had the least mineral N in 2015 and lower non-native density than compost + polymer. Also, mulch and polymer treatments combined had the lowest ratio of non-native to native species cover (Figure 7). Similarly, in the greenhouse, mulch and polymer amendments, which decreased plant-available N in the field, increased the growth of native *Elymus spp*. (rye) relative to non-native *Bromus spp*. (brome, Figure 9-10).

Polymer or compost incorporation did not increase soil moisture or native species establishment (Appendix 1, Table 2.1), although compost amendments increased plant growth in the greenhouse (Figure 9-10). This finding is surprising since organic matter does initially increase the water holding capacity of coarse textured soils; yet it can also displace the pore space for water as soils dry. In the greenhouse, although *B. tectorum* increased growth in compost compared to all other treatments, this was matched by *E. elymoides* growth. This demonstrates higher germination and establishment of annual or perennial non-native species in response to increased mineral N.

Increased N availability in the environment is tipping the balance towards non-native species establishment on roadsides (McCrae and Wilson 1992, Lee et al. 2012). Surface treatments with the highest plant-available N resulted in higher non-native species cover and density. Specifically, N released from yard-waste compost in the field benefited non-native species establishment over native perennial seeded species (Figure 6-8). Non-native density was higher in compost and compost/polymer treatments relative to mulch/polymer or mulch (Figure 8). Additionally, yard-waste compost incorporation significantly increased the biomass of native and non-native grasses in the greenhouse (Figure 9-10). Since both water and N were not limiting, grass species drastically increased growth in response to increased nutrients in the compost amendment.

Increased N availability favored establishment and competitive ability of non-native annual species and increased intraspecific competition. Nitro-phylic non-native perennials, like *B. inermis*, respond to this increased N with higher growth rate than native perennial grasses ($F_{1,61}$ =14.0, p=0.0003), like *E. canadensis*. Annuals respond to increased N with higher biomass than perennials (Claassen and Marler 1998, Lowe et al. 2003), but this is at greater amounts of increased N availability. Higher N additions increased foliar leaf N and biomass of invasive annual *B. tectorum* at significantly greater thresholds than perennial native *Bouteloua gracilis* (Lowe et al. 2003). In general, annual species respond to increased N with greater plasticity than perennial species (Roumet et al. 2006), especially during initial establishment (Marler and Claassen 1998).

Resource availability can be actively managed on trails, roads, parking lots, and other sites with initial increase in N availability that favors establishment of invasive annual species. Ecosystem-level thinking can prevent establishment of these invasive species. By linking soil resource availability and plant community establishment after disturbance, we reveal best management practices that can increase biodiversity and improve ecosystem function. Site conditions, visitor traffic, N deposition, and restoration methods influence availability of N (Steinfeld et al. 2007).

Initial excess soil N can be managed by stockpiling topsoil (Claassen 2012, Steinfeld et al. 2007), including early and late-season annual species in the seed mix (Herron et al. 2013, Hooper and Vitousek 1998), or application of amendments (Agaba et al. 2010, Brown et al. 2007, Curtis and Claassen 2007, Sanborn et al. 2004). In addition, by increasing soil water holding capacity after disturbance, we can favor establishment of perennial native species (Hamilton et al. 1999). Once established, these perennials have the potential to outcompete non-native annuals despite changes in nutrient availability (Corbin and D'Antonio 2004, McGlone et al. 2010).

FIGURES

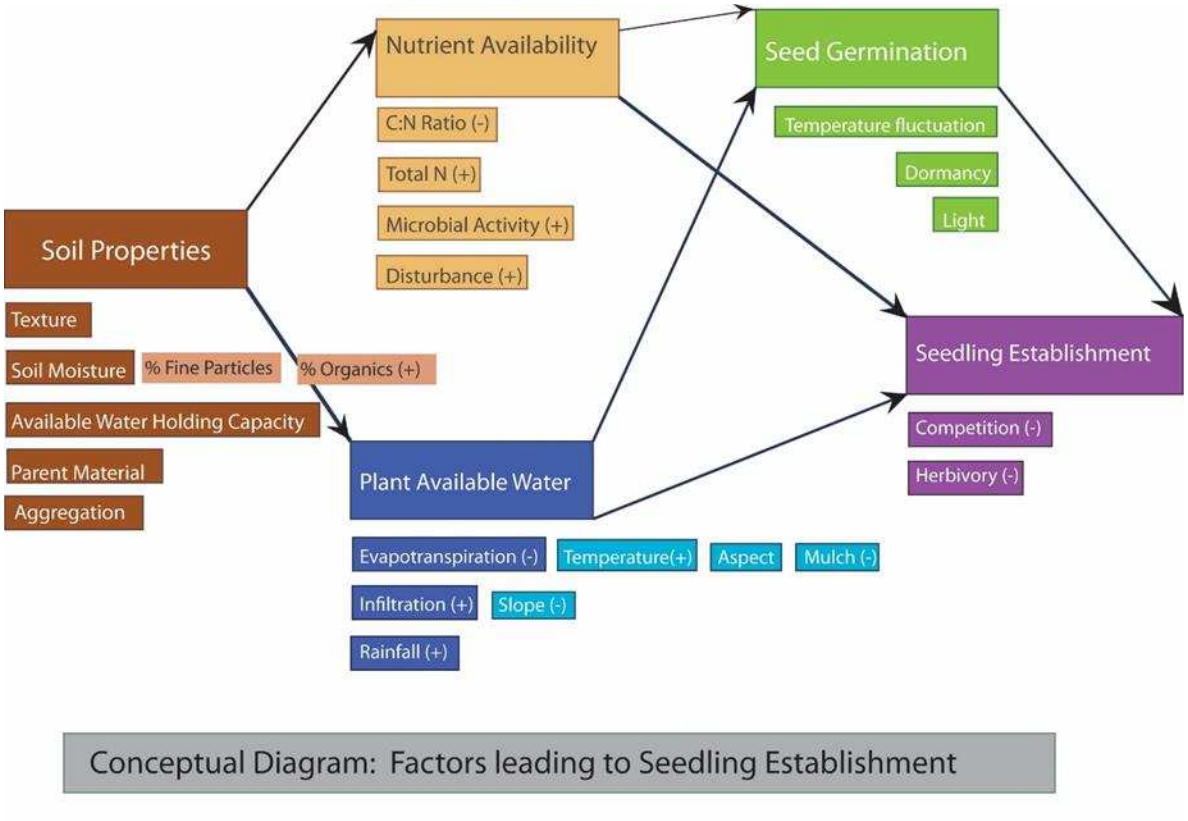

Figure 1. Relationships between soil properties and water and nutrient storage, which affect seedling establishment and plant growth. Variables with hypothesized direct positive relationships to plant growth seedling establishment and plant growth are indicated with a positive sign (+), like the influence of increased rainfall on plant available water. Environmental variables with a negative relationship, like how competition prevents seedling establishment, are indicated with a negative sign (-).

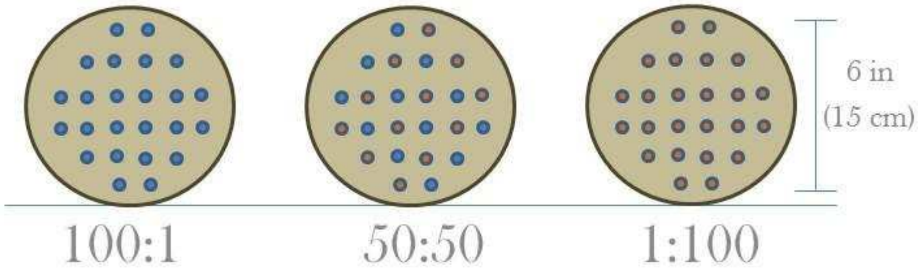

Figure 2. Diagram of the planting design for this greenhouse study with monocultures of each species and a polyculture at constant density of 30 plants per experimental unit.

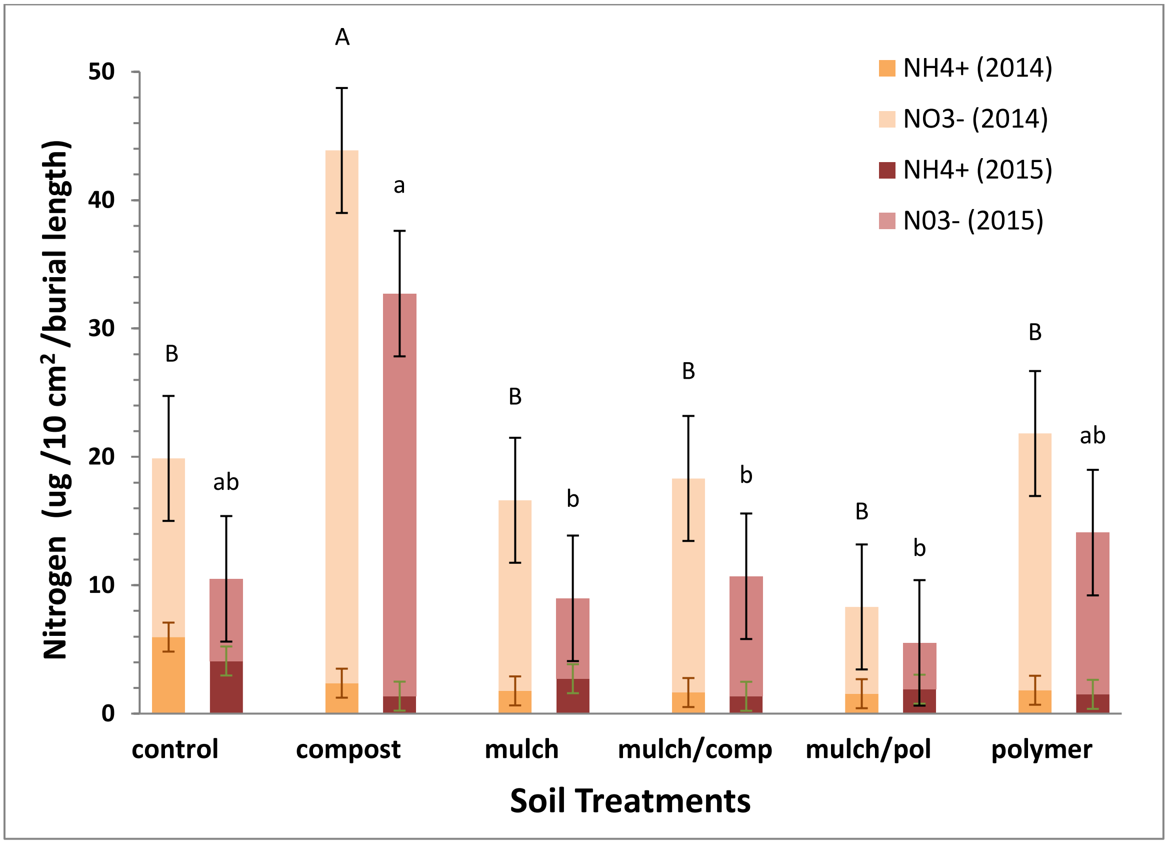

Figure 3. Plant-available nitrogen 6/15-7/15/2014 and 7/1-7/3/2015. Bars are means ± standard error of the mean. Means labeled with different letters are significantly different (α=0.05).

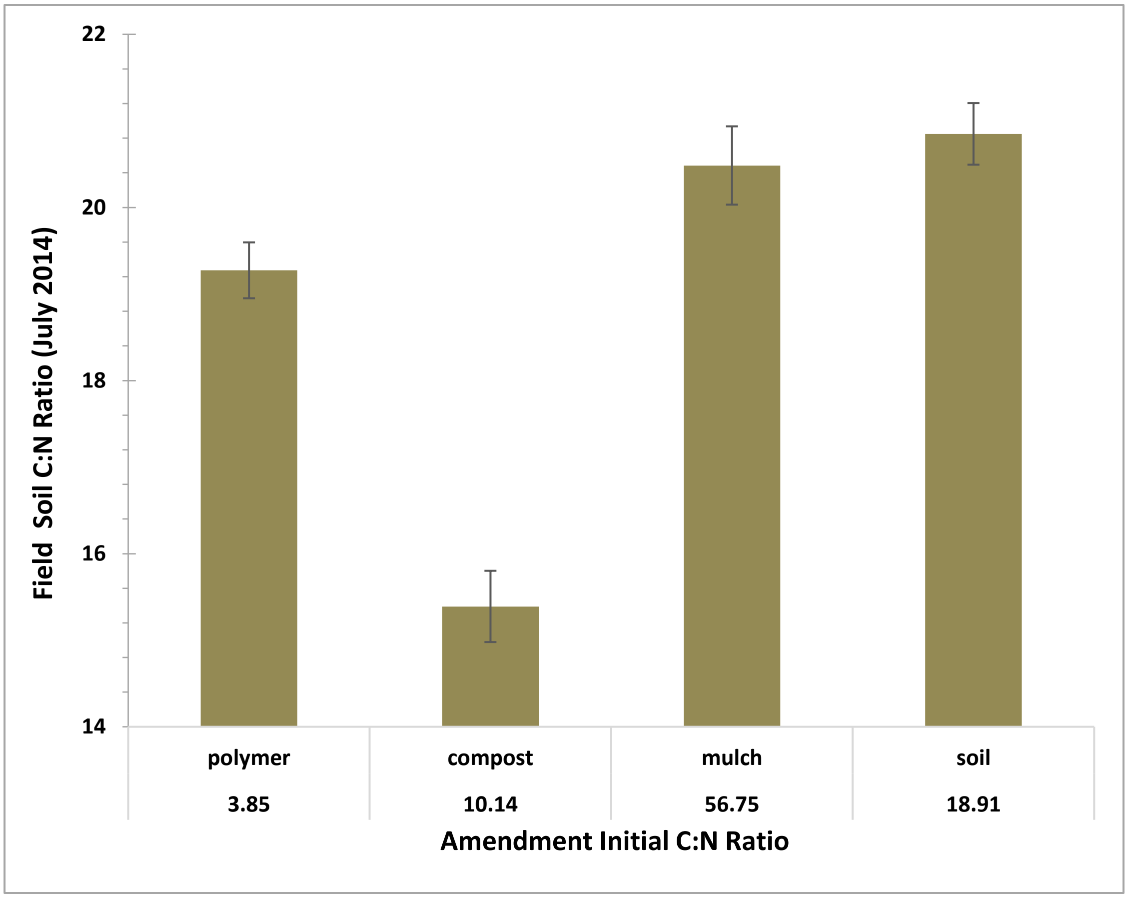

Figure 4. Carbon: nitrogen ratios of each of the amendments (text below bars) and bars are C:N ratios of field soils with amendments, one year after treatment . Soils samples were taken in July 2014. Bars are mean ± standard error of the mean.

