Improving forb establishment and restoring soil function in disturbed landscapes: Hitchhiking native forbs with white spruce

by

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Abstract

Changing requirements for land reclamation in Alberta has led to the need for revegetation of disturbed lands with native woody and herbaceous species. Our study involves "Hitchhiker Planting" which is similar to companion cropping in agriculture, with the goal of improving native forb establishment on reclamation sites through mixed-species plugs. This research examined growing white spruce (*Picea glauca*) in plugs with either fireweed (*Chamerion angustifolium*) or showy aster (*Eurybia conspicua*). The primary objective of this study was to determine if we could produce a mixed-species container stock that is comparable or better than single species stock in terms of spruce development without negatively affecting forb development. The secondary objectives were to study if the presence of a forb had an effect on vegetation dynamics aboveground, or microbial function belowground; with the comparison of microbial parameters to other landscape-level disturbance types (wildfire and forest harvesting). The main goal of developing a successful mixed-species (hitchhiker) stock type that did not inhibit spruce growth was successful. In general, the stock types in larger containers (615A) and where the forb was introduced 10 weeks or more after the spruce resulted in spruce growth that was comparable or greater than our standard white spruce stock. These stock types also demonstrated consistent performance in the hitchhiked native forb. A reduction in undesirable species cover was not statistically significant, but developing trends indicate we will see future significant effects. Belowground, no consistent hitchhiker effect was observed, which was potentially due to the vegetation community not having had sufficient time to impact the microbial community. Community level physiological profiles however are supported as an effective method for using microbial function to differentiate between sites based on disturbance level. Site conditions and characteristics were an important factor for all soil microbial parameters, with disturbance type having a notable effect, as expected. The potential impact of manganese on sites recovering from industrial disturbance warrants further investigation.

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List of Abbreviations

AIC: Akaike Information Criteria
ANOVA: Analysis of Variance
AS: Showy Aster
B: Boron
Ca: Calcium
CLPP: Community Level Physiological Profiles
Cu: Copper
DNA: Deoxyribonucleic Acid
EC: Electrical Conductivity
Fe: Iron
FW: Fireweed
K: Potassium
LFH: Litter-Folic-Humic Materal
MB-C: Microbial Biomass Carbon
MB-N Microbial Biomass Nitrogen
Mg: Magnesium
Mn: Manganese
MQ: Metabolic Quotient
MRPP: Multiple Response Permutations Procedure
MSIR: Multi-Substrate Induced Respiration
N: Nitrogen
NAIT: Northern Alberta Institute of Technology
NMDS: Non-Metric Multi-Dimensional Scaling
OSE: Oil Sands Exploration
P: Phosphorus
Pb: Lead
PCA: Principal Component Analysis
pH: Power of Hydrogen
RCD: Root Collar Diameter
S: Sulphur
Zn: Zinc

Chapter 1 – Introduction

1.1. Stand-level disturbances within the boreal forest

The boreal forest stretches across North America and Eurasia, generally north of the 50th parallel. It is the largest vegetation zone in Canada, covering 55% of the land mass from British Columbia and Yukon to Newfoundland and Labrador (La Roi & James-Abra, 2017). In Alberta, the mean annual temperature is near 0°C with an annual average precipitation of around 480mm (Alberta Parks, 2017). The primary stand-level disturbances within the boreal forest in Alberta are wildfires, forest harvesting, industrial disturbances (oil and gas development and mining) and insect outbreaks (Natural Resources Canada, 2017; Smreciu et al., 2003).

Wildfire is the dominant disturbance regime in the boreal forest, and is a necessary component, as it allows for forest renewal, nutrient release, canopy opening and growth stimulation (Natural Resources Canada, 2017). Serotinous cones (resin sealed, open following wildfire) of certain overstory trees, such as *Pinus contorta* (lodgepole pine) and *Pinus banksiana* (jack pine) are an example of one such adaptation to this disturbance regime. The other common species of the boreal forest, *Populous tremuloides* (aspen) reproduce primarily through suckering and wind dispersion of seeds; and *Picea glauca and mariana* (white and black spruce, respectively) through seeds distributed by cones during large masting events (Greene et al., 1998). Once a site is burned there is typically a flush of *Chamerion angustifolium* (fireweed), followed by intense growth of shrubs and suckering aspen, which is usually, but not always, replaced by a coniferous canopy (Rowe, 1983; Weber & Stocks, 1998). Peters et al. (2005) found that when a wildfire occurs in a mast year, the white spruce regeneration is significantly higher than that found in a non-mast year. Based on the number of squirrel pelts exported in 2011 (Alberta Trappers, 2012) it can be reasonably assumed that 2011 was a mast year in Alberta. In some instances of when fire occurred in a non-mast year, pine regeneration was present even though pine was not noted in the pre-disturbance over story (Peters et al., 2005), which delayed regeneration of white spruce 38-44 years after the fire, predominately on fire killed logs, as the quality of the seed bed quickly degrades. The higher the severity of the burn is, the higher the initial quality of the seedbed (Johnstone & Chapin, 2006); and the greater the period of ideal germination conditions. This seed bed creation is from the exposure of mineral soil due to intense fire (Greene et al., 2005), and generally allows for a correlation between fire intensity and post fire conifer abundance.

Forestry in Alberta is an important industry that has been present on the landscape since the early 1900's (Millar Western, 2017). Typical forestry operations consist of clear cutting, with small retention patches (Serrouya & D'Eon, 2004). Soil disturbance is primarily compaction, with the greatest increase in bulk density along the block roads (Block et al., 2002), and the main overall impact being the removal of over storywoody vegetation. Although an anthropogenic disturbance, forest harvesting has been shown to be similar to the effects of fire (Kishchuk et al., 2015). Hannam et al. 2006 showed that white spruce and a mixed aspen-white spruce forest microbial biomass and microbial community structure are not affected (with the exception of a fungal marker; 16:1ώ5) by partial or clear-cut harvesting, potentially due to the efforts by the forestry industry to minimalize soil compaction. Post-harvest management does have additional effects on the vegetation community, as vegetation management regimes are implemented to reduce aspen regeneration through suckering, to encourage white spruce development (Kabzems et al., 1998; Man et al., 2011).

Conventional oil and gas exploration in Alberta (excluding oil sands) is a driving force of the economy, and has a noticeable impact on the landscape, with over half a million barrels of oil being produced a day in 2015 (Alberta Energy, 2017). Infrastructure includes, but is not limited to, roads, pipeline, well pads and surface mines. Surface mining (oil sands) involves complete removal of the upper soil layers (Alberta Energy, 2017), and is beyond restoration measures, involving full reconstruction of ecosystems. Pipelines are revegetated following installation, and once decommissioned, their narrow footprint allows for natural ingress of woody species more readily than other disturbances (Salisbury, 2004). Roadways and well pads are more permanent, with roadways often remaining in place, as they allow for access to remote localities.

Wells pads for conventional oil and gas often remain in place for many years, while oil sands exploration (OSE) wells are drilled and surface reclaimed within a single winter (Osko & Glasgow, 2010). These well pads consist of the soils being stripped back, exploratory well drilled, and then removed before the soils are rolled back. A less common disturbance, that is similar to roadways, are airstrips. These features may or may not be paved, and typically remain in place for decades. Topsoil is stripped away and stock piled, while the sub soils are capped for the duration of the existence of the infrastructure.

1.2. Restoration of the boreal forest

Current reclamation guidelines for upstream oil and gas sites are the 2010 Reclamation Criteria for Wellsite's and Associated Facilities for Forested Lands (ESRD, 2013) as well as additional approvals under the Environmental Protection and Enhancement Act (AEP, 1993). These criteria list requirements for landscape, soil and vegetation parameters that will obtain equivalent land capability. These guidelines ensure that restoration actions are required for oil and gas sites, as the removal of the soil, and subsequent degradation of the seed bed prevents natural recovery from occurring easily (Bachmann, 2014; Frerichs, 2017). Natural ingress of surrounding vegetation occurs from the forest edges, but the overall success of natural ingress throughout the site depends on the development of other undesirable (largely agronomic) vegetation that often rapidly colonize the disturbed site (Landhäusser & Lieffers, 1994). To reduce restoration time, tree species (predominantly conifers such as white spruce) are generally planted instead of waiting for natural ingress.

Planting trees is currently the dominant and often only revegetation strategy undertaken on the majority of oil and gas restoration projects. Results are centered on forestry objectives, with guidelines based on tree presence and health as the basis of success (ESRD, 2013). Earlier methods involved seeding grass species across sites to rapidly introduce cover (Smreciu et al., 2003); however this has since been phased out due to the difficulty in later removing the grass (NAIT, 2012). Seeding of native forb species has been attempted, however it is difficult due to the lack of readily available seed in large quantities and the lack of understanding of effective seeding practices (Schoonmaker et al., 2014; Smreciu et al., 2003). Natural ingress of forbs is the primary method of establishment on reclamation sites currently.

Land reclamation in Alberta has historically been based on restoring “equivalent land capability”, as described in the current reclamation guidelines (ESRD, 2013). Talk has shifted to the idea of creating resilient ecosystems with this being incorporated in the criteria and indictors framework (Poscent & Charette, 2012) and the focus of a Summary of Resiliency of Reclaimed Boreal Forest Landscapes Seminar (Pyper et al., 2013). The concept of resilient ecosystems was first introduced by C.S. Holling and is defined as being “the capacity of an ecosystem to respond to a perturbation or disturbance by resisting damage and recovering quickly” (Pyper et al., 2013; Wikipedia, 2017). There is however no current measure of ecosystem resilience. It is logical to assume that areas affected by wildfire recover without human assistance and that natural resiliency could be an indicator of successful reclamation. Harvesting by forestry companies has achieved this resiliency (Kishchuk et al., 2015), but it is yet to be quantified in areas disturbed for oil and gas exploration. The current reclamation guidelines do not have any direct measures for soil microbial characteristics (ESRD, 2013; AEP, 1993), the understanding of which could allow for estimations of the ability of the ecosystem to recover from future disturbances (resiliency) and its trajectory.