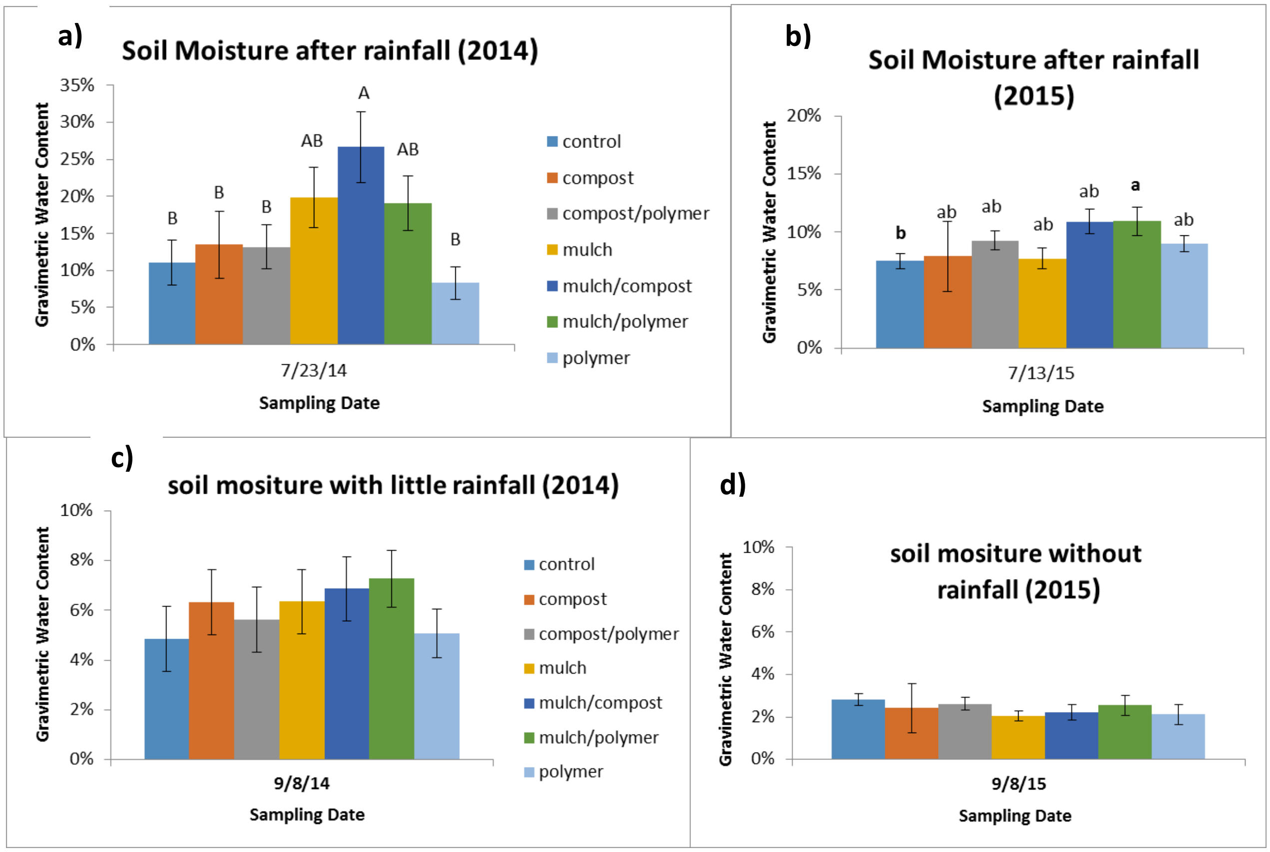

Figure 5. Mean gravimetric water content on four sampling dates in a) late July 2014 b) late July 2015 c) early September 2014 and d) early September 2015. Bars are means ± standard error of the mean. Means labeled with different letters are significantly different (α=0.05). Note that Y axis scales are different on each graph.

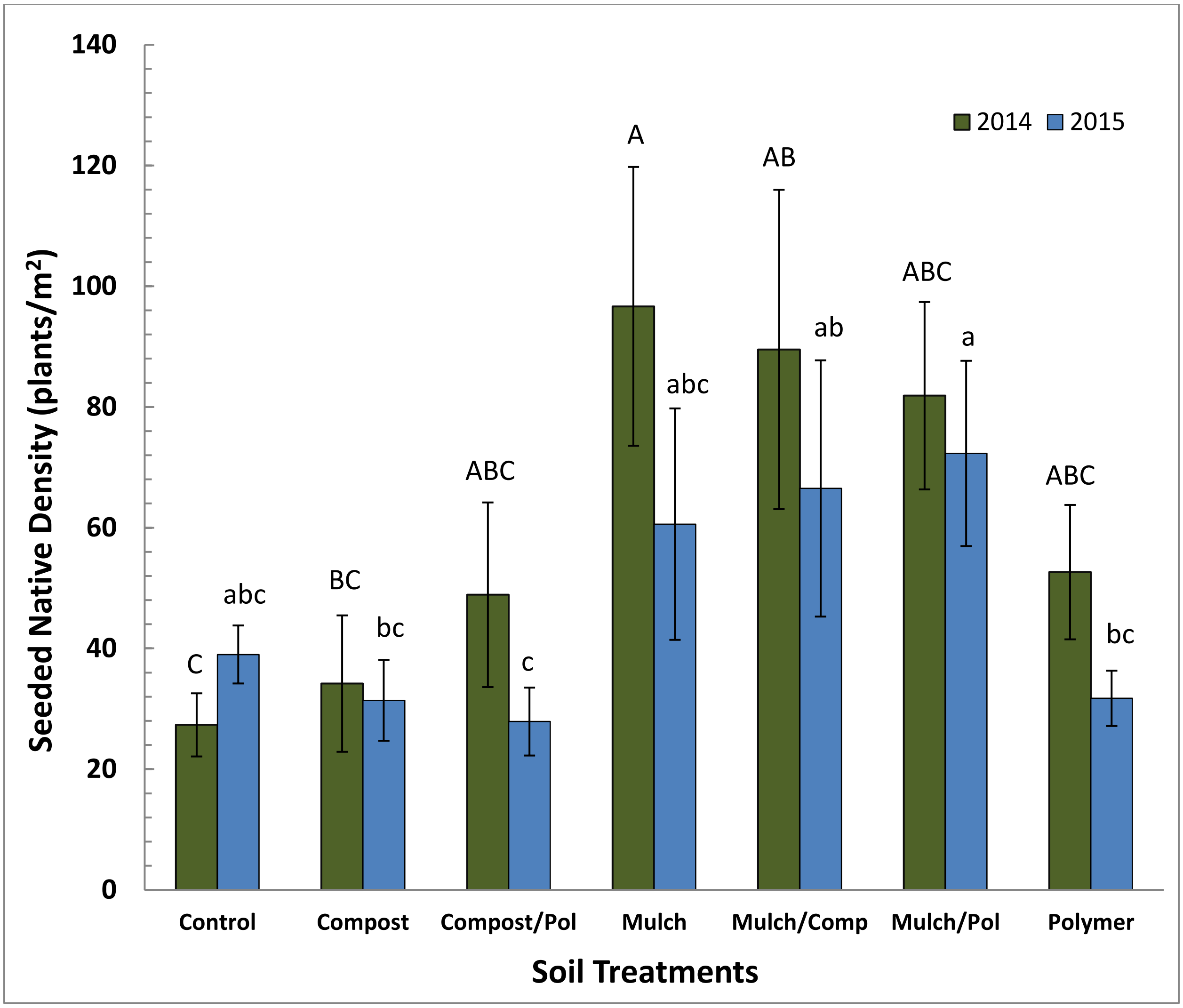

Figure 6. Bars are mean native plant density across six sites ±standard error of the mean. Means labeled with different letters are significantly different (α=0.05).

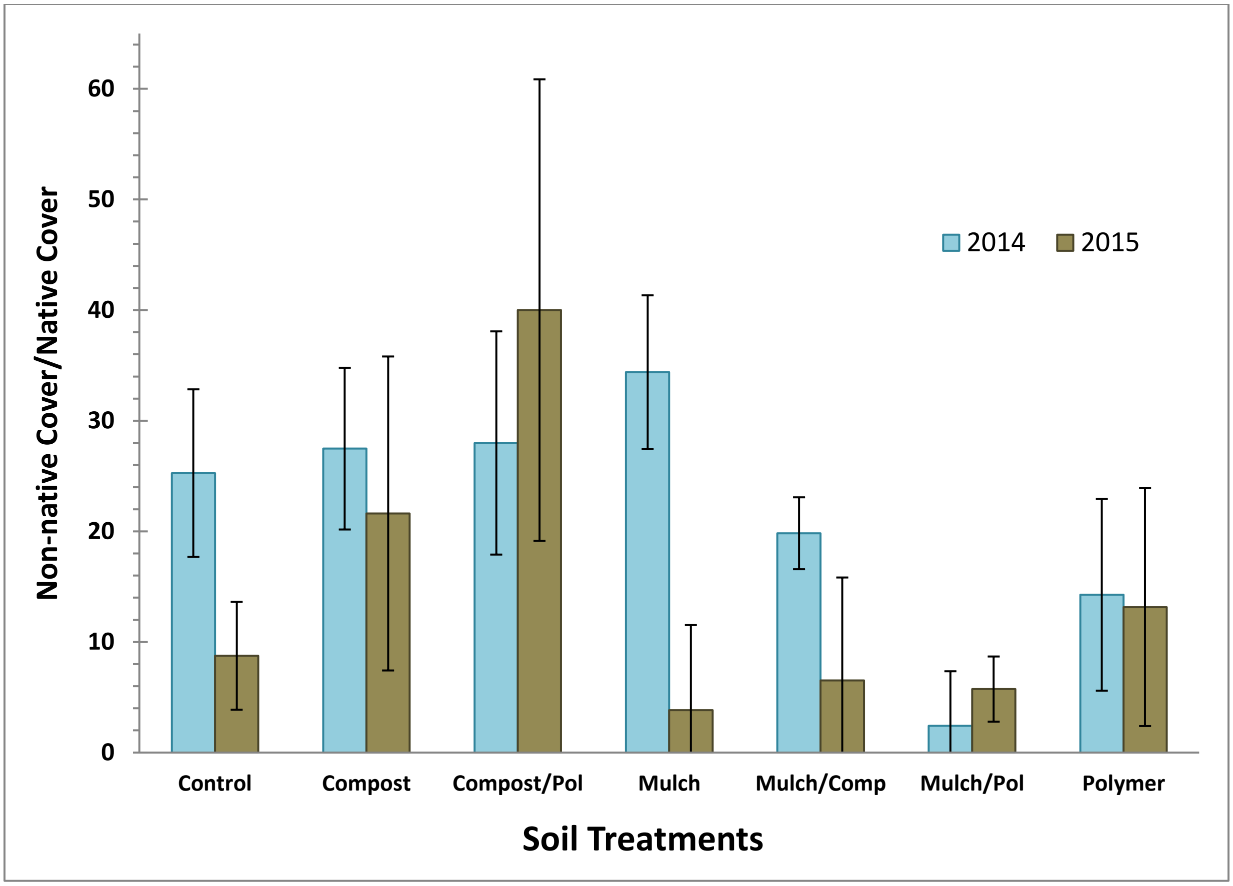

Figure 7. Proportion of observed cover of non-native species relative to cover of native species in each soil treatment in 2014 and 2015. Average values across all sites are shown as percentage of 100.

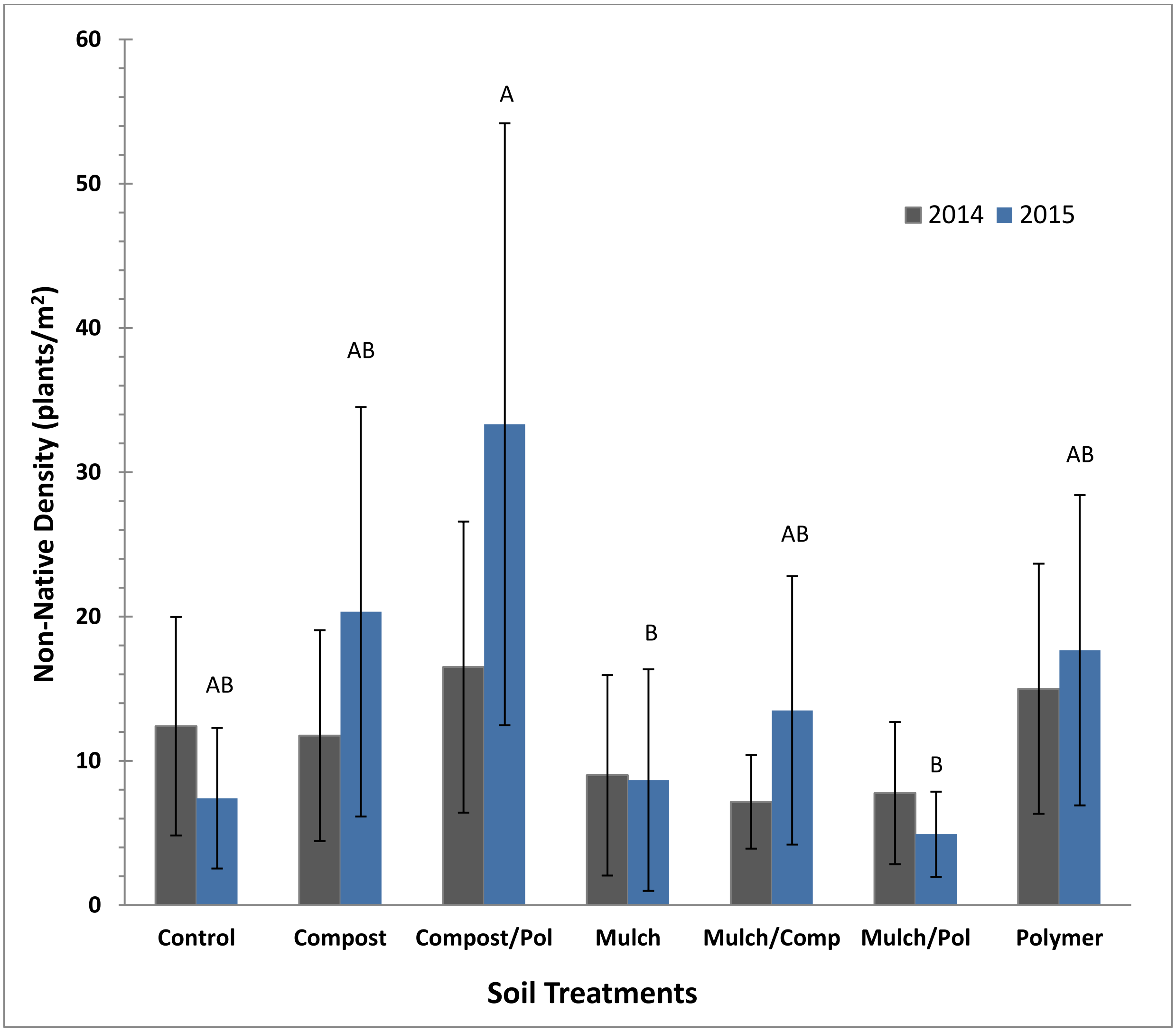

Figure 8. Bars are mean non-native plant density across six sites ± standard error of the mean. Means labeled with different letters are significantly different (α=0.05).

Table 1. Plant species cover in late July 2014 and 2015. Mean ± standard error of the mean.

Treatment	Non-native Cover (2014) (%)	Non-native Cover (2015) (%)	Native Cover (2014) (%)	Native Cover (2015) (%)
Control	3.4 ± 1.4	1.6 ± 2.5	9.6 ± 3.2	15.1 ± 3.7
Compost	5.6 ± 4.0	1.9 ± 4.0	10.7 ± 4.2	16.6 ± 3.3
Compost/Polymer	5.2 ± 2.8	1.2 ± 2.8	5.3 ± 2.6	17.8 ±5.1
Mulch	3.5 ±1.4	1.3 ± 1.4	8.5 ± 3.3	17.7 ± 5.9
Mulch/ Compost	2.3 ± 1.3	1.2 ± 1.3	8.9 ± 2.6	22.2 ± 6.1
Mulch/ Polymer	0.5 ± 0.4	0.5 ± 0.3	6.8 ± 2.9	15.1 ± 4.8
Polymer	1.2 ± 0.5	1.4 ± 0.5	15.4 ± 6.8	12.7 ± 2.2

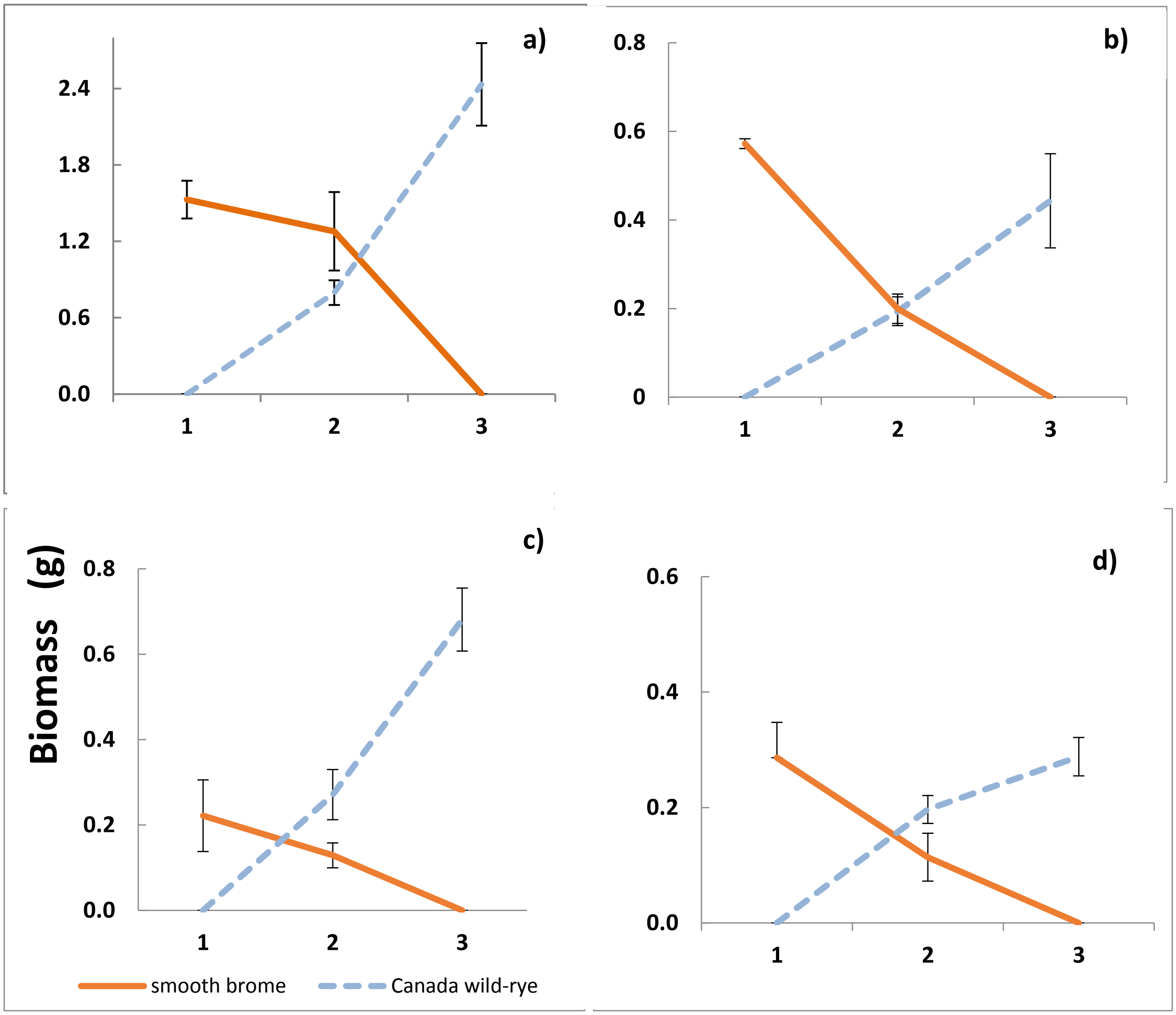

Figure 9a-d. Mean biomass of each species in monoculture (100:0), absent (0:100) and in competition (50:50). The figures show growth of perennials smooth brome and Canada wild-rye in a) yard-waste compost amended soil b) polymer amended soil c) control field soils and d) mulch blanket amended soil. Displayed are means ± 1 SE of the mean.

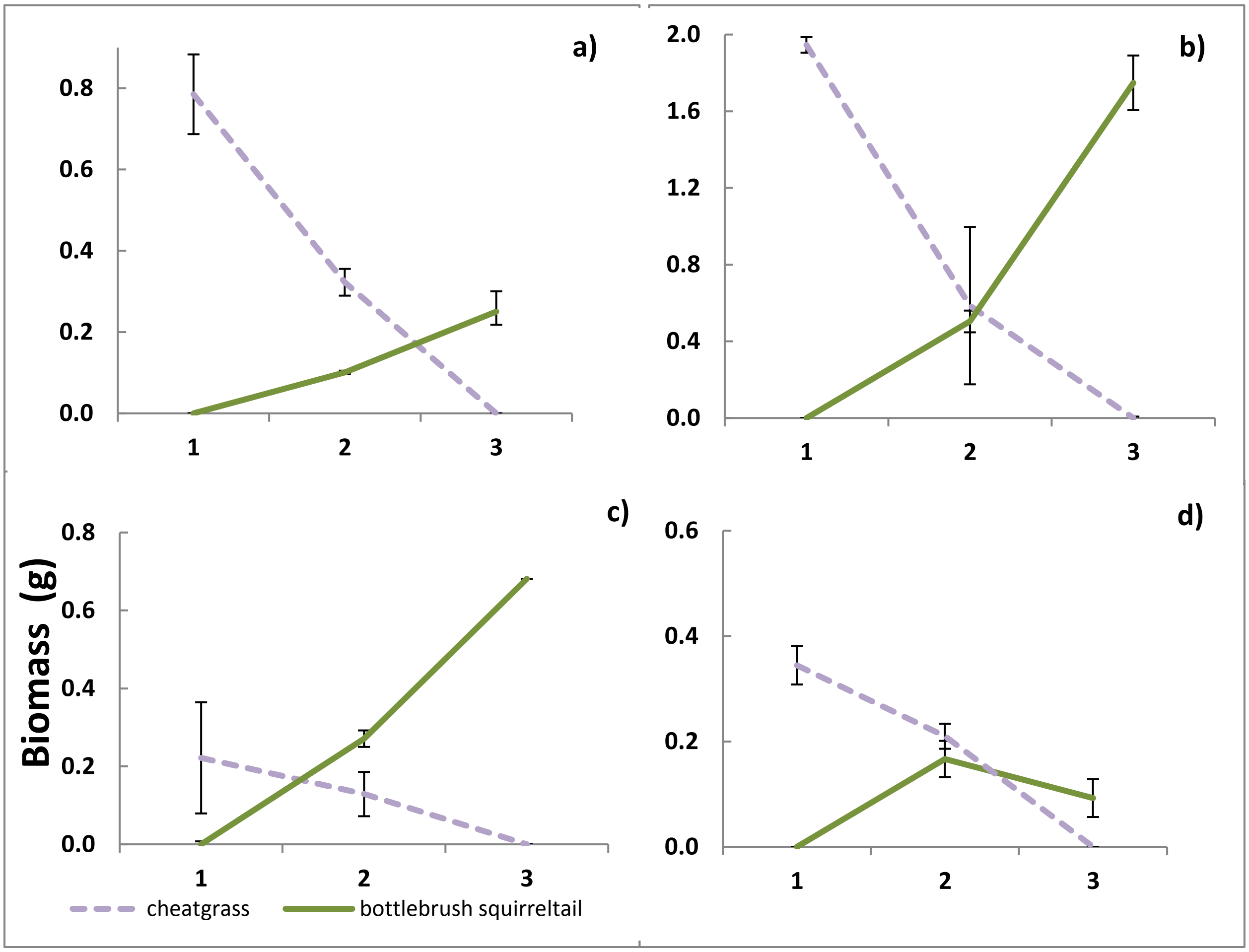

Figure 10a-d. Mean biomass of each species in monoculture (100:0), absent (0:100) and in competition (50:50). The figures show growth of short-lived cheatgrass and bottlebrush squirrel-tail a) polymer amended soil b) yard-waste compost c) control field soils and d) mulch blanket amended soil. Displayed are means ± 1 SE of the mean.

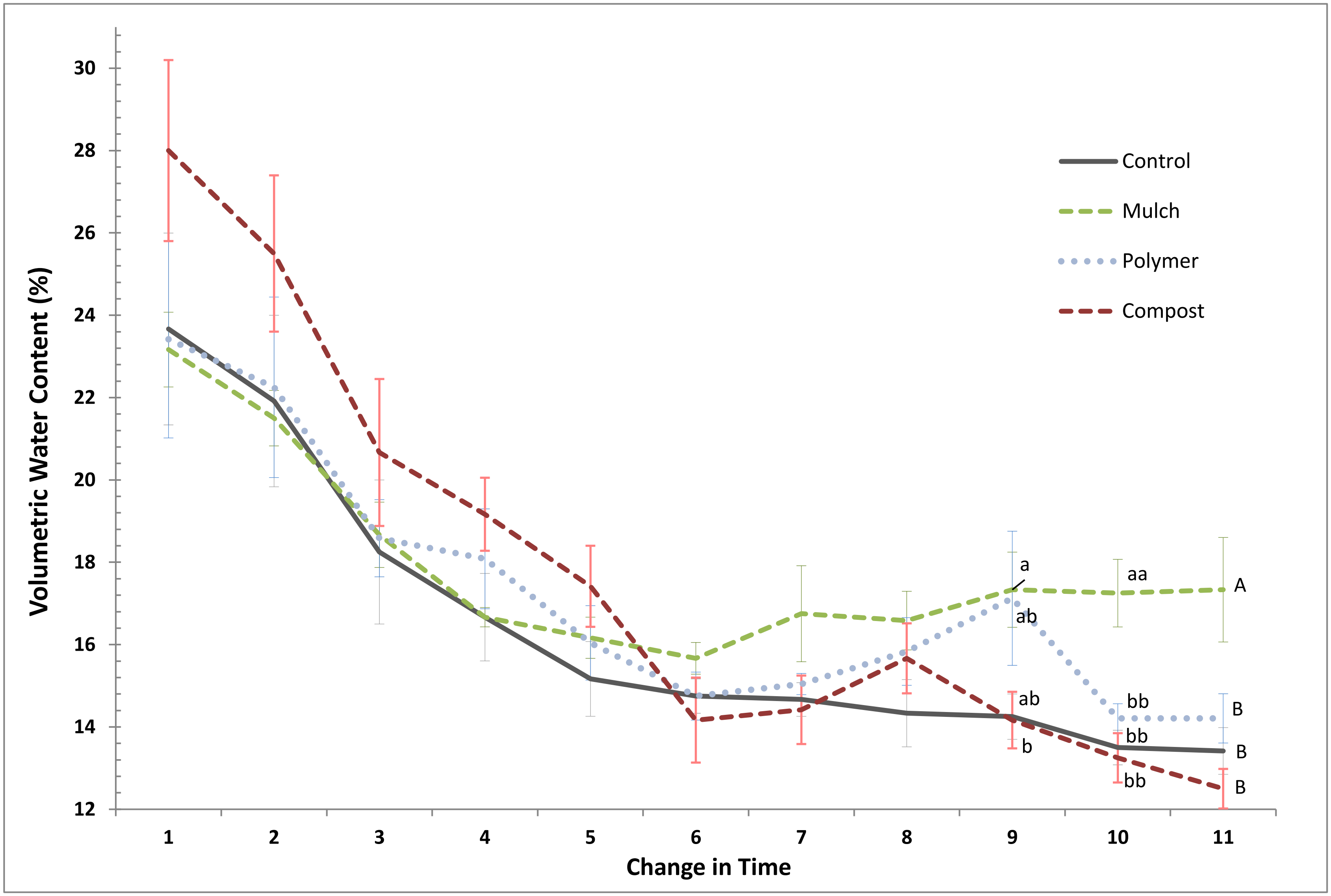

Figure 11. Soils watered to field capacity were measured twice daily for one week with 12 replicates per point. Experimental units were 15x15 cm diameter pots with 30 cm height. The soils were amended with polymer or yard-waste compost to 10 cm depth or with 2.5 cm of mulch blanket on the surface. There were approximately 20 grass seedlings in each pot. Treatments that are significantly different from each other are marked with different letters.

CHAPTER 3: SYNTHESIS AND RECOMMENDATIONS

Synthesis

Effective restoration after road construction requires ecosystem-level thinking. Road corridors have many negative impacts on ecosystem function, but the disturbance of road construction can be an opportunity to direct future plant establishment. Roadways occupy 1% of the surface of the United States (Forman and Alexander 1998), yet these corridors widely impact ecosystems by changing patterns of habitat fragmentation, pollution, hydraulic cycles, erosion and invasion (Steinfeld et al. 2007). The goal of this study was to manipulate soil characteristics by decreasing N availability and increasing water availability, in order to favor establishment of native perennial species over non-native annuals (Corbin and D'Antonio 2004, He 2011) and perennials on roadsides.

In the field study, native perennial germination and establishment increased in response to higher soil water content. The soil treatments significantly changed soil moisture over time (p=0.039, Figure 5), especially due to the mulch blanket treatments combined with polymer or compost incorporation. Mulch and polymer (p=0.006) or mulch and compost (p=0.03) increased native perennial germination rates in the field. Yet, mulch decreased the number of plants, a proxy for germination rates, in the greenhouse (p=0.01). Similarly, yard-waste compost incorporation in the field decreased plant establishment in comparison with mulch, but increased greenhouse germination (p=0.01, $F_{1,31}$=204) and growth (p<0.0001,$F_{1,31}$=557).