1.3. Hitchhiker planting

The placement of forb seedlings, as opposed to broadcast seeding, will ideally give the forbs enough of an advantage to outcompete the natural ingress of undesirable species, such as *Calamagrostis canadensis,* which is well studied, along with other less studied species. The seedlings are required as when forbs (eg. fireweed) and *Calamagrostis* colonize a site simultaneously, the fireweed will often be out-competed (Landhausser & Liefers, 1994). If the *Calamagrostis* cover on a site becomes too dense, it is difficult for the white spruce to establish (Hoggs & Lieffers, 1991), and removal of *Calamagrostis* and other undesirable species is difficult without canopy closure (Lieffers & Stadt, 1994). Non-native species are extremely difficult to remove from reclaimed areas, and some reclaimed sites still have 10% cover of non-native species after 20 years, compared to natural forests where there are no non- native species present (Pinno & Hawkes, 2015).

In addition to its ability to hopefully combat invasive species, the placement of seedlings over seeds is desirable in terms of success rate and economics. Broadcast seeding is unreliable (Schoonmaker et al., 2014; deBortoli, 2017; Nelson et al., 1969; Koniak, 1983), with many factors determining its success (wind, wildlife, etc.). The placement of seedlings conserves seed sources by controlling germination conditions and allows for consistent establishment. This consistency and reliability is important to the economic viability of revegetation methods. If a method’s success rate is low, it will likely be withheld from a restoration plan as budgets are often constrained. The planting of forb seedlings on their own is already a revegetation method (Smreci & Gould, 2015), however due to the individual plug requirement it is an expensive process, and requires twice the planting time (when tree planting is also a component of the plan).

Hitchhiker planting is a simple method, analogous to the concept of companion cropping, which has a longstanding history in agriculture (Davis, 1962). Some variations of it have been trialed previously (Garnett, 2003; Nelson, 2005), but it has never been attempted with these species, at this scale. White spruce seedlings are grown in the greenhouse, in plugs slightly larger than what the forestry industry standard is, to allow for the presence of forb roots. After a delay of x number of weeks, the forb seed is sown in, giving the spruce a “head start” before potential competition is introduced. The hitchhiker plants are grown together for one season in the greenhouse, before being frozen for storage before out planting the following year. This process (minus the forb inclusion) is comparable to industry standards for preparing coniferous species for reclamation planting. This idea can be incorporated into existing practices to allow for the inclusion of forb establishment in re-vegetation practices when desired, without increasing the planting requirements when tree planting is already part of the revegetation strategy.

1.4. The soil microbial community, its benefits and how to measure it

In order to support the future reclamation process, operators salvage the surface soils and stockpile them until it is time for reclamation (Osko & Glasgow, 2010). This process alters soil properties as well as its microbial community (Alberta Environment and Water, 2012; Mummey et al., 2002). A study of grasslands recovering from anthropogenic disturbance showed consistently lower aggregate stability than remnants and included an inoculation study which demonstrated that the degradation of the microbial community also contributes to the decline in soil aggregate stability in disturbed grasslands (Duchicela, 2012). With the understanding that microbial population dynamics can play a major role in plant species coexistence, it is expected that the reestablishment of the native soil community to be a limiting factor in restoration of native plant diversity and composition (Bever, 2010). The early specifics of this relationship are shown in a study by Zak (2003) that found microbial community biomass, respiration, and fungal abundance significantly increased with greater plant diversity, as did N mineralization rates. Changes in microbial community biomass, activity, and composition largely resulted from the higher levels of plant production associated with greater diversity, but nonetheless, greater plant production could not explain more rapid N mineralization, indicating that plant diversity affected this microbial process, which controls rates of ecosystem N cycling (Zak, 2003).

The distribution and dominance of plant species could drive the diversity of microhabitats available to the soil microbial community through mechanisms including complementarity in root foraging patterns and evenness in root exudate profiles (Lamb, 2011). In landscapes recovering from disturbance, the structure and dynamics of plant communities can be strongly influenced by historical factors, including assembly and priority effects (Lamb, 2011). These effects result when early occupancy of the site provides a substantial advantage to the initial colonizer, preventing or reducing the establishment of later colonists (Bever, 2010; Wubs et al., 2016). A study by Rodrigues et al. (2015) showed that overall, bacterial and fungal communities changed congruently with plant invaders, and supported the hypothesis that nitrogen cycling bacteria and their specific functions are important factors in plant invasions. Additionally studies in northern forests have shown that variation in microbial communities can be explained by variation in vegetation variables (Bach et al., 2008). Whether the changes in microbial communities are driven by direct plant-microbial interactions or a result of plant driven changes in soil properties remained to be determined (Bach et al., 2008); but the research in this field indicates that the early establishment of native herbaceous species will have both above and below ground affects.

Besides the effect of the herbaceous species, woody species also affect the soil microbial community, as shown by Tuason at al. (2009) which found that the rhizosphere soils of white spruce have a higher pH due to organic acid root exudates than the surrounding non-rhizosphere soils. This can influence the microbial populations within those rhizosphere soils (Anderson & Domsch, 1993). These types of affects can be seen at a stand level when Royer-Tardiff et al. (2010) compared the resistance of microbial communities to disturbance in pine, aspen and mixed-wood stands and found that mixed-wood stands had a higher resistance to disturbance. This higher resistance to disturbance in mixed-wood stands may be due to the presence of *Populus tremuloides*, as indicated by White et al. (2004) with research into the effect of stand type on the functional diversity of soil microbial communities. Overall the research is split on whether tree species such as white spruce is linked to the soil microbial community (Sorenson et al., 2011), or is not (White et al., 2004).

Various methods are available for measuring microbial characteristics, but in terms of microbial function, one method showing promise is multi-substrate induced respiration (MSIR) which has been shown to be an effective way of measuring microbial respiration, with the benefits of assessing the whole population and using a live soil sample, not an extract (Lalor et al., 2007). Additionally it has been shown to be substantially more accurate than the Degens and Harris approach (Degens and Harris, 1997), specifically for distinguishing between treatments

(Lalor et al., 2007). Community level physiological profiles (a specific adaptation of MSIR) allow us to obtain a measurement of carbon substrate utilization by microbes, or more simply their contribution to soil respiration (Chapman et al., 2007) and is an improvement over the previous BIOLOG® method, as it is not biased to fast growing bacteria (Campbell et al., 2003).

Measuring microbial properties to determine ecological function and resiliency is not a new technique, but it is one that has yet to be adopted into guidelines (AEP, 1993; ESRD, 2013; Poscent & Charette, 2012). Wardle (1992) shows how soil microbial biomass responds very rapidly to disturbance by anthromorphic activities, and indicates it is a highly useful ecological indicator. In 2000, Yin et al. (2000) published a paper stating that bacterial functional redundancy may be a useful indicator of soil quality and ecosystem functioning. These ideas of microbial properties being useful indicators of ecological function have begun to merge with ideas on resiliency. Stanturf & Madsen (2002) state that the rehabilitation of degraded forests increasingly relies on re-establish natural disturbance regimes, indicating a shift to incorporating resiliency into restoration discussions. Recently the ideas of ecological function and similarity to natural disturbance regimes is emerging in restoration research (Howell & MacKenzie, 2017; Banning et al., 2012; Howell et al., 2016; Poscent & Charette, 2012; Pyper et al., 2013; Kishchuk et al., 2015; Dimitriu et al., 2010; Griffiths & Philipot, 2013)

Using these measurements for microbial function will allow for the use of ordination analysis to compare resiliency of our reclamation sites to reference sites. Wildfire is the natural disturbance regime that shapes the boreal forest (Markham & Essery, 2015), and was a logical choice for reference sites. The 2011 fires allowed us to capture a mast year while the 2015 fires allowed us to sample sites that burned at the same time the study sites were planted. Forestry operations in Alberta have existed long enough to confirm that resilient ecosystems are restored following harvest (Kishchuk et al., 2015), similar to wildfire. It is this similarity to wildfire in terms of recovery from disturbance which makes them an optimum second reference site type for the experiment. The use of ordination space for comparisons is necessary to evaluate the similarity of the restoration and reference sites, without setting arbitrary benchmarks, and is readily used in existing research (Howell et al., 2016; Hogberg et al., 2017; Mukhopadhyay et al., 2014; Banning et al., 2012; Howell & MacKenzie, 2017; Ohtonen & Vare, 1998). It is based on the idea that a reasonable level of ecological function in forested lands differs from an agricultural context, where soil quality may be managed to maximize production (an easily measureable parameter) without adverse environmental effect. In a natural ecosystem, soil quality (or function) may be observed as a baseline value or set of values against which future changes in the system may be compared (Karlen et al., 2001).

1.5. Research objectives

The objective of this study was to examine the post out-planting effects and potential benefits of hitchhiker planting a native herbaceous species with a white spruce (*Picea glauca*) seedling. White spruce was the primary occupant of the plug, with the forb species fireweed (*Chamerion angustifolium*) and showy aster (*Eurybia conspicua*) hitchhiking. This study is the first of its kind with limited understanding both in terms of appropriate approaches to co-growing these species, out planting performance and secondary impacts on soil biological function. This study evaluates the viability of previously developed stock types under field conditions, as well as the potential for improved microbial properties on recently reclaimed industrial disturbances, with similarly aged disturbances recovering from wildfire or forest harvesting serving as a point of reference. More specifically, the following five objectives were identified and evaluated in this study:

(1) Can we produce mixed-container stock of white spruce that is comparable or better than singly produced white spruce seedlings?

(2) What is the effect of stock type on the growth of the native forb species, while co-grown with white spruce?

(3) Does the established forb impact vegetation dynamics (reduce competition) of other undesirable species in the immediate vicinity of the white spruce seedling?

(4) Will hitchhiker planting have an effect on soil microbial properties?

(5) How do soil microbial properties on the study sites compare to sites recovering from forestry harvesting and wildfire?

Chapter 2 - Ensuring plant diversity in disturbed landscapes: Hitchhiking native forbs with white spruce

2.1. Introduction

Land reclamation in Alberta is a constantly evolving industry, striving to be at the forefront with innovative ideas and practices. Vast amounts of northern boreal forest have been disturbed, with over 3,100 wells drilled across Alberta in Jan-Oct 2017 alone, in what could be considered a slow year (AER, 2017). Restoring these landscapes is an ever growing priority. Over the last 25 years, reclamation criteria (this is the criteria required by operator's in order to release liability of an upstream oil and gas site on forested lands) have evolved steadily with the most recent criteria, the 2010 Reclamation Criteria for Wellsite's and Associated Facilities for Forested Lands (ESRD, 2013), being the first to specify requirements for suitable native vegetation presence (stem counts and vegetation cover). Planting of woody species is a successful strategy for achieving sufficient woody stem counts (ESRD, 2013), however, increasing herbaceous cover is difficult due to both the lack of readily available seed in large quantities and understanding of effective seeding practices (Schoonmaker et al., 2014; Smreciu et al., 2003). This study will demonstrate an alternative approach to establishing native forbs that could be applied to many types of disturbed landscapes and in other regions outside Alberta.