These opposing trends can be explained by the interaction between water and nutrient availability; experimental units in the greenhouse were watered daily, while significant rainfall occurred every two weeks on Bear Lake Road (Appendix 1, Figure 18). Growth in mulch treatments likely was discouraged due to increased moisture and resulting microbial activity which immobilized N. Mulch blanket treatments maintained soil moisture between 21 and 18.5% when they were watered daily in the greenhouse. The higher water content in the greenhouse than the field caused rapid immobilization of N in mulch treatments. Soil moisture of 25-28% in compost amended soils in the greenhouse could have increased microbial decomposition rates of organic matter and release of mineral N for rapid plant growth, which was not observed in the field. These conclusions are speculative based on plant growth response because N was measured in the field but not the greenhouse study.

Water was much more limiting to plant establishment in the field than in the greenhouse. Native perennial species may be limited by water availability in competition with non-native annuals (Hamilton et al. 1999). Germinating plants experienced sporadic soil moisture during the growing season in the field due to length of time between rainfall events (Chapter 2, Figure 4 and Appendix 1, Figure 17). Gravimetric water content with mulch blanket ranged between 2 and 20% (Chapter 2, Figure 4) and control soils ranged between 2 and 11% (Chapter 2, Figure 4). Increased soil moisture through time increased native seeded establishment in mulch + compost and mulch + polymer treatments. Thin (2.5-5 cm) mulch treatments were shown to increase soil moisture in comparison to bare soil a week after rainfall events ($p=0.03$, $F_{1,29}=5.2$), which likely reduced plant stress and desiccation during drought. Compost incorporation can increase soil moisture and plant biomass in coarse textured, sandy road soils (Curtis and Claassen 2009), but we did not observe that it increased moisture or plant establishment. Of the soil treatments used, mulch blanket application alone and combined with polymer or compost caused the greatest increase in native seeded density (Chapter 2, Figure 7) and there was a slight trend that mulch + compost increased cover (Chapter 2, Table 1).

Increased N availability favored establishment and competitive ability of non-native annual species. Yard-waste compost increased mineral N in the field and plant growth in the greenhouse. In 2015, there were 83 cheatgrass plants/m^2 in compost treatments at site 5. In the greenhouse, although our non-native annual, cheatgrass, increased growth in compost compared to all other treatments, this was matched by the native annual grass, *E. elymoides*. Nitrophylic non-native perennials, like *B. inermis,* responded to this increased N with higher growth rate than native perennial grasses ($F_{1,61}$ =14.0, p=0.0003), like *E. canadensis*. Annuals respond to increased N with higher biomass than perennials (Marler and Claassen 1998, Lowe et al. 2003), but this is at greater amounts of increased N availability than this study was designed to test. For example, N addition increased foliar leaf N and biomass of invasive annual *B. tectorum* at significantly greater thresholds than perennial native *B. gracilis* (Lowe et al. 2003). In general, annual species respond to increased N with greater plasticity than perennial species.

To explore the effect of topographic features on plant establishment within the existing study framework, we ran a multivariate regression. For this model, predictor variables were treatment (amendment), aspect (south, south-east, east), percent slope (5-25%), and slope type (cut/fill). Response variables were either native or non-native density per m^2 in 2015. Non-native density was square root transformed to meet assumptions of normality. These data were not included in Chapter 2 because of our low field site replication, but further field data collection and multi-variate statistics could elucidate these trends.

Species establishment is highly dependent on topographic factors. Site aspect (p<0.001, $F_{3,3}$=18.7), percent slope (p=0.01, $F_{1,1}$=10.7), and slope type (p<0.001, $F_{1,1}$=33) influenced non-native plant density on roadsides more than amendment application (p=0.02, $F_{6,6}$=35.9) in 2015. The same trends were observed for native species establishment; using environmental co-variates like aspect, percent slope, and roadside type (cut/fill) improved the predictive ability of models for native and non-native density. East-facing roadsides had higher native density than south or south-west facing sites (p=0.0003, $F_{1,31}$=16.3). South-facing roadsides can have lower water potential and seedling germination than north-facing slopes (Bochet et al. 2007). Fill slopes had greater native density than cut slopes (p=0.025, $F_{1,31}$=5.5). South-facing slopes had much higher non-native species density than south-west, south-east or east facing sites (p=0.01, $F_{1,30}$=43.7). Non-native species densities averaged 26 plants/m^2 on fill slopes and 1.3 plants/m^2 on cut slopes (p=0.001, $F_{1,1}$=33).

Reduced N availability and increased soil moisture on Bear Lake road resulted in establishment of desired native plant communities. Water availability was limiting at field sites due to the sandy-loam soil texture, so germination in the field and greenhouse increased in response to higher soil water content. The highest seeded native plant density and cover occurred in the mulch and compost treatment, which significantly decreased N availability and increased water content in 2015. Amendments that decreased N availability, such as mulch and polymer also decreased non-native establishment.

By linking soil resource availability and plant community establishment after disturbance, we reveal best management practices that can increase biodiversity and ecosystem function. Our conclusions can apply to trails, roads, parking lots, and other sites with initial increase in N availability that favors establishment of invasive annual species. This increased N can be managed through species selection, topsoil management, and/or amendment selection and application. In addition, by increasing soil water holding capacity after disturbance, we can favor establishment of perennial native species (Hamilton et al. 1999). Once established, these perennials can outcompete non-native annuals despite changes in nutrient availability (Corbin and D'Antonio 2004, Marler and Claassen 1998, McGlone et al. 2012).

Management Considerations

The planning and decisions that determine management of topsoil, species selection, and soil resources can guide public agencies towards desirable restoration outcomes on roadsides.

Restoration goals and actions along roadsides should be site specific. These goals can consider the soils, species, and other environmental and social factors unique to the location. The soil texture, water holding capacity, and nutrients will define conditions for plant growth. Soils tested after construction could be compared to those from pre-construction for changes in organic matter content, carbon to nitrogen ratios, and plant-available N, as well as soil texture, bulk density, and rooting depth. Preexisting soil conditions can inform the selection of species for the seed mix and whether soil amendments, such as mulch, may be necessary to reduce growth of non-native species. To predict which invasive species may be a problem, roadside soils could be collected and the seedbank grown out in a greenhouse or under similar controlled conditions to identify the species in the existing seed bank. For example, some desirable native species may be known competitors of the resident non-natives, similar to the use of *E. elymoides* as a strong competitor with *B. tectorum* in the Bear Lake Rd. Phase II project.

In addition to soil testing, managers can analyze the proportion of the roadway that is south or east facing in comparison with north or west facing, and restore these differently. South-facing slopes will be warmer and have greater water stress that limits plant growth in water limited environments and are likely to benefit more from amendments like a wood mulch blanket. Warmer temperatures on south-facing slopes may lead to earlier germination of seed in the spring (Bochet et al. 2007). South-facing slopes are more likely to have higher non-native species density and mulch could suppress these species (Rhoades et al. 2011).

Topsoil management practices will improve outcomes for roadside restoration and other restoration projects. Topsoils from Bear Lake Rd. were stockpiled at the Upper Fisherman's parking lot and replaced on roadside soil to 10 cm depth (as in Claassen 2012), as available. For some portions of the roadway, topsoil was salvaged from Moraine Park, which has higher percentage of clay and organic matter (Claassen 2012). Planting stockpiled topsoil with annual cover crops can increase organic matter, microbial activity and decrease N availability, which will prepare the soil for use in restoration (Paschke 2014). Maintaining heterogeneity in application of topsoil by creating microsites can favor biodiversity composed of both annual and perennial native species (Molofsky and Augspurger 1992). Often topsoil management is not a priority for road contractors, which is why implementation of the topsoil management plan for each project is critical to achieve plant establishment.

Species can be carefully selected to create functionally intact communities that are resistant to invasion. The differences in phenology of species are an opportunity for directed restoration planning and methodology. Addition of annual species to perennial seed mixes can decrease exotic annual cover post-disturbance (Herron et al. 2013). There were no annual species seeded in this study, which is of concern for competition with annual invasives, like *B. tectorum,* although *E. elymoides* is a short-lived perennial which can act like an annual after disturbance. Establishment of annual seed into established perennial grasses can be difficult, but annual forbs can be established through transplanting (Brown and Bugg 2001). Leguminous N-fixing species in the seed mix can increase initial vegetation cover (Staab et al. 2015) and improve soil fertility.

Oxytropis lambertii compromised 11% of the seed mix and had very successful establishment on Bear Lake Rd. in 2014 and 2015.

In general, species richness can be reduced by the proportion of grass biomass on restored roadsides (Staab et al. 2015). In this study, *E. canadensis* and *E. elymoides* were the most abundant species observed because they made up 45% of the seed mix. These species were seeded at this rate to compete with *B. tectorum*, but their abundance may have reduced establishment of native forbs. By carefully timing phases of restoration and selecting species through research, it is much easier to achieve a diverse plant community.

Amendments can meet re-vegetation goals of species establishment, nutrient-cycling, or water availability. Soil amendments high in organic matter can reduce bulk density (Curtis and Claassen 2009), improve water holding capacity in damp conditions, and increase soil aggregation after disturbance (Brady and Weil 2004). Yard-waste compost is an amendment that is relatively high in organic matter and low in N (Yost 2012, Appendix 1, Fig 13) and is an alternative to compost made from manures, bio-solids or food waste feedstocks. Granitic field soils typically have very low N content, so incorporation of yard-waste compost could encourage establishment of non-natives (Curtis and Claassen 2012) that can benefit from increased N availability.

Amendments with recalcitrant carbon, like wood mulch, can reduce soil N (Rhoades et al. 2015), evaporation rates, and increase soil water content, depending on the application depth (Fornwalt and Rhoades 2011, Rhoades et al. 2015). Similar mulch application rates of 4-6 cm in the Front Range of Colorado have decreased ammonium and nitrate and increased seeded grass cover (Fornwalt and Rhoades 2011) in pile burn scar restoration. In contrast, thick 10 cm mulch application suppresses both native and non-native establishment through reduction of temperatures and soil N (Rhoades et al. 2015). Other studies have demonstrated no effect of 10 cm mulch blanket on seeded native density or cover (Elseroad et al. 2003). Rainfall patterns, landform, and soil texture of the field site will drastically change how mulch impacts soil nutrients and plant growth over time, so application rate should be adjusted to these factors. An alternative restoration method to mulch blanket as a surface amendment is mulch chip incorporation (Eldridge et al. 2012). Wood mulch incorporation can reduce bulk density (Sanborn et al. 2004), non-native establishment (Eldridge et al. 2012) and increase C:N ratio and native perennial cover (Brown et al. 2007).

Naturally, there are tradeoffs among management decisions, including the selection of soil amendments. Amendments may change soil temperature in addition to water and nutrient availability and microbial activity. Reduced soil temperature due to mulch may favor germination of specific functional groups. Warm-season (C4) grasses, which require warmer temperatures for germination, may germinate much later in mulch than cool season (C3) grasses (Lodge and Whalley 1981). Yet, in our greenhouse study, we observed similar germination and plant biomass in mulch for both cool and warm-season grasses.

The cost and feasibility of amendment application as a restoration method depends on the specific amendments that are chosen, the appropriate amendment and incorporation depth, the application method, and the local supplier’s price. A soil scientist, ecologist or botanist who understands plant-soil relationships would be able to find a local weed-free source of amendments and recommend what depth is warranted by the pre-construction soils testing.

The amendment application methods will determine installation and labor costs. Amendment application costs for our experiment are estimated (Appendix 1, Table 6) based on the purchase of compost, mulch and polymer at A1 Organics (Eaton, Co). In this restoration study, amendments were loaded into the back of a truck, transferred using three gallon buckets and spread across the surface of each treatment plot. A mechanized sprayer or skid loader would significantly reduce the labor hours required to amend soils.

Amendments are expensive, but there are creative solutions that could be used to obtain this organic material. Amendment application costs for the amendment combinations varied from slightly over $1,000/acre to nearly $8,000/acre. Mulch blanket (2.5 cm) application cost approximately $4,200/acre while yard-waste compost (1.2 cm) cost $1,050/acre. As a result, combined mulch and compost incorporation would cost approximately $5,250/acre. Amendment costs could be reduced through collaboration and outreach with invested partners. For example, mulch can be chipped from trees that were cut down to build the roadway or generated elsewhere during fuel management activities. Private supply companies might be willing to discount materials if they are able to advertise that it was used in a public restoration project.

Gaps in Scientific Knowledge

More research should be funded on the impacts of road corridors on native habitats in the United States. These findings could help determine priorities for conservation of roadside habitat. Many non-native species on road corridors have the potential to become invasive species, but only a few will have the seed dispersal capabilities to do so. Few studies are examining the potential for non-native species to become invasive or how restoration methods can prevent invasion. These studies should ideally be long-term (20+ years) and at multiple spatial scales. At a minimum, plant establishment trials should run 10 years, with sampling in year 1, 3, 5 and 10. Roadsides were surveyed in Australia to demonstrate abundance of naturalized invasives, agricultural weeds, and those that are dispersed by animals (Sullivan et al. 2009). In this study, they did not observe that roads were linear dispersal corridors, but reflected species composition of adjacent lands.

More understanding is needed about the mechanisms and processes that drive organic matter decomposition and how its water holding capacity changes with time. Little is known about the mechanisms for improved water holding due to organic matter decomposition and fractionation (Cotrufo et al. 2013). Organic matter results from dynamic abiotic and biotic cycling of detritus (Campbell and Paustian 2015). The rate of its decomposition is dependent on both form and composition of the organic material (Paul 1978). The environmental process that determine substrate availability to enzymes, which are a proxy for microbial activity, can be researched within the context of climate change (Davidson and Janssens 2006). This research can inform best management practices for management of soil organics in the face of climate change.

Climate change is predicted to change organic matter dynamics and speed decomposition of soil organic carbon by up to 10% (Hararuk et al. 2015). This loss of organic carbon will impact the function of water and nutrient cycling in terrestrial ecosystems. There has been a lot of research about global C and N cycling and impacts of the loss of permafrost. What is lacking is more work that examines how microbial activity and nutrient cycling are altered by interactions between disturbance, climate change, and change in species composition. Further studies should be conducted to understand how the lignin, phenol, C:N ratio, and surface area of carbon amendments impact decomposition rates and nutrient and water availability across ecosystem types. This modeling of microbial activity post-disturbance (Claassen 1993) can be achieved by monitoring soil temperature and moisture through time to model respiration rates. In addition, microbial C:N ratios, fungal:bacterial ratio, or microbial biomass can be used to assess the function of the microbial species community.

Another gap in scientific knowledge is how beneficial species relationships post-disturbance affect availability of water and nutrients. In arid ecosystems, plant species alter between facilitation and competition with other species, dependent on water availability (Jankju 2008).

This switch from beneficial to competitive relationships is also driven by changes in resource availability. As plant-essential nutrients become limiting, species share nutrients through arbuscular mycorrhizal fungi (Sylvia et al. 2005). For example, facultative beneficial relationships between *B. tectorum* and neighboring native grasses and forbs may be occurring throughout the growing season. When resources are abundant, there is no need for facultative relationships between species. Science that uncovers beneficial species relationships may be a key towards better management of both invasive and rare species.

In conclusion, changes in resource availability do direct successional outcomes of species establishment. This study demonstrates that soil amendments can manipulate resource availability and therefore competitive dynamics between non-native and non-native species. With pine duff wood mulch blanket, it is possible to decrease N and increase water availability to favor perennial native grasses over non-native species. By understanding mechanisms behind how germination, establishment and competition between plant species are tied to soil resources availability, we can find solutions in a changing world.

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APPENDIX 1: FIELD STUDY

Table 2. Field Study Hypotheses. Summary of the rationale behind each of our hypotheses, which response variables were compared, and the results observed are in yellow text.

Hypothesis	Comparisons	Expected Outcomes	Alternate Hypothesis	Alternate Outcomes
H 1: Polymer and/or compost applications will improve soil moisture and germination and establishment of native species.	Plant cover, density, and soil moisture in polymer (P), compost (Cm), mulch + compost (MCm) and mulch+polymer (MP) compared to control (Ct)	Native density and cover- P, Cm, MP, MCm > Ct Soil Moisture- P, Cm, MCm, MP > Ct	**H1B:** Increased soil moisture from compost and polymer will discourage establishment of native species. **H1C:** Increased soil moisture from compost and polymer not effect native species establishment.	Native cover and density- **H1B:** Cm , P < Ct **H1C:** Cm, P = Ct
H 2: Mulch application will increase native species establishment by reducing water loss from evaporation.	Plant cover, density, soil moisture in M, MCm ,and MPm compared to control Soil temperature in M, MCm compared to Ct	Native density and cover, soil moisture- M, MCm, MP > Ct Soil Temperature- M, MCm, MP < Ct	**H2B:** Mulch application will decrease germination and establishment of native species by decreasing water infiltration **H2C:** Mulch application will not affect native species establishment.	Native cover and density- **H2B:** M, MCm, MP < Ct **H2C:** M, MCm, MP = Ct
H 3: Mulch application will reduce available N and create a barrier to decrease invasive species establishment.	Non-native density and cover in M, MCm, Cm, MP compared to Ct	Non-native density and cover- M, MCm, MP < Ct Soil N- M, MCm, MP < Ct	**H3B:** Mulch application will increase both non-native and native establishment. **H3C:** Mulch application alone and combined with polymer reduces non-native density relative to compost/polymer.	Non-native density and cover- **H3B:** M, MCm and MP > Ct **H3C:** M, MP < Cm/P
H4: Compost will increase slowly-available N and native seeded establishment, but not non-native establishment.	seeded, non-seeded native, and non-native species cover and density, and soil cover and density, and soil water and plant available N in Cm, CmP, MCm compared to C	Native cover and density- Cm, MCm > Ct Soil moisture, soil N- Cm, CmP, MCm > Ct	**H4B:** Non-native species will benefit more than native seeded species from increased soil moisture and nitrogen in compost.	Non-native density and cover- **H4B**: Cm, CmP > M, M/P

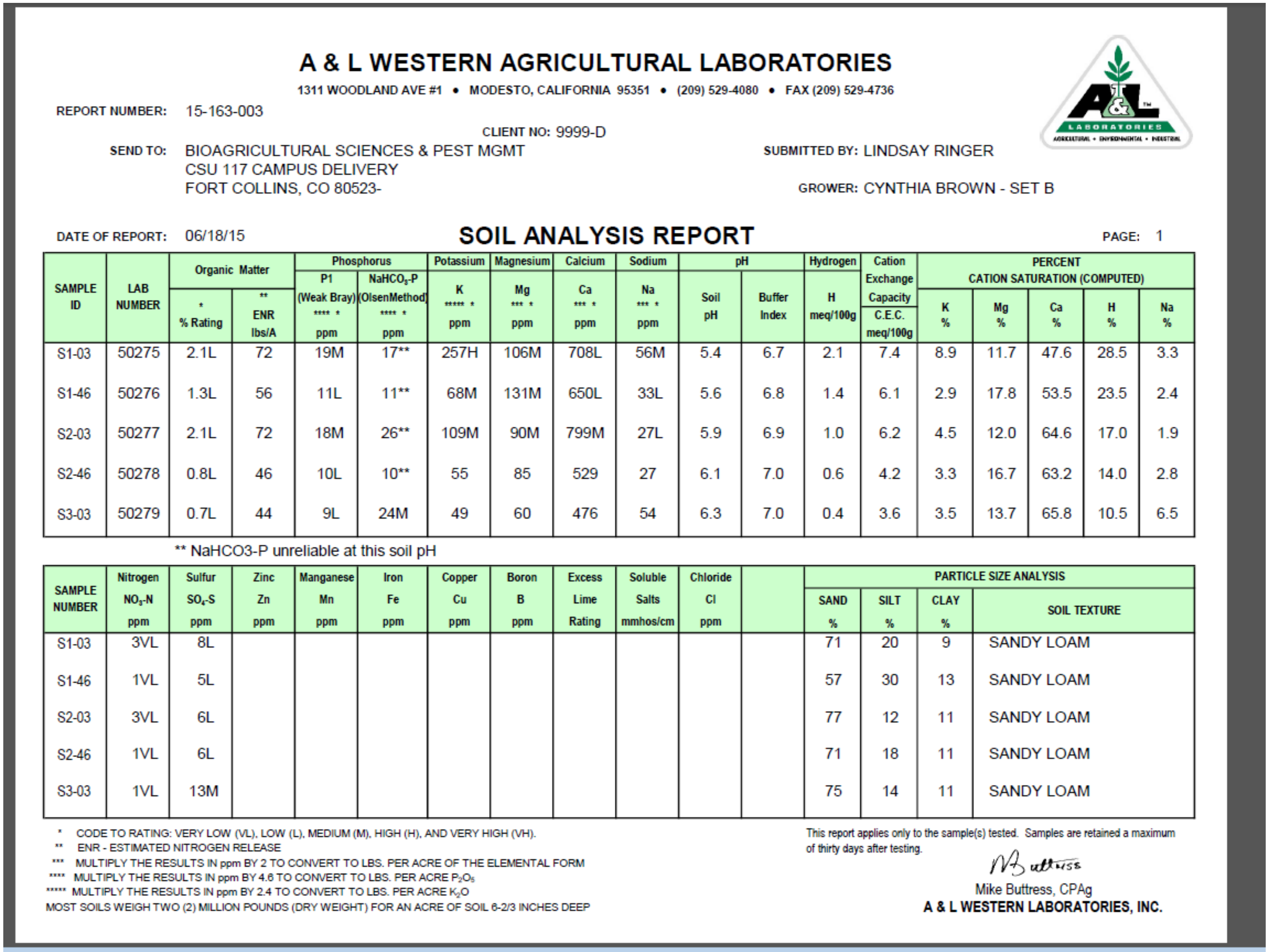
Table 3. Field Site Description. The site location, aspect, slope type, grade, declination, size, soil texture, elevation and estimated rooting depth.

Study Site	**Location**	**Aspect**	**Slope**	**Grade** (%)	**Decli-nation** (◦)	**Distance 1** (Perp, m)	**Distance 2** (Parallel, m)	**Area** (m^2)	**Texture** (% sand, silt, clay)	**Elevation** (meters)	**Rooting Depth** (cm)
1	Upper Road Obliteration	E/SE	cut	30	100	1.8	7.6	9.7	71:20: 9	2614	15-20
2	Realignment	SE	cut	35	145	1.5	6	8.13	77:12:11	2591	20-25
3	Lower Road Obliteration	SE	fill	10	125	3.3	4.8	10.2	75:14:11	2569	8-20
4	Hollowell Park	S	cut	23	200	1.2	9.1	6.5	77:12:11	2550	15-23
5	Moraine Park	S/SE	fill	25	200	2.4	7.6	10.2	77:12:11	2486	10-15
6	Moraine Park	SW	cut	30	150	1.2	6.7	6.7	75:14:11	2516	10-15

Table 4. Total Carbon and Nitrogen of Amendments with values for each replicate.

Sample ID	**Sample Amount (mg)**	**Weight [%] Nitrogen**	**Weight [%] Carbon**	**C:N**
polymer (1)	0.551	8.936	33.979	3.802484
polymer 2	0.493	9.087	35.075	3.85991
polymer 3	0.522	10.25	39.733	3.87639
compost 1	23.091	0.876	10.174	11.61416
compost 2	25.343	1.139	10.92	9.587357
compost 3	9.07	1.109	10.226	9.22092
Mulch:				
Mulch_5	2.965	0.417	23.338	55.96643
Mulch-6	3.544	0.497	28.314	56.96982
Mulch_7	3.332	0.341	19.549	57.32845

Table 5. Soil Analysis. Organic matter, nutrients, pH, CEC, and texture of field soils collected in Fall 2013 from 0-7.5 and 7.5-15 cm at site 1 (S1) through site 6 (S6) and where greenhouse soils were collected at glacier basin (GB).



A & L WESTERN AGRICULTURAL LABORATORIES

1311 WOODLAND AVE #1 • MODESTO, CALIFORNIA 95351 • (209) 529-4080 • FAX (209) 529-4736

REPORT NUMBER: 15-163-003

CLIENT NO: 9999-D

SEND TO: BIOAGRICULTURAL SCIENCES & PEST MGMT
CSU 117 CAMPUS DELIVERY
FORT COLLINS, CO 80523-

SUBMITTED BY: LINDSAY RINGER

GROWER: CYNTHIA BROWN - SET B

DATE OF REPORT: 06/18/15

SOIL ANALYSIS REPORT

PAGE: 1

SAMPLE ID	LAB NUMBER	Organic Matter		Phosphorus		Potassium	Magnesium	Calcium	Sodium	pH		Hydrogen	Cation Exchange Capacity	PERCENT CATION SATURATION (COMPUTED)				
		* % Rating	** ENR lbs/A	P1 (Weak Bray) **** * ppm	NaHCO$_3$-P (OlsenMethod) **** * ppm	K ***** * ppm	Mg *** * ppm	Ca *** * ppm	Na *** * ppm	Soil pH	Buffer Index	H meq/100g	C.E.C. meq/100g	K %	Mg %	Ca %	H %	Na %
S1-03	50275	2.1L	72	19M	17**	257H	106M	708L	56M	5.4	6.7	2.1	7.4	8.9	11.7	47.6	28.5	3.3
S1-46	50276	1.3L	56	11L	11**	68M	131M	650L	33L	5.6	6.8	1.4	6.1	2.9	17.8	53.5	23.5	2.4
S2-03	50277	2.1L	72	18M	26**	109M	90M	799M	27L	5.9	6.9	1.0	6.2	4.5	12.0	64.6	17.0	1.9
S2-46	50278	0.8L	46	10L	10**	55	85	529	27	6.1	7.0	0.6	4.2	3.3	16.7	63.2	14.0	2.8
S3-03	50279	0.7L	44	9L	24M	49	60	476	54	6.3	7.0	0.4	3.6	3.5	13.7	65.8	10.5	6.5

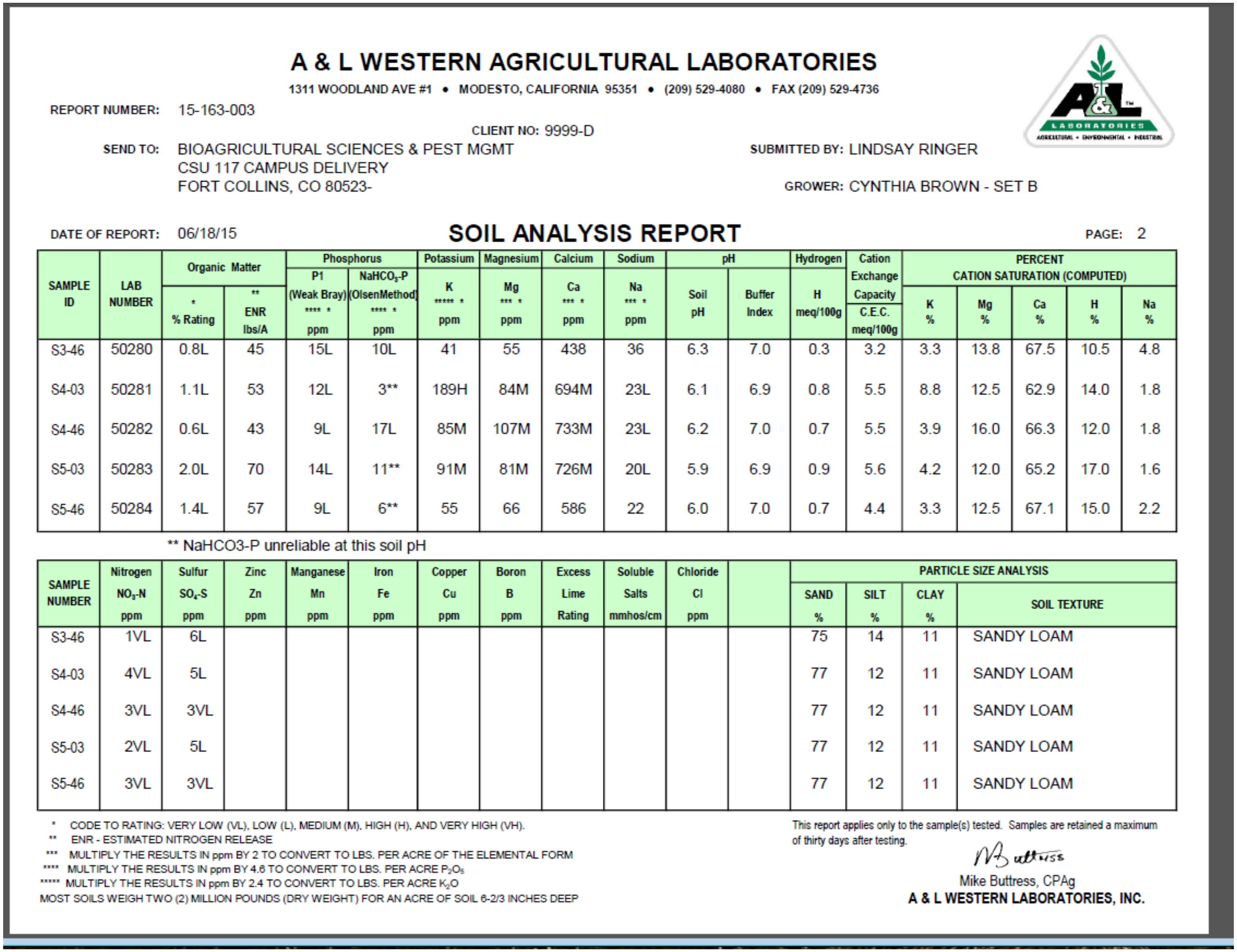
** NaHCO3-P unreliable at this soil pH

SAMPLE NUMBER	Nitrogen NO$_3$-N ppm	Sulfur SO$_4$-S ppm	Zinc Zn ppm	Manganese Mn ppm	Iron Fe ppm	Copper Cu ppm	Boron B ppm	Excess Lime Rating	Soluble Salts mmhos/cm	Chloride Cl ppm		PARTICLE SIZE ANALYSIS			
												SAND %	SILT %	CLAY %	SOIL TEXTURE
S1-03	3VL	8L										71	20	9	SANDY LOAM
S1-46	1VL	5L										57	30	13	SANDY LOAM
S2-03	3VL	6L										77	12	11	SANDY LOAM
S2-46	1VL	6L										71	18	11	SANDY LOAM
S3-03	1VL	13M										75	14	11	SANDY LOAM

* CODE TO RATING: VERY LOW (VL), LOW (L), MEDIUM (M), HIGH (H), AND VERY HIGH (VH).
** ENR - ESTIMATED NITROGEN RELEASE
*** MULTIPLY THE RESULTS IN ppm BY 2 TO CONVERT TO LBS. PER ACRE OF THE ELEMENTAL FORM
**** MULTIPLY THE RESULTS IN ppm BY 4.6 TO CONVERT TO LBS. PER ACRE P_2O_5
***** MULTIPLY THE RESULTS IN ppm BY 2.4 TO CONVERT TO LBS. PER ACRE K_2O
MOST SOILS WEIGH TWO (2) MILLION POUNDS (DRY WEIGHT) FOR AN ACRE OF SOIL 6-2/3 INCHES DEEP

This report applies only to the sample(s) tested. Samples are retained a maximum of thirty days after testing.