Presently, native forb species are introduced on reclaimed industrial sites mainly through broadcast seeding or natural ingress (Schoonmaker et al., 2014; Smreciu et al., 2003). Broadcast seeding can be accomplished manually or with mechanical methods and is effective for distributing seed across large areas (Smreciu & Gould, 2015). The primary constraint to broadcast seeding is the lack of consistent results, which occurs from various factors including improper seed prep, variable seed bed conditions, post seeding conditions, wind removal and animal removal (Schoonmaker et al., 2014; deBortoli, 2017; Nelson et al., 1969; Koniak, 1983).

The other commonly practiced method is allowing for natural ingress of native species. This method can achieve the desired result, however the timeframe is often longer (years to decades) and there is no guarantee all the desired species will return (Schoonmaker et al., 2014; Smreciu & Gould, 2015).

An alternative approach to direct seeding native forbs or waiting for natural recovery is to plant nursery stock seedlings of native forbs. This would be a significant additional cost and likely have limited adoptability as most resources for operational planting on reclaimed sites are focused around trees and shrubs (Schoonmaker et al., 2014). However, a native forb could be co-planted or 'hitchhiked' in the same nursery container as a tree or shrub species intended for out planting on a reclaimed site. This novel concept, hitchhiker planting, would be conceptually similar to companion cropping in agriculture where one species, oats for example, is planted for rapid establishment, soil stabilization and weed suppression while alfalfa plants develop (Curran et al., 1993; Davis, 1962). Choosing species for hitchhiker planting is a balance between a forb that is aggressive enough to colonize a site, but will not substantially hinder development of the primary woody species. This balance could ideally be achieved by using native species that are natural colonizers of sites following disturbance.

While native herbaceous cover will establish over time from the seed bank and surrounding forest (Schoonmaker et al., 2014), rapid establishment via hitchhiker planting is desirable to combat the establishment of undesirable species (both non-native species and species not typical of a forest community) around planted tree and shrub species (Landhäusser et al., 1996). While there are many different species, one that is extensively studied, and has quantifiable impacts representative of undesirable species, is the grass *Calamagrostis canadensis*. The presence of *Calamagrostis* is greatly increased following disturbance, as the grass will persist in the understory of the mature forest in scattered locations, and then following disturbance the expansion of the clones allows for rapid site domination (Lieffers & Stadt, 1994). This initial expansion has cascading effects, as the expansion by *Calamagrostis*, or any undesirable species, can limit growth and nitrogen uptake by white spruce, to a greater extent than competition by aspen or forbs such as fireweed (Hangs et al., 2002). In addition to limiting nitrogen uptake, undesirable species introduce significant competition for light, moisture and other nutrients, negatively impacting growth of desired species (Balandier et al., 2006; Bauman et al., 2015; Eis, 1981). In spruce plantations, use of agronomic species as replacement vegetation demonstrated that they can successfully overtake a site and prevent the establishment of native forest species (Negrave & Kabzems, 1993)

Fireweed (*Chamerion angustifolium*) is one of the first species in the boreal forest to recolonize an area following natural disturbance (Royer & Dickinson, 2007; Phillips, 2012). Its presence is a part of natural ecosystem recovery, and has made it an ideal reclamation species for upland areas (Pinno et al., 2015). The widespread presence of this species is attributable to a number of a factors including being a prolific seed producer (a single plant can produce 20,000 – 80,000 seeds per year), immense seed dispersal distances over 300 km and germination typically occurring within the first year (Royer & Dickinson, 2007; Phillips, 2012; Bianco, 1990). Once established, reproduction primarily occurs from spreading roots and suckering (average spread rate of 1m per year) until maximum density is reached in 2-5 years (Bianco, 1990).

Showy aster (*Eurybia conspicua)* is another native herbaceous perennial boreal forest species in western Canada. It spreads by an extensive system of rhizomes and forms large, loose clumps with plants 30-100cm tall. This aster generally forms a leafy ground cover, sprouts and flowers abundantly following wildfire and though less prevalent than fireweed, is a relatively common herbaceous species following natural disturbances (Royer & Dickinson, 2007; Phillips, 2012).

White spruce is a dominant tree species in the boreal forest, regenerates almost entirely from seed (driven by large dispersal events), has low mortality rates and is shade tolerant which allows it to persist in the forest understory. The seedlings are most vulnerable until they are approximately 30cm tall, and are then considered established (Gärtner et al., 2011). It is during the establishment stage that undesirable species can have the greatest damaging effects on white spruce growth (Gärtner et al., 2011). While the presence of native herbaceous vegetation, including fireweed, could slow also down woody growth of white spruce, it is thought to be less of a concern (Eis, 1981).

The objective of this study was to determine the post out-planting effects of hitchhiker planting a native herbaceous species with a white spruce seedling. For this experiment *Picea glauca* (white spruce) was chosen as the primary tree species due to its abundant use in reforestation and knowledge around its propagation and early growth. The forb species chosen were *Chamerion angustifolium* (fireweed) and *Eurybia conspicua* (showy aster) which are both native species and early colonizers of disturbed areas but with contrasting aboveground growth and root development, features which may impact the development hitchhiker container stock and field performance of white spruce. This study will answer the following questions:

(1) Can we produce mixed-container stock of white spruce that is comparable or better than singly produced white spruce seedlings?

(2) What is the effect of stock type on the growth of the native forb species, while co-grown with white spruce?

(3) Does the established forb impact vegetation dynamics (reduce competition) of other undesirable species in the immediate vicinity of the white spruce seedling?

2.2. Methodology

2.2.1. Greenhouse propagation of hitchhiker stock

In March 2014, a greenhouse trial was initiated at the NAIT Boreal Research Institute (Peace River, Alberta, N56.235680, W117.330994) which created and evaluated nine stock types of white spruce (*Picea glauca*) grown with either showy aster (*Eurybia conspicua*) or fireweed (*Chamerion angustifolium*). The stock types consisted of two different sizes of Styroblock™ containers (512A or 615A) with the forb sown six, eight, ten or twelve weeks after the white spruce was sown (Figure 2-1). A control group, which was intended to represent a typical or conventionally grown white spruce seedling, was produced in a 412A Styroblock™ (typical size used by the forest industry). The control stock type was grown in the industry standard plug in order to provide comparative representation of the 'business as usual' approach to establishing white spruce. Spruce were not grown individually in the larger stock sizes as the effects of a larger plug on spruce growth are well documented (increase in height of ~10-30%, Scarratt, 1972; Government of British Columbia, 2015) and was the not the focus of this study. The purpose of this approach is to determine if the growth of hitchhiker stock is viable (in terms of survival) and how it compares to the industry standard approach in terms of growth and survival. The knowledge that the inclusion of forbs increases the number of roots in the plug also negated the need to evaluate hitchhiker planting in the same size plug as the industry standard, as negative effects on spruce growth and survival would be apparent.

Seedlings were grown under greenhouse conditions (see Appendix A for details) until lifting in November 2014 and were stored frozen at -4°C until out-planting in May 2015. A random subset of 12 seedlings (n=12 per stocktype) were destructively harvested following seedling lifting and the following measurements collected: (i) root collar diameter (RCD) measured with calipers in millimeters (mm) at the base of the seedling and (ii) total height of the seedlings measured in centimeters (cm) from where the seedling interfaced with the soil to the tip of the tallest leader. Shoots were separated from root plugs and dried at 70°C for 24 hours in paper bags. Dry biomass of needles and stems were weighed separately. Root plugs were manually washed and soil sieves (1 mm mesh screen) were used to capture loose root fragments. As the root systems were heavily entangled, they were manually separated by immersing the washed root system in a bowl of water and gently pulling the root systems of each plant apart. Loose roots were manually separated; herbaceous roots were easily distinguished from white spruce roots. Washed roots were dried at 70°C for 24 hours in paper bags and weighed. A subsample of roots from each washed plant was scanned on a flatbed scanner with image analysis software (WinRHIZO Regent Instruments Inc., Canada) to determine root length and surface area. These values were extrapolated with respect to total and subsampled root dry mass to obtain whole plant root length and surface area.

2.2.2. Study Sites

The reclamation sites were located within the boreal forest of northern Alberta. Please refer to chapter 1 for more details on the climate of the boreal forest. Four sites were selected for evaluation of the hitchhiker container stock produced in the greenhouse in 2014. The sites varied in level of disturbance (short-term vs long-term) to allow for an assessment of the suitability of hitchhiker planting across varying disturbance types. Two sites (OSE1 and OSE2) were former oil sands exploration (OSE) wells while the other two sites (BP1 and AS1) were located within a former airstrip (Table 2-1). The BP1 site was a topsoil storage area for the airstrip, the soils were coarse textured, and following removal of the stored topsoil for airstrip reclamation the site was prepared with a McNabb RipPlow (McNabb et al., 2013) prior to planting in order to create surface heterogeneity. AS1 was an area formerly covered by the airstrip (A horizon was stripped away and stored on BP1 during airstrip operation when the site was covered by asphalt), had finer textured soils and the topsoil (previously stored at the BP1 location) were spread with a D7 cat before planting. The OSE1 site was a cut & fill (A horizon stripped back and stored, remaining soil used to level site, during reclamation site is re-contoured and A horizon spread back across final grade) OSE well site, the soils were fine textured and the topsoil was spread with a D7 cat before planting. The OSE2 site was also a cut & fill OSE well site, the soils were coarse textured and the site was prepared with a McNabb RipPlow (McNabb et al., 2013) before planting.

Container stock seedlings were planted at each site in late May 2015. Each site followed the same experimental design where three seedlings (subsamples) of each stock type (17 stock types in total) were planted into each of seven replicate lines (21 seedlings per stock type planted at each site). The order of planting of each stock type was randomized within each line. Individual seedlings were planted at 2 m spacing with 2 m spacing between lines (Figure 2-2).