M Buttress
Mike Buttress, CPAg
A & L WESTERN LABORATORIES, INC.



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FORT COLLINS, CO 80523-

SUBMITTED BY: LINDSAY RINGER

GROWER: CYNTHIA BROWN - SET B

DATE OF REPORT: 06/18/15

SOIL ANALYSIS REPORT

PAGE: 2

SAMPLE ID	LAB NUMBER	Organic Matter		Phosphorus		Potassium	Magnesium	Calcium	Sodium	pH		Hydrogen	Cation Exchange Capacity	PERCENT CATION SATURATION (COMPUTED)				
		* % Rating	** ENR lbs/A	P1 (Weak Bray) **** * ppm	$NaHCO_3$-P (OlsenMethod) **** * ppm	K ***** * ppm	Mg *** * ppm	Ca *** * ppm	Na *** * ppm	Soil pH	Buffer Index	H meq/100g	C.E.C. meq/100g	K %	Mg %	Ca %	H %	Na %
S3-46	50280	0.8L	45	15L	10L	41	55	438	36	6.3	7.0	0.3	3.2	3.3	13.8	67.5	10.5	4.8
S4-03	50281	1.1L	53	12L	3**	189H	84M	694M	23L	6.1	6.9	0.8	5.5	8.8	12.5	62.9	14.0	1.8
S4-46	50282	0.6L	43	9L	17L	85M	107M	733M	23L	6.2	7.0	0.7	5.5	3.9	16.0	66.3	12.0	1.8
S5-03	50283	2.0L	70	14L	11**	91M	81M	726M	20L	5.9	6.9	0.9	5.6	4.2	12.0	65.2	17.0	1.6
S5-46	50284	1.4L	57	9L	6**	55	66	586	22	6.0	7.0	0.7	4.4	3.3	12.5	67.1	15.0	2.2

** NaHCO3-P unreliable at this soil pH

SAMPLE NUMBER	Nitrogen NO_3-N ppm	Sulfur SO_4-S ppm	Zinc Zn ppm	Manganese Mn ppm	Iron Fe ppm	Copper Cu ppm	Boron B ppm	Excess Lime Rating	Soluble Salts mmhos/cm	Chloride Cl ppm		PARTICLE SIZE ANALYSIS			
												SAND %	SILT %	CLAY %	SOIL TEXTURE
S3-46	1VL	6L										75	14	11	SANDY LOAM
S4-03	4VL	5L										77	12	11	SANDY LOAM
S4-46	3VL	3VL										77	12	11	SANDY LOAM
S5-03	2VL	5L										77	12	11	SANDY LOAM
S5-46	3VL	3VL										77	12	11	SANDY LOAM

* CODE TO RATING: VERY LOW (VL), LOW (L), MEDIUM (M), HIGH (H), AND VERY HIGH (VH).
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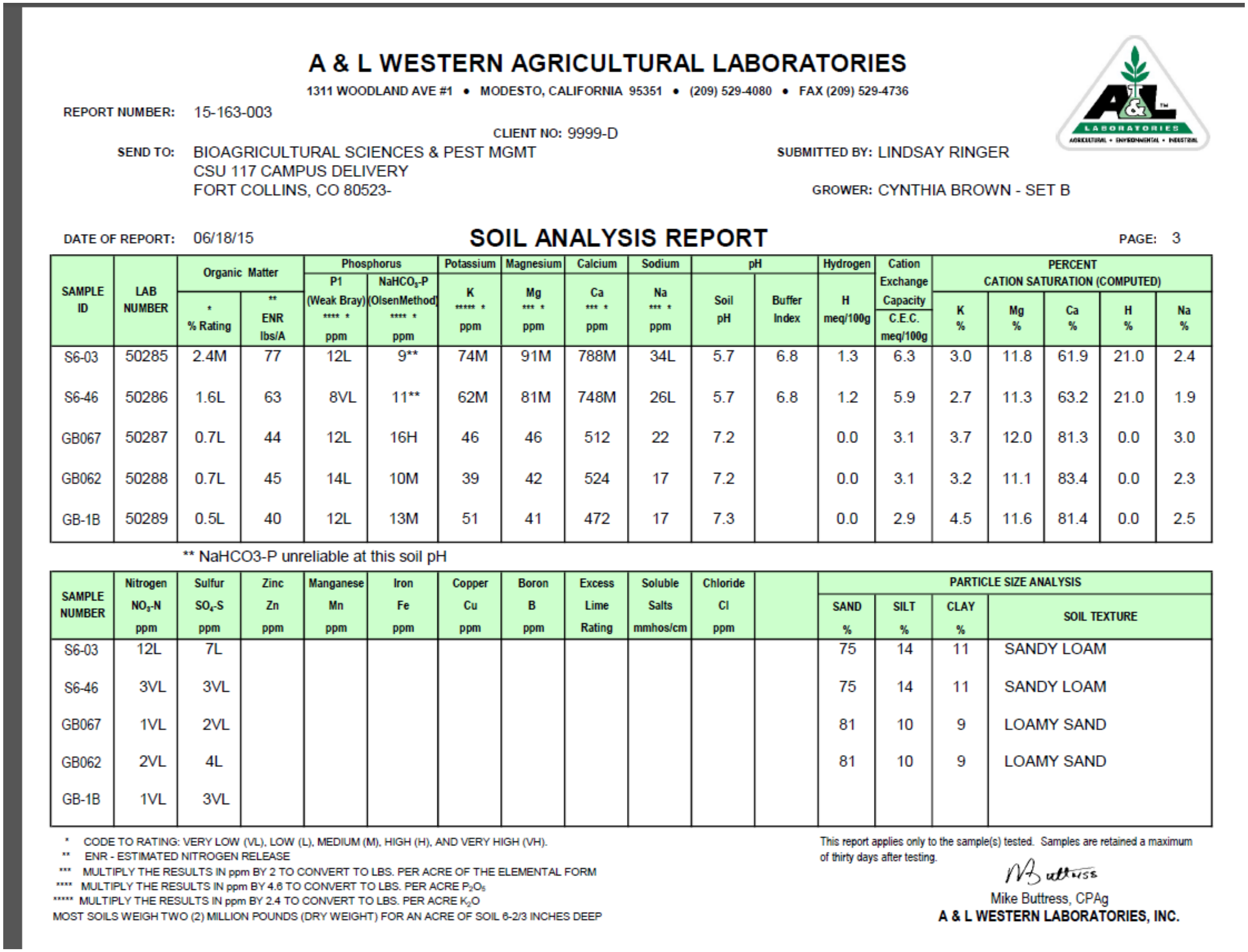
Mike Buttress, CPAg
A & L WESTERN LABORATORIES, INC.



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FORT COLLINS, CO 80523-

SUBMITTED BY: LINDSAY RINGER

GROWER: CYNTHIA BROWN - SET B

DATE OF REPORT: 06/18/15

SOIL ANALYSIS REPORT

PAGE: 3

SAMPLE ID	LAB NUMBER	Organic Matter		Phosphorus		Potassium	Magnesium	Calcium	Sodium	pH		Hydrogen	Cation Exchange Capacity	PERCENT CATION SATURATION (COMPUTED)				
		* % Rating	** ENR lbs/A	P1 (Weak Bray) **** * ppm	NaHCO$_3$-P (OlsenMethod) **** * ppm	K ***** * ppm	Mg *** * ppm	Ca *** * ppm	Na *** * ppm	Soil pH	Buffer Index	H meq/100g	C.E.C. meq/100g	K %	Mg %	Ca %	H %	Na %
S6-03	50285	2.4M	77	12L	9**	74M	91M	788M	34L	5.7	6.8	1.3	6.3	3.0	11.8	61.9	21.0	2.4
S6-46	50286	1.6L	63	8VL	11**	62M	81M	748M	26L	5.7	6.8	1.2	5.9	2.7	11.3	63.2	21.0	1.9
GB067	50287	0.7L	44	12L	16H	46	46	512	22	7.2		0.0	3.1	3.7	12.0	81.3	0.0	3.0
GB062	50288	0.7L	45	14L	10M	39	42	524	17	7.2		0.0	3.1	3.2	11.1	83.4	0.0	2.3
GB-1B	50289	0.5L	40	12L	13M	51	41	472	17	7.3		0.0	2.9	4.5	11.6	81.4	0.0	2.5

** NaHCO3-P unreliable at this soil pH

SAMPLE NUMBER	Nitrogen NO$_3$-N ppm	Sulfur SO$_4$-S ppm	Zinc Zn ppm	Manganese Mn ppm	Iron Fe ppm	Copper Cu ppm	Boron B ppm	Excess Lime Rating	Soluble Salts mmhos/cm	Chloride Cl ppm		PARTICLE SIZE ANALYSIS			
												SAND %	SILT %	CLAY %	SOIL TEXTURE
S6-03	12L	7L										75	14	11	SANDY LOAM
S6-46	3VL	3VL										75	14	11	SANDY LOAM
GB067	1VL	2VL										81	10	9	LOAMY SAND
GB062	2VL	4L										81	10	9	LOAMY SAND
GB-1B	1VL	3VL													

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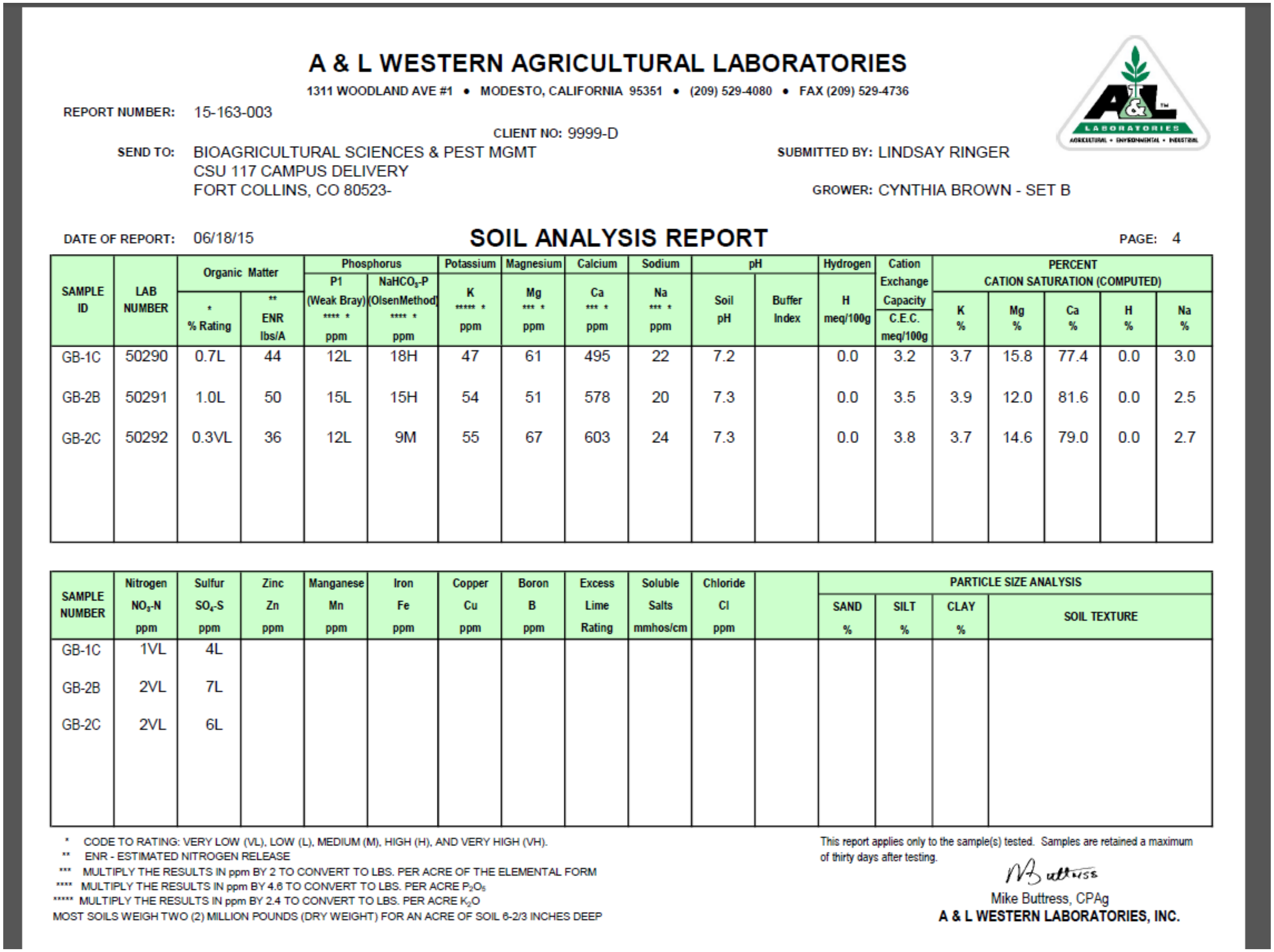

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FORT COLLINS, CO 80523-

SUBMITTED BY: LINDSAY RINGER

GROWER: CYNTHIA BROWN - SET B

DATE OF REPORT: 06/18/15

SOIL ANALYSIS REPORT

PAGE: 4

SAMPLE ID	LAB NUMBER	Organic Matter		Phosphorus		Potassium	Magnesium	Calcium	Sodium	pH		Hydrogen	Cation Exchange Capacity	PERCENT CATION SATURATION (COMPUTED)				
		* % Rating	** ENR lbs/A	P1 (Weak Bray) **** * ppm	$NaHCO_3$-P (OlsenMethod) **** * ppm	K ***** * ppm	Mg *** * ppm	Ca *** * ppm	Na *** * ppm	Soil pH	Buffer Index	H meq/100g	C.E.C. meq/100g	K %	Mg %	Ca %	H %	Na %
GB-1C	50290	0.7L	44	12L	18H	47	61	495	22	7.2		0.0	3.2	3.7	15.8	77.4	0.0	3.0
GB-2B	50291	1.0L	50	15L	15H	54	51	578	20	7.3		0.0	3.5	3.9	12.0	81.6	0.0	2.5
GB-2C	50292	0.3VL	36	12L	9M	55	67	603	24	7.3		0.0	3.8	3.7	14.6	79.0	0.0	2.7