2.2.3. Aboveground Measurements

In August 2016, for each white spruce seedling on every study site (n=21) the following was measured: (i) total height from where the base of the tree intersected the soil to the tip of the tallest leader in cm, (ii) increment growth was also determined in cm as the distance from the previous year's terminal bud scar to the tip of the longest leader and (iii) RCD (root collar diameter) was determined in mm using calipers at the base of the tree. For the hitchhiker forb, the following was quantified: (i) total height in centimeters from where the forb intersected the ground to its tallest point and (ii) vegetation % cover surrounding the white spruce seedling using a 50cm by 50cm quadrat that was centered over each white spruce. In addition, the percent cover within each quadrat was collectively determined for grass species, non-native species and other native forbs (fireweed and showy aster were assessed separately). Refer to Figure 2-3 for a timeline of planting and sampling events.

2.2.4. Destructive Harvesting

Destructive harvesting of aboveground biomass was conducted on a subset of seedlings within each of the study sites (n=7). At each line (7 total per site), the 3rd replicate for each stock type was clipped and separated by species (white spruce, fireweed or showy aster). For fireweed and showy aster, all aboveground biomass within the quadrat was collected except where belowground root development was determined, in that instance, all aboveground biomass that had egressed outside the quadrat could be collected as the root harvesting ensured that the aboveground material had originated from the target individual. Biomass samples were placed in paper bags and returned to the lab where they were oven dried at 70˚C for 24 hours. Dried samples were weighed to nearest 0.0001 grams.

Due to time constraints, determination of belowground root development was only conducted at two of the study sites (AS1 and OSE2). When it was collected the belowground biomass was meticulously extracted from the third replicate, in a minimum of five lines. This was accomplished by tracing the root systems out from the hitchhiker plant using hand exposure with the assistance of small trowels. Maximum root length and forb suckering data was collected before the roots were placed in large plastic bags and frozen at -4˚C until further processing could be completed. Root systems were manually washed and soil sieves (1 mm mesh screen) were used to capture loose root fragments. If the root systems were heavily entangled, they were manually separated by immersing the washed root system in a bowl of water and gently pulling the root systems of each plant apart. The root systems were separated into two classifications: roots that were present in the original plugs, and roots that had egressed from the plug. All plant material was dried at 70˚C for 24 hours. Dried samples were weighed to nearest 0.0001 grams.

2.2.7. Statistical Analysis

Statistical analysis and modelling was completed using R software (R Core Team, 2015). A linear mixed-effects analysis of variance (ANOVA) was performed using "lme4" package to analyze the fixed effect of stock type on each of the seedling growth parameters. Assumptions of ANOVA were checked with diagnostic plots prior to the analysis for all sets of data. Model-estimated β coefficients of fixed effects were derived using "effects" package. The post hoc comparison of treatment means was compared using Tukey post-hoc tests (Tukey, 1949) and the comparisons with the control were evaluated using Dunnett's test ("lsmeans" package) with a significant result having a p-value<0.05 (Dunnett, 1955). Non-linear regression was completed using the "lme4" package to analyze vegetation cover comparisons. Assumptions of non-linear regression were checked with diagnostic plots prior to the analysis for all sets of data and Akaike Information Criteria (AIC) (Akaike, 1974) values were used to determine the model of best fit (exponential).

2.3. Results

2.3.1 Greenhouse

Height, root collar diameter, needle mass, stem mass and root mass in white spruce was significantly lower (relative to the control group) for fireweed stock types with earlier sow dates (6-10 week for 512A, 6-8 week for 615A) (Table 2-2). Where the fireweed was sown later, however, most spruce growth characteristics were comparable to, or in some cases significantly greater than the control (Table 2-2). Spruce root:shoot ratio was significantly increased for nearly all sow dates (with exception of FW 615-6), while root length and surface area significantly decreased for all sow dates (Table 2-2).

A similar pattern to that described above for fireweed stock types was observed in spruce grown with aster but was limited to the earliest sow dates and/or small stock sizes. Height, root collar diameter, needle mass, stem mass and root mass were significantly lower in spruce only for the 6 week sow dates (both stock sizes) and 8 week sow date (512A stock size), while these characteristics were not significantly different (or were larger) from the control for all other stock types (Table 2-2). Spruce root:shoot ratio was significantly higher, while root length and surface area was lower, in all stock types with the exception of the 8-12 week 615A stock types (Table 2-2).

2.3.1. Spruce Survival, Total Height, RCD and Growth Increment

Overall survival for white spruce after two growing seasons was 92%, with no significant variation between stock sizes or forb species. After two growing seasons, at all study sites, total height of spruce in most hitchhiker stock (fireweed or aster types) was similar to or significantly taller than the control spruce (Table 2-3). The only types that were significantly shorter than the control stock were FW 512-6 and FW 615-6 stock types (Table 2-3). Growth increment of spruce was not significantly lower from the single grown (control) spruce in any of the aster stock types (at any site) except for AS 512-6 and AS 615-6 on the AS1 site (Table 2-3). However, growth increment was consistently lower for stock types grown with fireweed on the OSE2 site though this was generally not the case for the other three sites (Table 2-3). Root collar diameter was similar or greater than the control spruce in most fireweed and aster stock types across the study sites with the exception of FW/AS 612-6, 512-8 and FW 615-6 which tended to be significantly smaller (Table 2-3).

2.3.2. Above and Belowground Biomass

For all study sites, spruce aboveground biomass was not significantly less than the control spruce in any fireweed stock types, except in the FW 512-6 stock types on the AS1 and OSE2 sites as well as the FW 615-6 stock type at the OSE2 site (Table 2-3). In the aster stock types, spruce aboveground biomass was not significantly different from the control spruce in any stock types except in the AS 512-6 and AS 615-6 stock types at OSE2 and in the AS 512-6 and AS 512-8 stock types at AS1 (Table 2-3).

Spruce belowground biomass was significantly lower than the control spruce in the FW 512-6 and 612-6 as well as FW 512-6 and FW 615-6 stock types on the OSE2 site only (Table 2-3), while no significant differences were detected between the control group and either the aster or fireweed stock types at AS1 (Table 2-3).

Neither fireweed nor aster aboveground or belowground biomass was significantly different between stock types (Figures 2-4 to 2-7). In addition, the number of suckers and longest lateral roots were not significantly different between stock types in aster or fireweed (Figures 2-8 & 2-9).

2.3.3. Target Forb Survival and Cover, Undesirable Species and Total Percent Cover

Overall survival for aster was 70% after two growing seasons, with it ranging from 80% in the 6 week sow dates, to 60% in the 12 week sow dates, and averaging at 70% in the 8 and 10 week sow dates. Overall survival for fireweed was lower at 65% overall, with it being highest at 68% in the 6 week sow dates and 60% in the 12 week sow dates.

There were no significant differences between stock types for target forb percentage cover in either species, when the control stock type was excluded (data not shown). When the control was included, fireweed cover was not significantly different to the control on the BP1, OSE2 and OSE1 sites. On the AS1 site no fireweed was recorded in control plots, but cover varied from 10-20% in the hitchhiker plots (Table 2-3). Aster cover was not significantly different in the hitchhiker plots relative to the control plots on the OSE1 site; however on the other sites there was no aster noted in the control plots, while it averaged 10-20% in the hitchhiker plots (Table 2-3).

Over all sites, only the FW 615A-8 and FW 615-10 stock types on the OSE1 site had significantly different (less) undesirable species cover relative to the control (Table 2-3). There was no detectable difference in undesirable cover in the aster stock types compared with the control (Table 2-3).

Total cover (all species except the target forb) was generally not significantly different from the control in fireweed stock types with the exception of one or two stock types (with no consistent pattern) across study sites that were significantly lower (Table 2-3). In the aster stock types total cover was comparable to the control across all stock types on the BP1 and OSE2 sites, but was significantly less on the AS 512-6 stock type on AS1 and in the AS 512-6, AS 615-10 and AS 615-12 stock types on the OSE1 site (Table 2-3).

Non-linear regression analysis of cover measurements was used to assess trends not appearing as significant results in earlier ANOVA analysis (Appendix A). Non-linear regression analysis of target forb (aster and fireweed) cover, compared to non-native species cover, native species cover, total cover and grass species cover individually indicates that as target forb cover increases, percent cover of non-native species cover, native species cover, total cover and grass species cover was reduced (Figure 2-10 & 2-11).

2.4. Discussion

2.4.1. Spruce Development

The main goal of developing a successful mixed-species (hitchhiker) stock type that did not inhibit spruce survival or growth was successful. In general, the stock types in larger containers (615A) and where the forb was introduced 10 weeks or more after the spruce resulted in spruce growth that was comparable or greater than our standard white spruce stock. There were not hitchhiker effects on spruce survival. These stock types also consistently resulted in robust development of the hitchhiked native forb. The tertiary goal of reducing undesirable species cover was not apparently obvious, but developing trends indicate we will see future effects.

In general, singly grown spruce and fireweed stock types were comparable in total height after two growing seasons though earliest sow date resulted in decreased height on all sites in the smaller (512A) stock size and most sites in the larger (615A) stock size. Fireweeds' aggressiveness in expansion is well documented (Province of British Columbia, 1997; Pinno et al., 2015; Pinno et al., 2017) and was part of the reason it was chosen. The spruce were comparable in height to the control in the latest sow dates coming out of the greenhouse, and in the year two results an increase in spruce height relative to the control was observed only in the largest stock size for two sow dates on one site, and negative effects on height were only observed in the earliest sow dates, which suggest intraspecific competition between fireweed and spruce generally did not inhibit spruce growth, but also did not allow it to utilize the extra space in the greenhouse (Tables 2-2 & 2-3).

Similar results as observed for height were seen with increment and RCD data; however increment data appears to be driven more by site conditions than by stock type, as a negative result was observed across all stock types on one site. RCD data is generally the most sensitive to competition (Bokalo et al., 2007; Hangs et al., 2002), and this was observed with the RCD data having the most negative results compared to the control (Table 3-2), however, it generally was similar in response to the increment data, which was to be expected based on previous research (Groot, 1999), but without the obvious site conditions effect. The overall neutral to positive comparison of spruce stock type characteristics to our control relieved concerns that on sites with excellent conditions for forb establishment and expansion, white spruce may not be able to compete with the forbs dominance (Province of British Columbia, 1997; Frey et al., 2003; Pinno et al., 2015; Pinno et al., 2017), especially with fireweed being considered a forestry issue in certain situations (Bianco, 1990; Province of British Columbia, 1997). The variation between the different measurements confirms the need for multiple measurements to accurately capture the effect of hitchhiker planting on spruce development.

2.4.2 Fireweed Success

The lack of significant difference between stock types in terms of fireweed cover was unexpected, but is likely explained primarily by ineffective measurement techniques. The small (0.5 meter by 0.5 meter) quadrat used in this study was not accurately capturing cover as fireweed after two years had spread from the original planting location substantially, which was noted in our root egression data and has also been observed previously (Province of British Columbia, 1997; Bianco, 1990).

The belowground root egression, and aboveground biomass data was highly variable between stock types, and the earlier sow dates (with the greatest growth levels coming out of the greenhouse) thrived on the ripped OSE2 site. When the fireweed was able to expand, it did so rapidly, and this was strongly influenced by site conditions (Bianco, 1990; Dona & Galen, 2007;

Messier & Kimmins, 1992), rather than by stock type. The level of root egress observed on the ripped OSE2 site, where soil conditions were favorable to growth and expansion (Province of British Columbia, 1997; Pinno et al., 2015; Pinno et al., 2017), was ten times greater than that observed on the non-ripped AS1 site (Figure 2-8). The expansion of the fireweed was greater than that observed for aster, and is typical of its expansion strategy (Province of British Columbia, 1997; Royer & Dickinson, 2007; Phillips, 2012). Site trends were also observed when looking at the cover data, with AS1 showing significant increases in fireweed cover relative to the control, and BP1 and OSE2 showing a trend of increasing cover, even though it was not a significant result. The only site where no visible increase was observed, OSE1, also had the most competition. This increased competition prevents the hitchhiker plants from expanding, and will leave the spruce susceptible to increased competition and stress (Lieffers & Stadt, 1994; Landhausser & Lieffers, 1994; Hangs et al., 2002). The increased background levels of fireweed were not surprising, based on it being the early colonizer it is (Pinno et al., 2015; Pinno et al., 2017). The rapid fireweed expansion is important since its survival success is lower than that of aster (65% vs 70%), it is important for site success that fireweed expands rapidly when it does survive.

2.4.3 Aster Success

With the aster results, we saw very similar results to that observed with fireweed, but with more comparability overall between the singly grown spruce, and spruce in our aster stock types in terms of total spruce height, RCD and increment growth. There were less instances were aster caused a negative effect on white spruce and more cases in which results were increased relative to the control group. This less aggressive nature of aster is a positive sign for spruce growth short term, but may not be ideal for combatting invasive and non-native species long term (Royer & Dickinson, 2007; Phillips, 2012; Strong & Sidhu, 2005). By staying close to the point of planting it will provide protection; however it may also compete with the spruce long term, and by not colonizing the site will still allow the ingress of undesirable species.

Aster cover was significantly higher than the control in all stock types on all but the OSE1 site, with no significant differences within the stock types themselves. This increase relative to the control showed that hitchhiker planting improved aster establishment compared to natural ingress on all but the OSE1 site, where background levels could not be distinguished from the hitchhiker plants. It is not believed that measurement techniques failed to accurately capture aster cover as aster does not egress at the same rate as fireweed (Royer & Dickinson, 2007; Phillips, 2012).

Aboveground biomass of aster was extremely variable between stock types and between sites. Some sites followed the expected trend where biomass increases as stock size increases and sow date decreases, but other sites had exceptions to that trend, which was potentially the result of site conditions and microsites providing ideal conditions for expansion (Bauman et al., 2015; Brown & Naeth, 2014; NAIT, 2017), however there is no literature on this for showy aster, as there was for fireweed. Root egression appears to be similar between all stock types and across all sites, which suggests aster is not spreading across the site at the same rates as fireweed, but instead staying close to where it was planted, which could have long term implications on spruce success through increased competition (Man et al., 2008). Aster is known to form an inconspicuous green blanket across sites, which only becomes noticeable when it blooms following fire (Royer & Dickinson, 2007; Phillips, 2012), so it is possible the aster will spread out from its initial planting area in following years. Asters preference to stay where it was planted may be influencing its survival numbers, as it had 70% survival overall, with a high of 80% in the early sow dates, and this likely is the reasons for its consistent increase in cover.

2.4.4 Undesirable Species Cover

Undesirable (non-native and invasive) species cover was variable by both site and stock type, regardless of hitchhiker forb species. The OSE2 and BP1 sites (both surface ripped) showed the lowest number of undesirable species, which correlates with existing research (Bauman et al., 2015; Boher et al., 2017), and clearest trend that hitchhiker plants could be reducing non-native species cover. When all sites and stock types were combined together for non-linear regression analysis, it was observed that the hitchhiker plants were occupying physical space that might otherwise have been occupied by undesirable and grass species, on the first-come first-serve basis (Errington & Pinno, 2016; Frerichs, 2017). While no stock type trends developed, it is promising to see an indication that the basic principle of reducing undesirable species cover through physical occupation of space is working in the early years and continued monitoring will be important to confirm if these trends continue into years 3-5.

2.5. Conclusion

Overall when it comes down to viability of the spruce in the hitchhiker plants, it is consistently viable in the larger stock size and later sow dates. To produce a spruce that is at least comparable in size and growth to a standard sized seedling, the recommended stock size is the 615A for fireweed, and either the 512A or 615A for aster, with a sow date of 10-12 weeks. Achieving forb cover was consistently observed with the 615A stock size, and the 10 week sow date having some advantages over 12 week sow dates (specifically in terms of forb survival) though the importance of this distinction tends to be site specific. While there is no statistically significant results on whether the hitchhiker planting can reduce undesirable species, the clear trends developing strongly suggest that physical occupation of space by the hitchhiker plants is having an effect, and that we should see a more pronounced (and statistically significant) effect in later years.

Site conditions play a large role in success of hitchhiker planting, and in determining which forb species to use. The ripped sites allow for easy egression of roots, particularly for the fireweed, which can reduce competition with the spruce. Microsites may play an important role in both spruce and forb success, and should be considered when planning future studies.

Tables

Table 2-1: Site conditions and history of the four reclamation sites on which hitchhiker planting occurred. Soil texture is hand texture assessment based on the Canadian System of Soil Classification (Soil Classification Working Group, 1998). Land use refers to the former land use prior to restoration. All sites were forested prior to initial disturbance. Dates disturbed and reclaimed refer to initial dates and are generalized with emphasis being on the years scale. Topography is a visual assessment from site visitation. Soil moisture and temperature are averages from the 2016 field season. More detailed information on those measurements is in Chapter 3.

Site	**AS1**	**BP1**	**OSE1**	**OSE2**
Soil Texture	Fine (silt loam - clay loam)	Coarse (clay loam - sandy clay loam)	Fine (silt loam - clay loam)	Coarse (clay loam - sandy clay loam)
Land Use	Former airstrip	Borrow pit/ topsoil storage for former airstrip	Oil sands exploration well	Oil sands exploration well
Date Disturbed - Reclaimed	1970s - 2014	1970s - 2014	Winter 2013/14 – reclaimed within 30 days	Winter 2013/14 – reclaimed within 30 days, ripped fall 2014
Topography	Level	Level	Rolling	Rolling
Soil Moisture ($m^3 \cdot m^{3-1}$)	0.363	0.247	0.255	0.231
Soil Temperature (°C)	13.291	13.837	12.957	14.061

Table 2-2: Spruce and forb greenhouse growth characteristics for each developed stock type. Stock types on the left are represented as Hitchhiker Forb Species-Stock Size-Sow Date where FW = Fireweed, AS= Showy Aster, 512 = 512A stock size, 615 = 615A stock size, 6 = 6 week sow date, 8 = 8 week sow date, 10 = 10 week sow date and 12 = 12 week sow date. A ↔ means there was no significant difference between the hitchhiker stock type and the control stock type, ↑ means the stock type had a significantly higher result than the control and ↓ means the stock type had a significantly lower result than the control. For each parameter for each stock type n=12 for destructive measurements and n=96 for non-destructive measurements.

	FW512-6	FW512-8	FW512-10	FW512-12	FW615-6	FW615-8	FW615-10	FW615-12
Spruce Height (cm)	↓	↓	↔	↔	↓	↓	↔	↔
Spruce Root Collar Diameter (mm)	↓	↓	↓	↔	↓	↓	↔	↔
Spruce Needle Mass (g)	↓	↓	↓	↔	↓	↓	↔	↑
Spruce Stem Mass (g)	↓	↓	↓	↔	↓	↓	↔	↔
SpruceRoot Mass (g)	↓	↓	↓	↓	↓	↓	↓	↔
Spruce Root:Shoot Ratio	↑	↑	↑	↑	↔	↑	↑	↑
Spruce Root Length (cm)	↓	↓	↓	↓	↓	↓	↓	↓
Spruce Root Surface Area (cm^2)	↓	↓	↓	↓	↓	↓	↓	↓

	AS512-6	AS512-8	AS512-10	AS512-12	AS615-6	AS615-8	AS615-10	AS615-12
Spruce Height (cm)	↓	↔	↔	↔	↔	↔	↑	↑
Spruce Root Collar Diameter (mm)	↓	↓	↔	↔	↓	↔	↔	↑
Spruce Needle Mass (g)	↓	↓	↔	↔	↓	↔	↔	↑
Spruce Stem Mass (g)	↓	↔	↔	↔	↓	↔	↔	↑
SpruceRoot Mass (g)	↓	↓	↓	↓	↓	↔	↔	↔
Spruce Root:Shoot Ratio	↑	↑	↑	↑	↑	↑	↔	↔
Spruce Root Length (cm)	↓	↓	↓	↓	↓	↔	↔	↔
Spruce Root Surface Area (cm^2)	↑	↓	↓	↓	↓	↔	↔	↔

Table 2-3: Spruce and Forb Growth Characteristics from the 2016 field sampling event on the four reclamation sites. Stock types on the left are represented as Hitchhiker Forb Species-Stock Size-Sow Date where FW = Fireweed, AS= Showy Aster, 512 = 512A stock size, 615 = 615A stock size, 6 = 6 week sow date, 8 = 8 week sow date, 10 = 10 week sow date and 12 = 12 week sow date. The results are separated by site, in the order BP1, AS1, OSE2, OSE1. A ↔ means there was no significant difference between the hitchhiker stock type and the control stock type, ↑ means the stock type had a significantly higher result than the control and ↓ means the stock type had a significantly lower result than the control. Refer to Appendix A for detailed graphs for each parameter. For each stock type on each site n=21 for non-destructive measurements and n=7 for destructive measurements.