SAMPLE NUMBER	Nitrogen NO_3-N ppm	Sulfur SO_4-S ppm	Zinc Zn ppm	Manganese Mn ppm	Iron Fe ppm	Copper Cu ppm	Boron B ppm	Excess Lime Rating	Soluble Salts mmhos/cm	Chloride Cl ppm		PARTICLE SIZE ANALYSIS			
												SAND %	SILT %	CLAY %	SOIL TEXTURE
GB-1C	1VL	4L													
GB-2B	2VL	7L													
GB-2C	2VL	6L													

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M Buttress

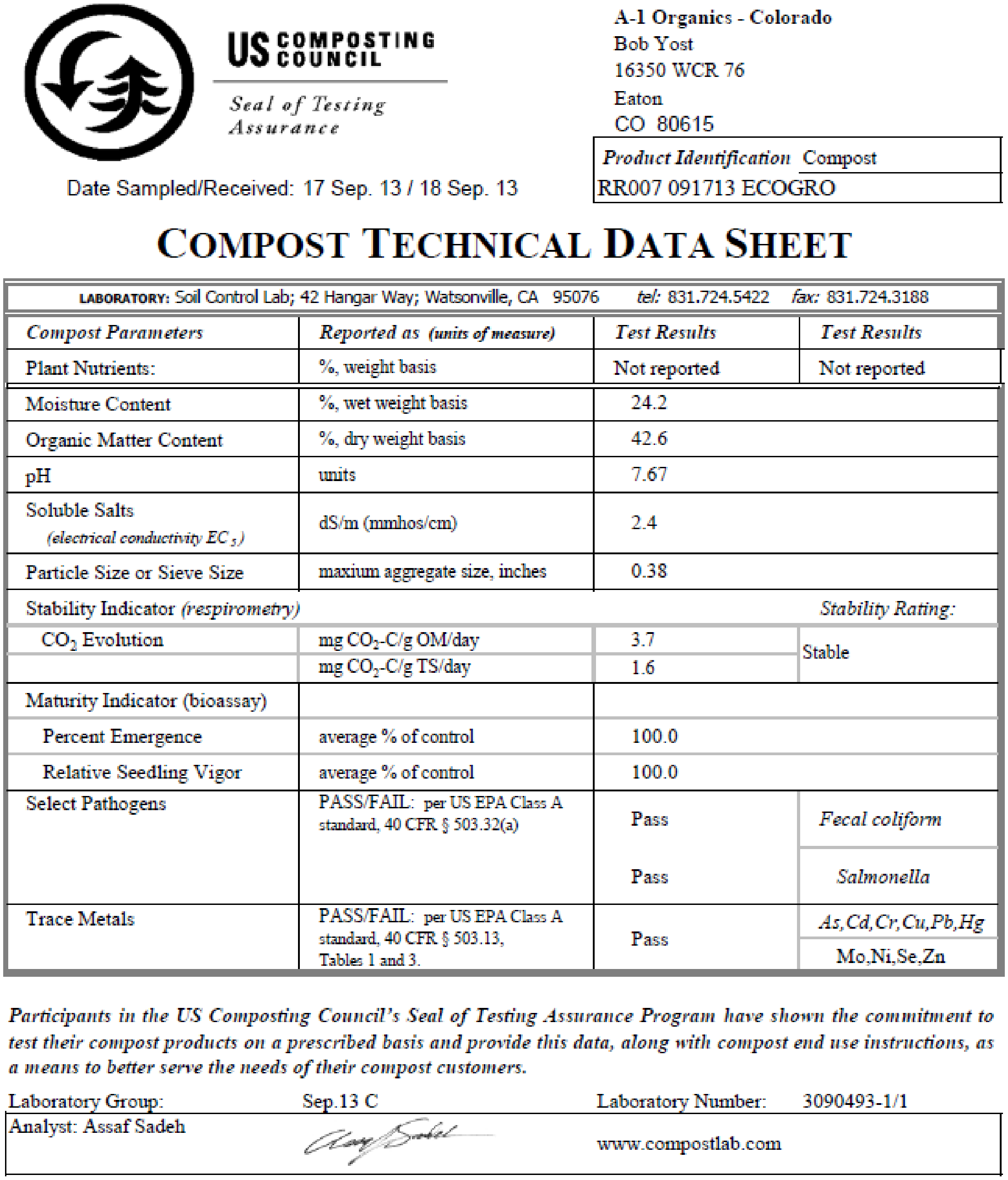
Mike Buttress, CPAg
A & L WESTERN LABORATORIES, INC.

Table 6. Estimated application costs per acre are based on application rates per 92 m^2 (1000 ft^2), which is the total area to which each amendment was applied on Bear Lake Road.

Wood mulch (2.5 cm depth)	2.5 m^3	3 yd^3	\$32/yd^3	\$4,181.76	350
Compost (1.3 cm depth)	1.25 m^3	1.5 yd^3	\$16/yd^3	\$1,045.44	350
Polymer	492 grams	1 pound	\$86.95/10 lbs.	\$3,787.54	262
Mulch + Compost	2.5 m^3 + 1.2m^3	3 yd^3 + 1.5 yd^3	\$48/ 1.5 yd^3	\$5,227.10	700
Mulch + Polymer	2.5 m^3 + 492 g	3 yd^3 + 1 lb.	\$118.95	\$7,969.30	611

Material:	pine mulch	yard-waste compost	polyacrylamide gel
Apl Rate:	2.5cm	1.25 cm	490 g/m^2
Method:	blanket	incorporated	incorporated
Depth:	N/A	10 cm	10 cm
C:N:	57:1	10:1	4:1

Figure 12 Photograph of pine-duff wood mulch, yard-waste compost, and super-absorbent polymer applied to field and greenhouse studies.



US COMPOSTING COUNCIL
Seal of Testing Assurance

A-1 Organics - Colorado
Bob Yost
16350 WCR 76
Eaton
CO 80615

Product Identification	Compost
RR007 091713 ECOGRO	

Date Sampled/Received: 17 Sep. 13 / 18 Sep. 13

COMPOST TECHNICAL DATA SHEET

LABORATORY: Soil Control Lab; 42 Hangar Way; Watsonville, CA 95076 *tel:* 831.724.5422 *fax:* 831.724.3188

Compost Parameters	*Reported as (units of measure)*	*Test Results*	*Test Results*
Plant Nutrients:	%, weight basis	Not reported	Not reported
Moisture Content	%, wet weight basis	24.2	
Organic Matter Content	%, dry weight basis	42.6	
pH	units	7.67	
Soluble Salts *(electrical conductivity EC_5)*	dS/m (mmhos/cm)	2.4	
Particle Size or Sieve Size	maxium aggregate size, inches	0.38	
Stability Indicator *(respirometry)*			*Stability Rating:*
CO_2 Evolution	mg CO_2-C/g OM/day	3.7	Stable
	mg CO_2-C/g TS/day	1.6	
Maturity Indicator (bioassay)			
Percent Emergence	average % of control	100.0	
Relative Seedling Vigor	average % of control	100.0	
Select Pathogens	PASS/FAIL: per US EPA Class A standard, 40 CFR § 503.32(a)	Pass	*Fecal coliform*
		Pass	*Salmonella*
Trace Metals	PASS/FAIL: per US EPA Class A standard, 40 CFR § 503.13, Tables 1 and 3.	Pass	*As,Cd,Cr,Cu,Pb,Hg*
			Mo,Ni,Se,Zn

Participants in the US Composting Council's Seal of Testing Assurance Program have shown the commitment to test their compost products on a prescribed basis and provide this data, along with compost end use instructions, as a means to better serve the needs of their compost customers.

Laboratory Group:	Sep.13 C	Laboratory Number:	3090493-1/1
Analyst: Assaf Sadeh		www.compostlab.com	

Figure 13 Yard-waste compost analytics conducted by contract for A1 Organics in Eaton, CO.

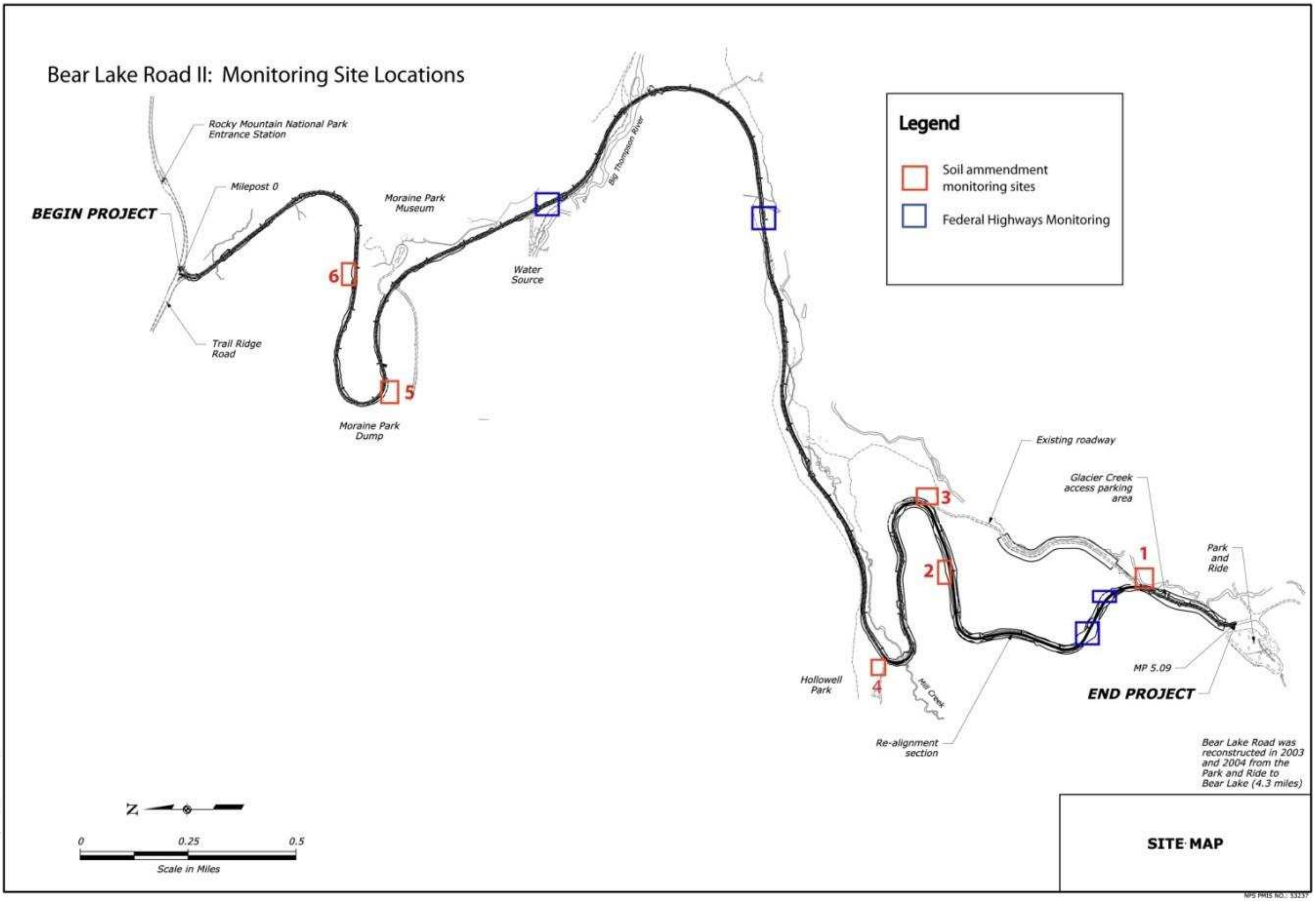


Figure 14 Two sites were chosen at each of three elevation ranges. Sites 5 and 6 in Moraine Park are from 2,470 – 2,500 m (8,100-8,200 ft), Sites 3 and 4 near Hollowell Park are from 2,530-2,560 m (8,300-8,400 ft) and sites 1 and 2 at the road re-alignment are from 2,590- 2,620 m (8,500-8,600 ft).

Table 7. Native, hydro-seeded species with scientific name, common name, percent composition, functional group, rooting, and phenology characteristics.

Species	Common Name	%	Habit	Functional group	Rooting	Season	Other
Antennaria spp.	Pussytoes	1.0	Perennial	Forb	Stolons, veg. reprod.	May-July	
Artemisia frigida	Fringed sagebrush	6.0	Perennial	subshrub	Taproot, grows adventitious roots	July-Oct	
Bouteloua gracillis	Blue grama	6.25	C4 grass	Grass	Sod forming with tillers, 3-6' rooting depth	July-Oct	C4
Elymus canadensis	Canada wildrye	22.5	perennial	Grass	Fibrous, symbiotic mycorrhizae	June-Sept	C3
Elymus elymoides	Bottlebrush squirreltail	22.5	C3 grass	Grass	fibrous	May-Aug	C3
Koeleria macrantha	Junegrass	14.0	C3 grass	Grass	fibrous	May-Aug	C3
Muhlenbergia montana	Mountain muhly	14.6	C4 grass	Grass	fibrous	July-Sept	C4
Heterotheca villosa	Harry golden aster	1.5	Perennial forb	Forb	taproot	May-Oct	
Oxytropis lambertii	Purple locoweed	10.8	Perennial forb	Forb	Taproot	April-Aug	N-fixing
Thermopsis divaricarpa	Foothill golden banner	0.08	Perennial forb	Forb	Taproot	May-Aug	N-fixing