	FW512-6				FW512-8				FW512-10				FW512-12				FW615-6				FW615-8				FW615-10				FW615-12			
	BP1	AS1	OSE2	OSE1	BP1	AS1	OSE2	OSE1	BP1	AS1	OSE2	OSE1	BP1	AS1	OSE2	OSE1	BP1	AS1	OSE2	OSE1	BP1	AS1	OSE2	OSE1	BP1	AS1	OSE2	OSE1	BP1	AS1	OSE2	OSE1
Spruce Height (cm)	↓	↓	↓	↓	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↓	↓	↔	↓	↔	↔	↔	↔	↔	↔	↔	↑	↔	↔	↔	↑
Spruce Root Collar Diameter (mm)	↔	↓	↓	↓	↔	↓	↓	↔	↔	↔	↓	↔	↓	↔	↔	↔	↔	↓	↓	↔	↔	↔	↓	↔	↔	↔	↔	↔	↑	↔	↔	↑
Spruce Growth Increment (cm)	↔	↓	↓	↔	↔	↔	↓	↔	↔	↔	↓	↔	↔	↔	↓	↔	↔	↓	↔	↔	↔	↔	↓	↔	↔	↔	↓	↔	↔	↔	↓	↔
Spruce Aboveground Biomass (g)	↔	↓	↓	↔	↔	↓	↔	↔	--	↔	↔	↔	↔	↔	↔	↔	↔	↔	↓	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↑
Spruce Belowground Biomass (g)	--	↔	↓	--	--	↔	↔	--	--	↔	↔	--	--	↔	↔	--	--	↔	↓	--	--	↔	↔	--	--	↔	↔	--	--	↔	↑	--
Target Forb Cover	↔	↑	↔	↔	↔	↑	↔	↔	↔	↑	↔	↔	↔	↑	↔	↔	↔	↑	↔	↔	↔	↑	↔	↔	↔	↑	↔	↔	↔	--	↔	↔
Invasive Species Cover	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↓	↔	↔	↔	↓	↔	↔	↔	↔
Total Herbaceous Species Cover	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↑	↔	↔	↓	↔	↔	↔	↔	↔	↔	↔	↓	↔	↔	↔	↓

Table 2-3 (Continued): Spruce and Forb Growth Characteristics from the 2016 field sampling event on the four reclamation sites. Stock types on the left are represented as Hitchhiker Forb Species-Stock Size-Sow Date where FW = Fireweed, AS= Showy Aster, 512 = 512A stock size, 615 = 615A stock size, 6 = 6 week sow date, 8 = 8 week sow date, 10 = 10 week sow date and 12 = 12 week sow date. The results are separated by site, in the order BP1, AS1, OSE2, OSE1. A ↔ means there was no significant difference between the hitchhiker stock type and the control stock type, ↑ means the stock type had a significantly higher result than the control and ↓ means the stock type had a significantly lower result than the control. Refer to Appendix A for detailed graphs for each parameter. For each stock type on each site n=21 for non-destructive measurements and n=7 for destructive measurements.

	AS512-6				AS512-8				AS512-10				AS512-12				AS615-6				AS615-8				AS615-10				AS615-12			
	BP1	AS1	OSE2	OSE1	BP1	AS1	OSE2	OSE1	BP1	AS1	OSE2	OSE1	BP1	AS1	OSE2	OSE1	BP1	AS1	OSE2	OSE1	BP1	AS1	OSE2	OSE1	BP1	AS1	OSE2	OSE1	BP1	AS1	OSE2	OSE1
Spruce Height (cm)	↓	↓	↓	↓	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↓	↔	↓	↔	↔	↔	↔	↔	↔	↔	↑	↑	↔	↔	↑
Spruce Root Collar Diameter (mm)	↔	↓	↓	↓	↔	↓	↓	↔	↔	↔	↓	↔	↑	↔	↔	↔	↔	↓	↓	↔	↔	↔	↓	↔	↔	↔	↔	↔	↑	↔	↔	↑
Spruce Growth Increment (cm)	↑	↓	↔	↔	↑	↔	↔	↔	↑	↔	↔	↔	↑	↔	↔	↔	↔	↓	↔	↔	↑	↔	↔	↔	↑	↔	↔	↔	↑	↔	↔	↔
Spruce Aboveground Biomass (g)	↔	↔	↓	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↓	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↑	↔	↑
Spruce Belowground Biomass (g)	--	↔	↓	--	--	↔	↔	--	--	↔	↔	--	--	↔	↔	--	--	↔	↓	--	--	↔	↔	--	--	↔	↔	--	--	↑	↑	--
Target Forb Cover	↑	↑	↑	↔	↑	↑	↑	↔	↑	↑	↑	↔	↑	↑	↑	↔	↑	↑	↑	↔	↑	↑	↑	↔	↑	↑	↑	↔	↑	↑	↑	↔
Invasive Species Cover	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔
Total Herbaceous Species Cover	↔	↓	↔	↓	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↓	↔	↔	↔	↓

Figures

Figure 2-1: Visual representation of stock type development for the 16 treatments. The 16 treatments consisted of two forb species, showy aster or fireweed. The two forb species were then grown in two separate container sizes, 512A or 615A. For each forb species, in each container size, 4 different sow dates, or "head starts for the spruce" were utilized, 6, 8, 10 and 12 weeks. The 17th stock type is the control or benchmark spruce, grown individually, in the industry standard 412A container size. In the surrounding pictures: As = Aster, Fw = Fireweed, 6wk = 6 week sow date, 8wk = 8 week sow date, 10wk = 10 week sow date, 12wk = 12 week sow date, 512A refers to the 512A stock size, 615A refers to the 615A stock size and Control is the benchmark spruce grown singly in the industry standard 412A stock size.

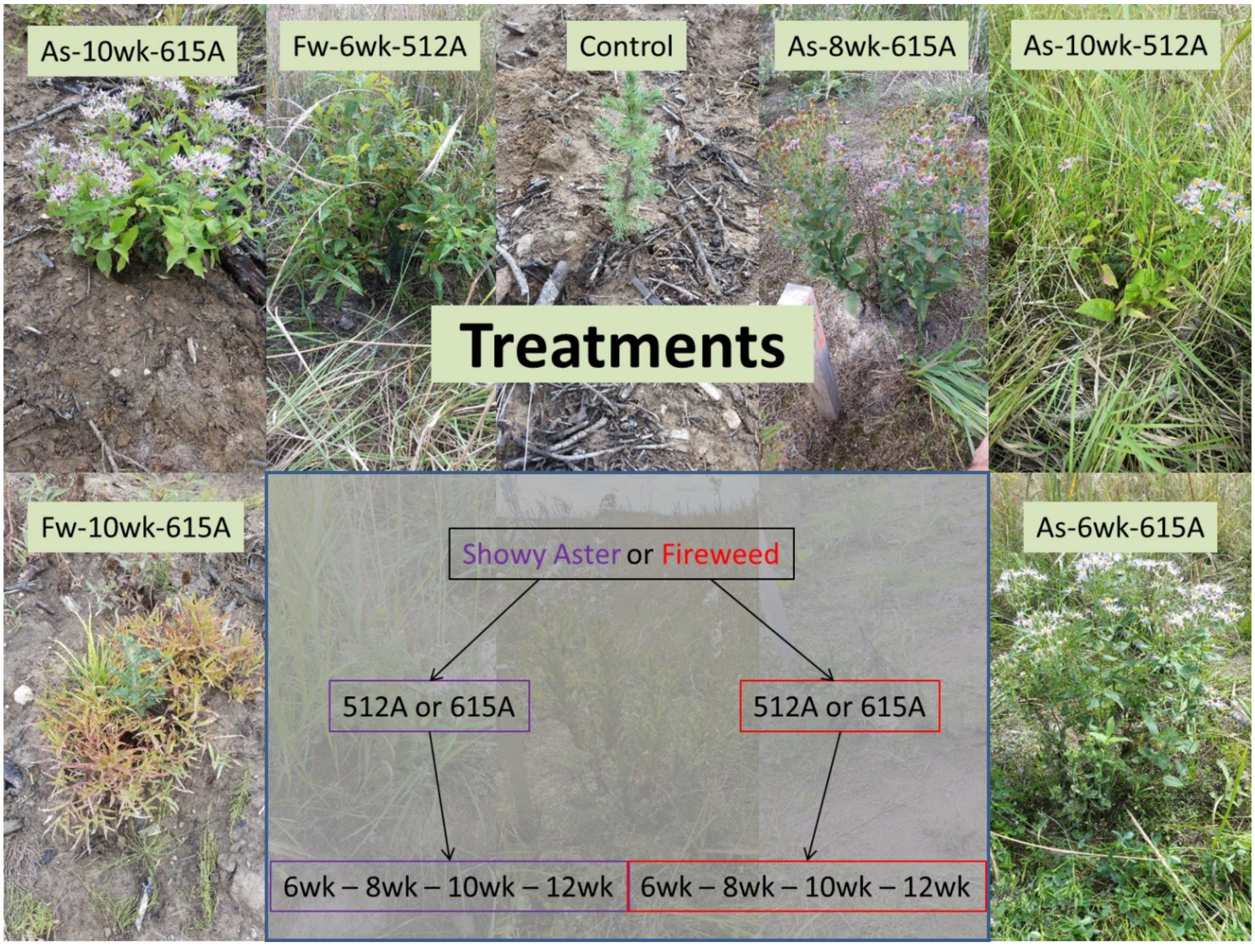


Figure 2-2: Site layout for the planting of the reclamation sites with the hitchhiker stock types. All 17 stock types (16 hitchhiker stock types plus control/benchmark) were randomized in order and out planted in 7 lines on each site. Each line is spaced 2 m apart. Within each line every stock type is made up of 3 replicates, for a total of 51 trees in each line, each planted 2 m apart and 21 individual plants per reclamation site. The overall size of the layout was 16m wide (including setbacks of 2m from the edges) by 102m long. The summarized treatment codes are listed below the layout.