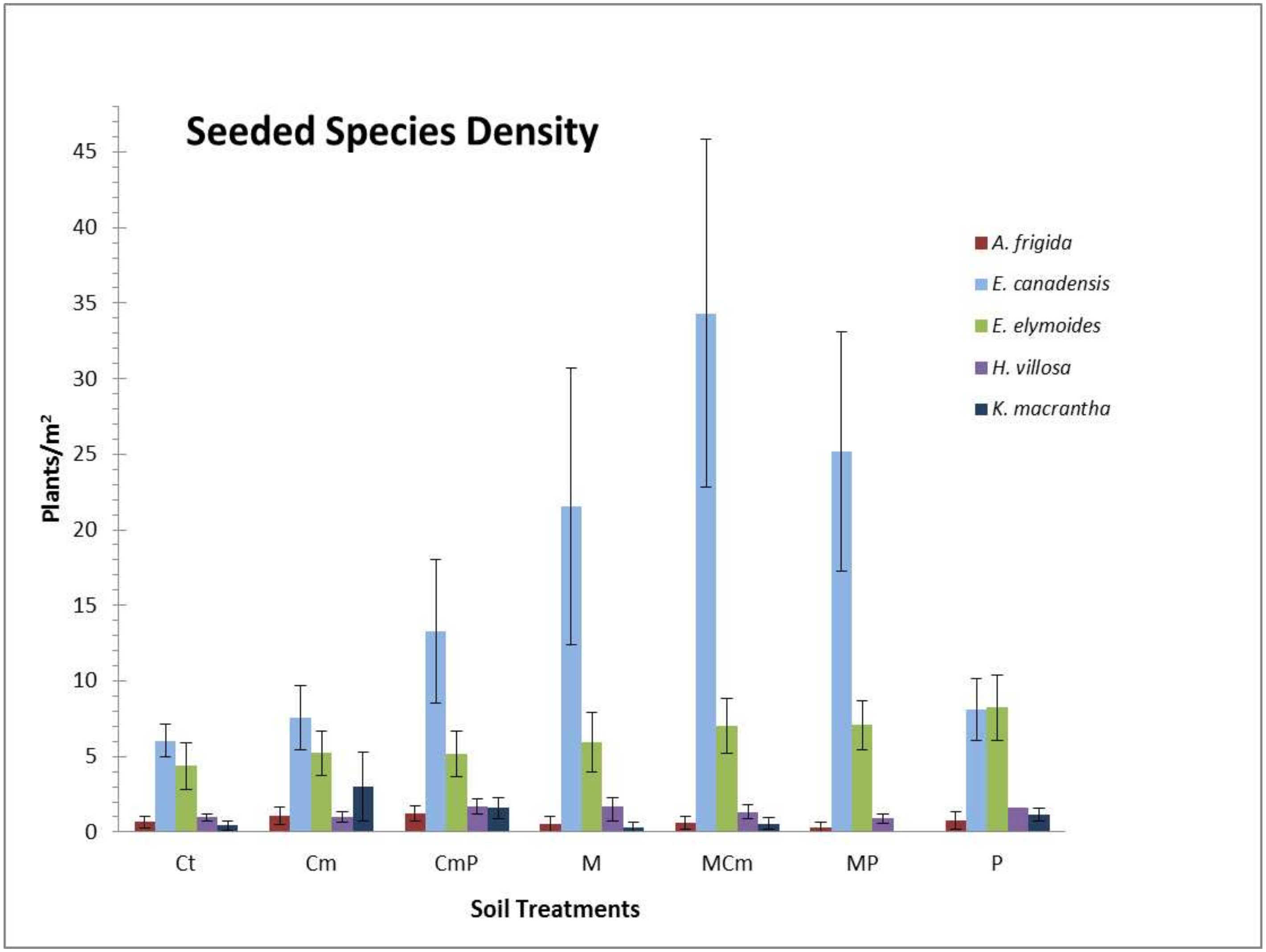


Figure 15 Seeded Density by Species. Mean plant number per m^2 ± standard error of the mean observed in summer 2014. Most species observed in the first year were grasses, such as bottlebrush squirreltail (*E. elymoides*) and Canada wild-rye (*E. Canadensis*). Seeded species not shown (pussytoes, mountain muhly, golden banner and locoweed) were not in a large enough abundance at all sites to be graphed here.

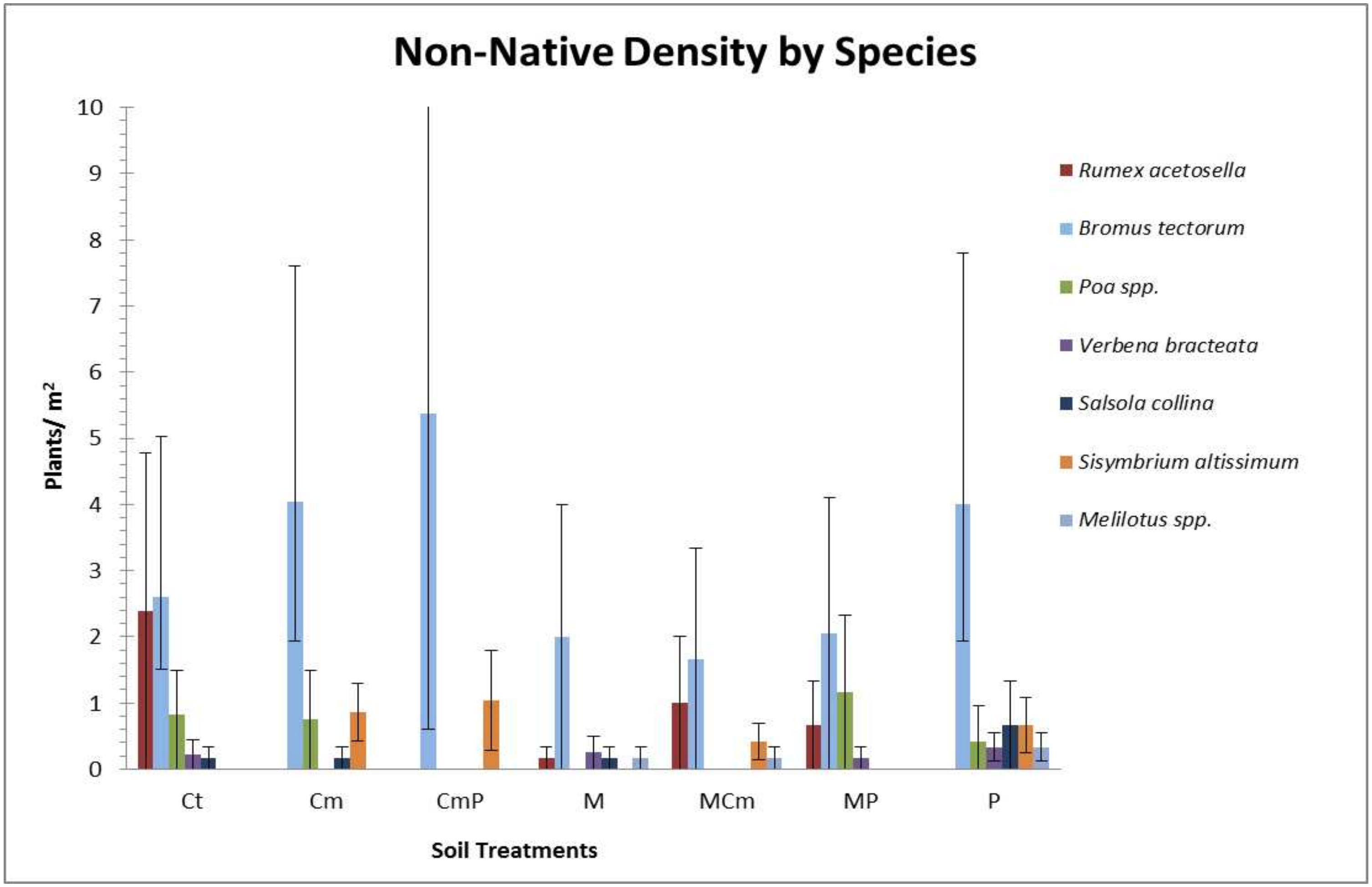


Figure 16 Non-Native Density by Species. The number of non-native species in 2014 per m^2 ± standard error of the mean. Cheatgrass (*Bromus tectorum*) and sheep sorrel (*Rumex acetosella*) were the most abundant non-native species observed at Bear Lake Rd. restoration sites in 2014. There was a trend that non-native density was lower in mulch treatments. Treatments from left to right are control, compost, compost + polymer, mulch, mulch + compost, mulch + polymer, and polymer.

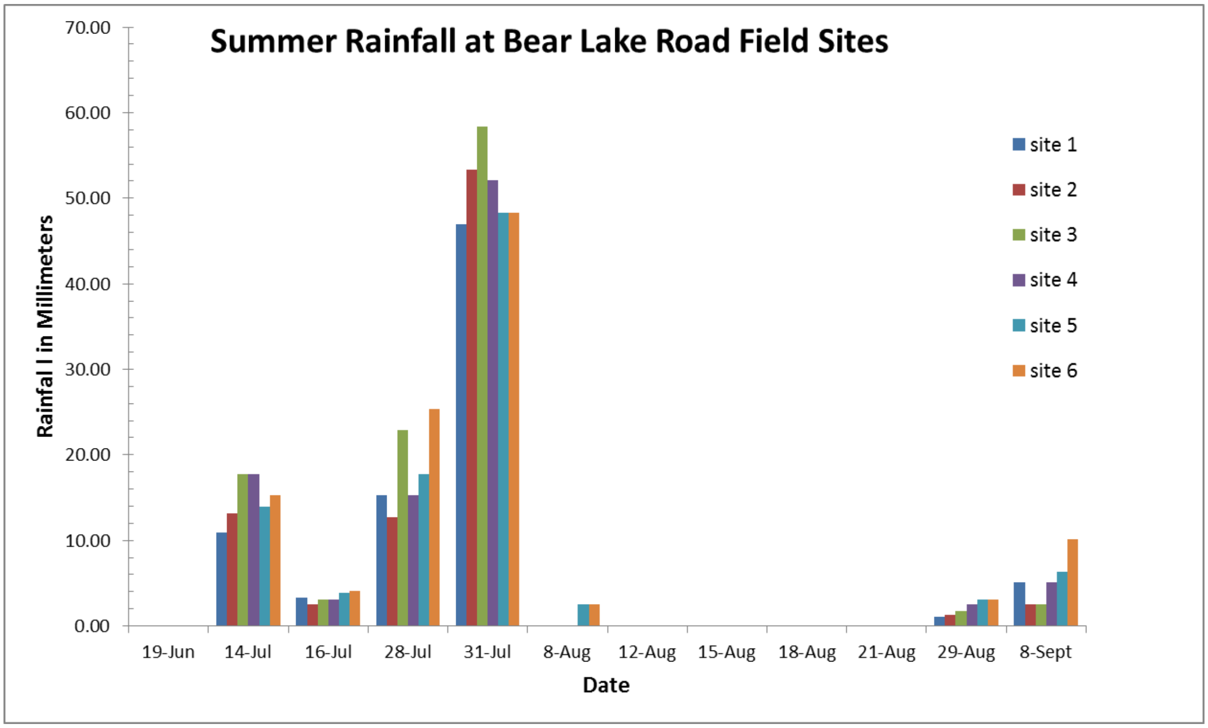


Figure 17 Rainfall measured in Taylor gauges at each site in Jun through September 2014.

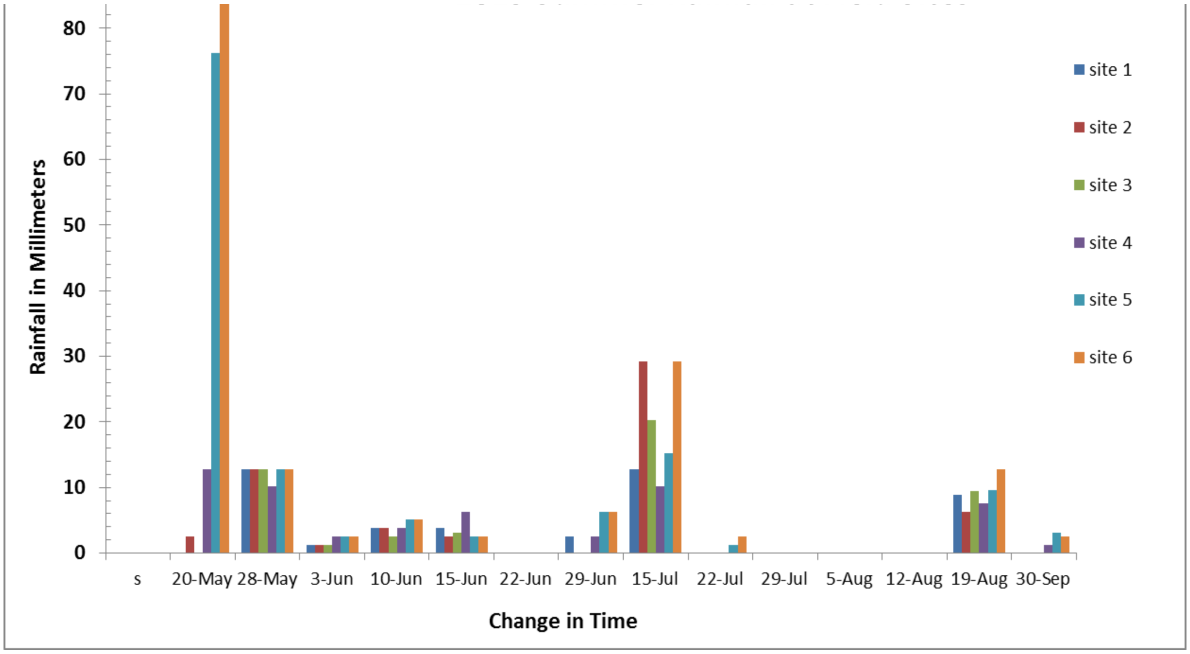


Figure 18 Rainfall measured in Taylor rain gauges in June through September 2015. Gauges at site 1-4 broke from freezing in winter 2014 and were replaced in early June.

APPENDIX 2: GREENHOUSE STUDY

Table 8. Hypotheses of Greenhouse Study. Summary of the rationale behind each of our hypotheses, how plant growth response variables can be compared, and the results we expect if our hypotheses or their alternatives are supported.

Hypotheses	**Comparisons**	**Expected Outcomes**	**Alternative Hypothesis**	**Outcomes**
H 1: Polymer incorporation will decrease soil N and increase soil water, favoring native over non-native establishment.	Plant growth rate, biomass, and relative yield total in polymer (P) relative to control (Ct)	Native growth, biomass, RYT, RII in comparison to non-native 1) P > Ct	*H1B: Improved soil moisture and decreased N from polymer will discourage establishment of native species.* *H1C: Improved soil moisture from polymer will encourage establishment of non-natives.*	1B) Native species growth rate, biomass, RYT Ct > P 1C) Non-native species growth rate, biomass, RYT P > Ct
H 2: Compost incorporation will increase soil N and increase soil water, favoring non-native growth over native.	Plant growth rate, biomass, and relative yield total in Compost (Cm) relative to control (Ct)	Non-native growth, biomass, RYT, RII in comparison to native 1) Cm > Ct	*H2B: Increased soil N and water from compost will discourage establishment of non-native species.* *H2C: Compost application will increase establishment of natives relative to non-natives.*	2B) Non-native species growth rate, biomass, etc. Ct > Cm 2C) Native growth, biomass, etc. in comparison to non-native Cm > Ct
H 3: Mulch application will decrease soil N and increase soil water, favoring native over non-native establishment.	Plant growth rate, biomass, and relative yield total in mulch (M) relative to control (Ct)	Native growth rate, biomass, RYT, RII in comparison to non-native 1) M > Ct	*H3B: Mulch application will increase both non-native and native establishment relative to control.* *H3C: Mulch application will have no effect on non-native growth.*	3B) All species growth rate, biomass, etc. M > Ct 3C) Non-native growth rate, biomass, etc. M=Ct

Figure 19 Spring 2015 soil collection and sieving at Glacier Basin, Rocky Mountain National Park.

Figure 20 Plants were harvested from 12 in (20 cm) tall pots eight weeks after planting. We measured plant height but also dried and weighed grass roots and shoots to determine total biomass of each species in each experimental unit.