Randomization of planting lines in each study site (block)						
line 1	**line 2**	**line 3**	**line 4**	**line 5**	**line 6**	**line 7**
15	7	15	7	13	3	7
11	14	1	2	15	10	11
3	11	13	15	12	16	17
14	16	14	1	8	11	1
10	15	5	13	11	17	10
4	10	6	9	1	1	2
8	1	12	16	10	15	13
17	17	16	17	16	6	4
6	8	9	6	3	2	12
16	6	2	3	14	4	5
9	3	3	8	4	8	16
7	13	11	14	7	5	14
1	2	7	12	5	9	3
2	9	4	5	9	12	6
13	5	10	11	2	13	9
5	12	17	4	17	14	8
12	4	8	10	6	7	15

trt code	forb	Sow date	Stock size
1	aster	6wk	512A
2	aster	8wk	512A
3	aster	10wk	512A
4	aster	12wk	512A
5	fireweed	6wk	512A
6	fireweed	8wk	512A
7	fireweed	10wk	512A
8	fireweed	12wk	512A
9	aster	6wk	615A
10	aster	8wk	615A
11	aster	10wk	615A
12	aster	12wk	615A
13	fireweed	6wk	615A
14	fireweed	8wk	615A
15	fireweed	10wk	615A
16	fireweed	12wk	615A
17	control	none	412A

Figure 2-3: Timeline of planting and sampling events. Each event occurred over an approximately two week timeframe.

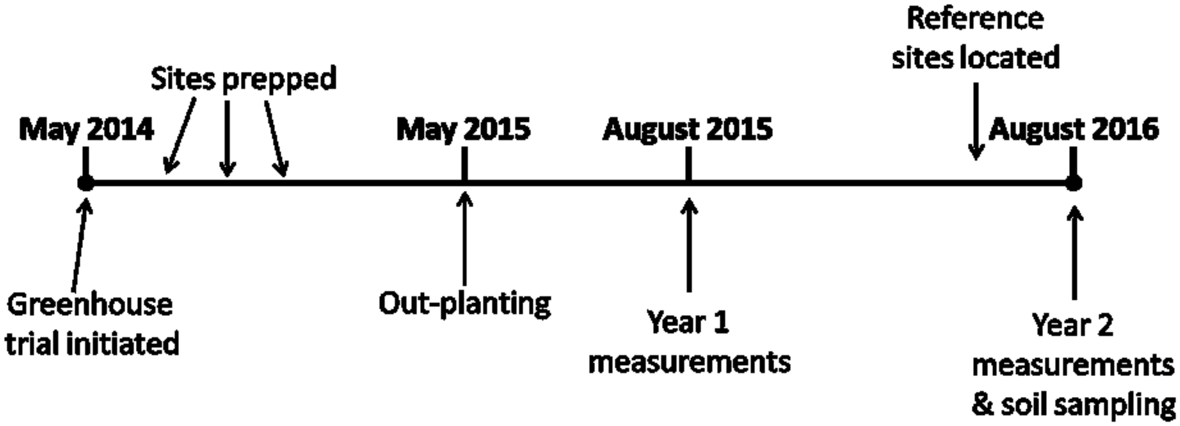


Figure 2-4: Target forb (fireweed) aboveground biomass (g) results from the 2016 growing season. Forb sowing period on the x axis refers to the time delay from sowing the spruce seed to sowing the forb seed. The 512A and 615A groupings refer to the two different stock sizes used. Error bars represent the 95% confidence interval of the mean (n=7). Significance is determined by Tukey's post-hoc test with a p-value<0.05. Note the variation in axis scale required to see stock type differences between the sites. Panel (a) has results from the BP1 site, (b) OSE2, (c) AS1 and (d) OSE1.

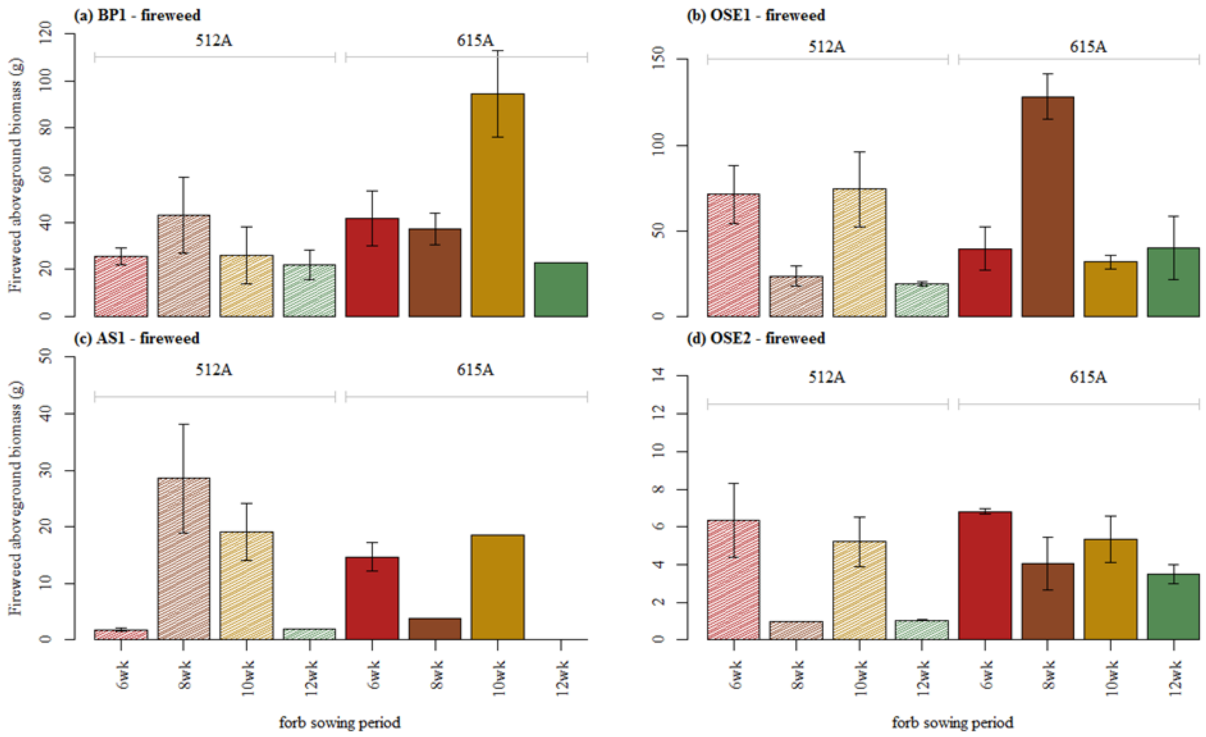


Figure 2-5: Target forb (fireweed) belowground biomass (g) results from the 2016 growing season. Forb sowing period on the x axis refers to the time delay from sowing the spruce seed to sowing the forb seed. The 512A and 615A groupings refer to the two different stock sizes used. Error bars represent the 95% confidence interval of the mean (n=7). Significance is determined by Tukey’s post-hoc test with a p-value<0.05. Note the variation in axis scale required to see stock type differences between the sites. Panel (a) has results from the OSE2 site and (b) from AS1.

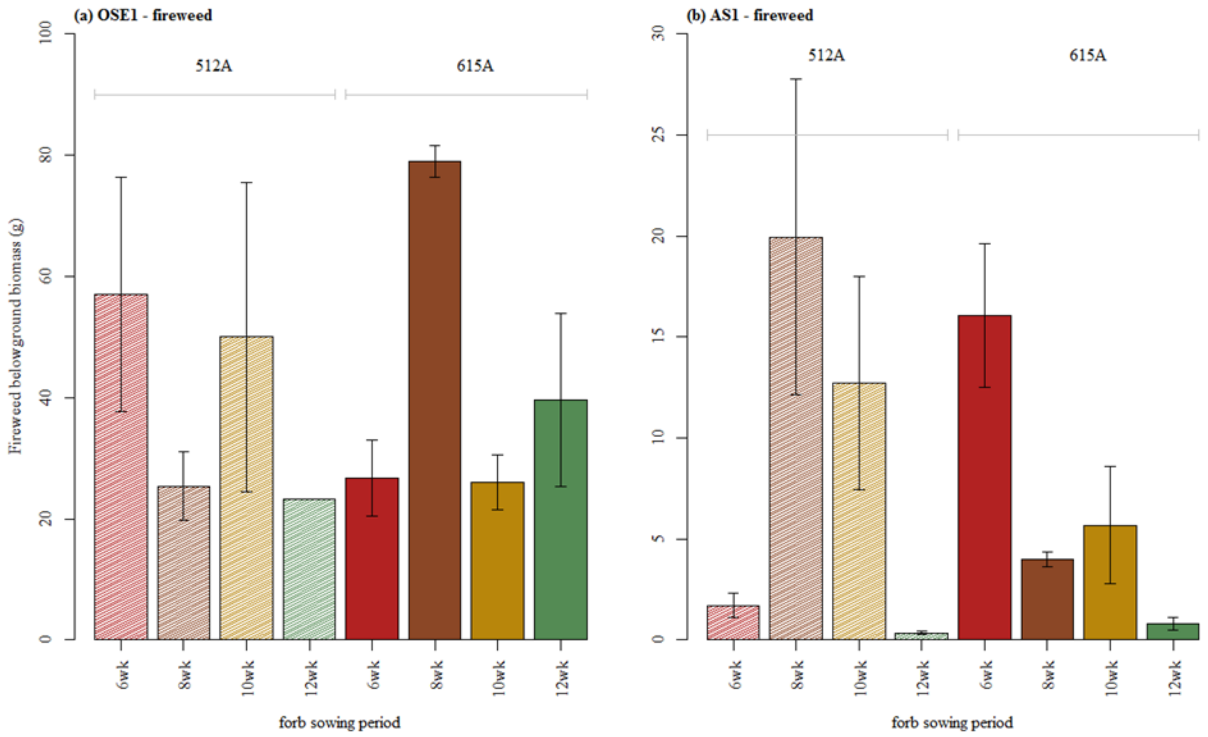


Figure 2-6: Target forb (aster) aboveground biomass (g) results from the 2016 growing season. Forb sowing period on the x axis refers to the time delay from sowing the spruce seed to sowing the forb seed. The 512A and 615A groupings refer to the two different stock sizes used. Error bars represent the 95% confidence interval of the mean (n=7). Significance is determined by Tukey's post-hoc test with a p-value<0.05. Note the variation in axis scale required to see stock type differences between the sites. Panel (a) has results from the BP1 site, (b) OSE2, (c) AS1 and (d) OSE1.

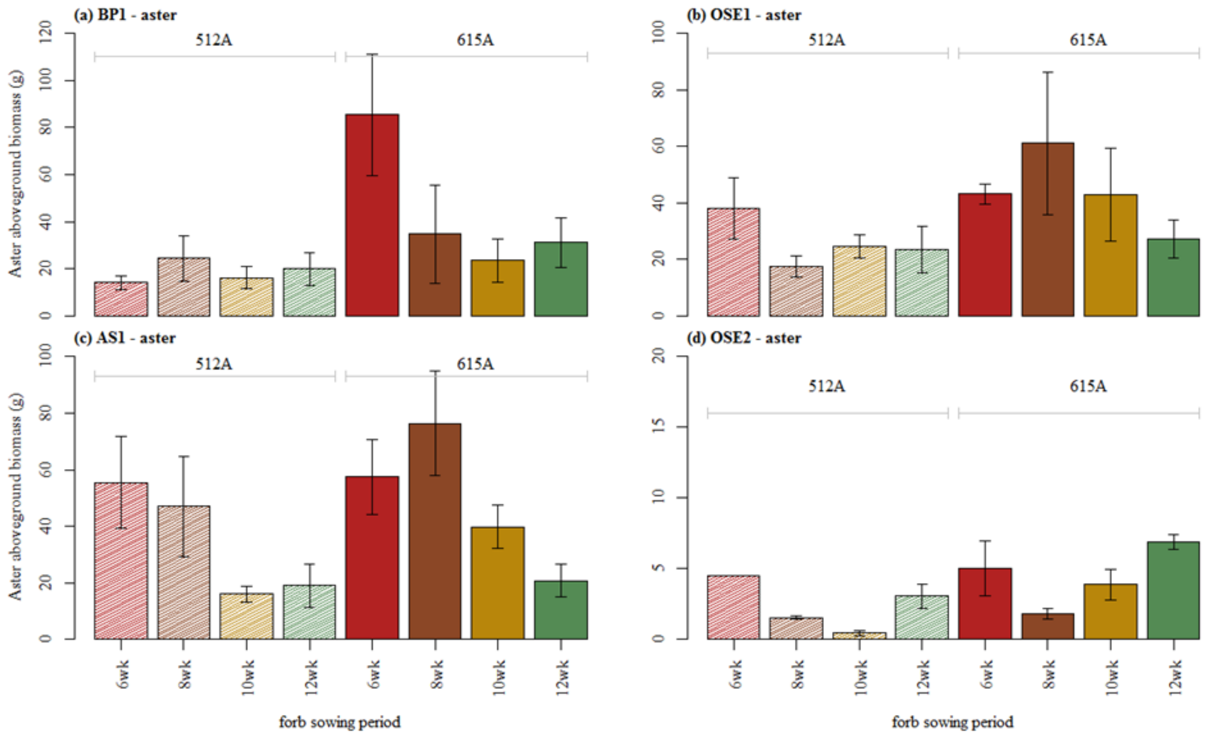


Figure 2-7: Target forb (aster) belowground biomass (g) results from the 2016 growing season. Forb sowing period on the x axis refers to the time delay from sowing the spruce seed to sowing the forb seed. The 512A and 615A groupings refer to the two different stock sizes used. Error bars represent the 95% confidence interval of the mean (n=7). Significance is determined by Tukey's post-hoc test with a p-value<0.05. Panel (a) has results from the OSE2 site and (b) from AS1.

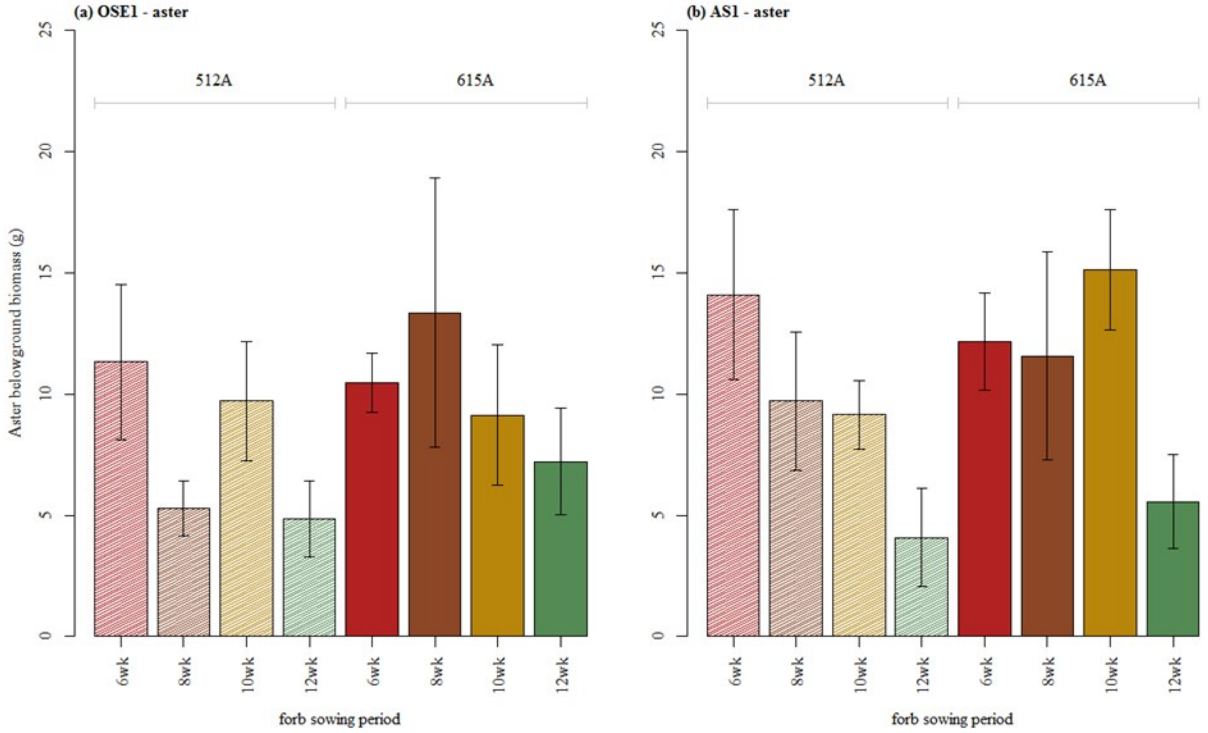


Figure 2-8: Target forb (fireweed) root expansion in terms of longest lateral root and number of suckers results from the 2016 growing season. Forb sowing period on the x axis refers to the time delay from sowing the spruce seed to sowing the forb seed. The 512A and 615A groupings refer to the two different stock sizes used. Error bars represent the 95% confidence interval of the mean (n=7). Significance is determined by Tukey's post-hoc test with a p-value<0.05. Note the variation in axis scale required to see stock type differences between the sites. Panels (a) and (c) have results from the OSE2 site, while (b) and (d) from AS1.

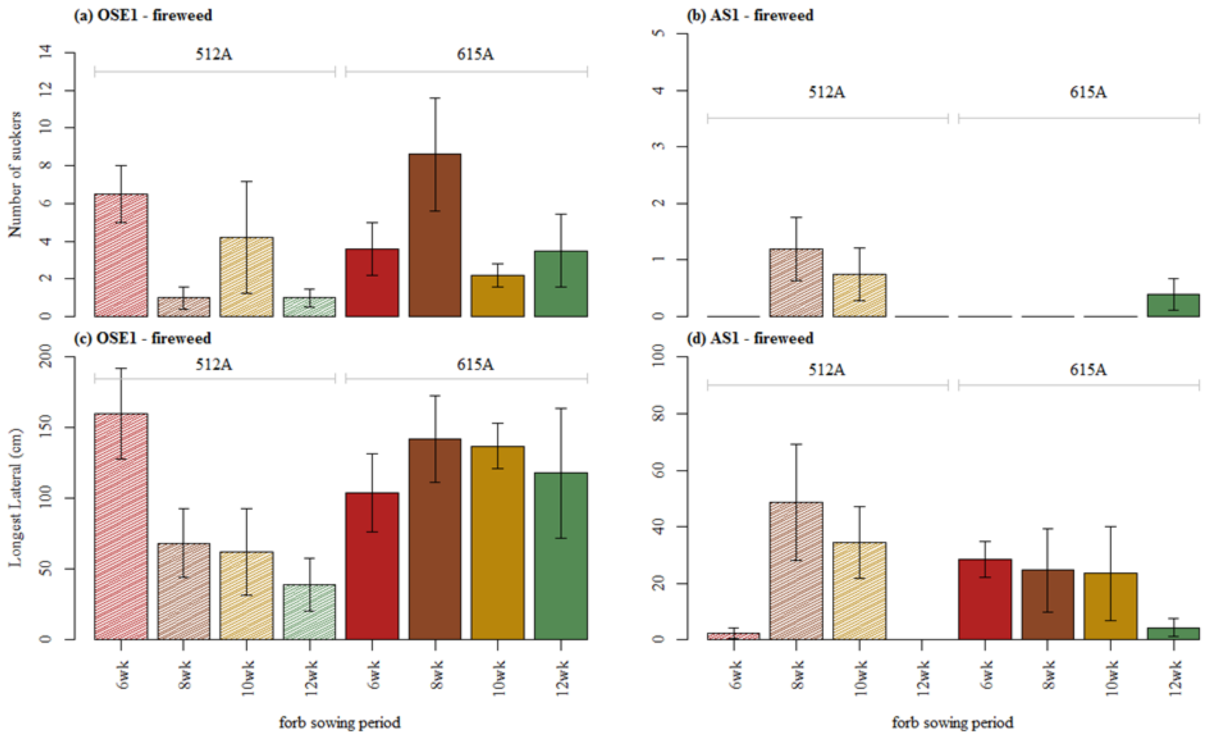


Figure 2-9: Target forb (aster) root expansion in terms of longest lateral root and number of suckers results from the 2016 growing season. Forb sowing period on the x axis refers to the time delay from sowing the spruce seed to sowing the forb seed. The 512A and 615A groupings refer to the two different stock sizes used. Error bars represent the 95% confidence interval of the mean (n=7). Significance is determined by Tukey's post-hoc test with a p-value<0.05. Panels (a) and (c) have results from the OSE2 site, while (b) and (d) from AS1.

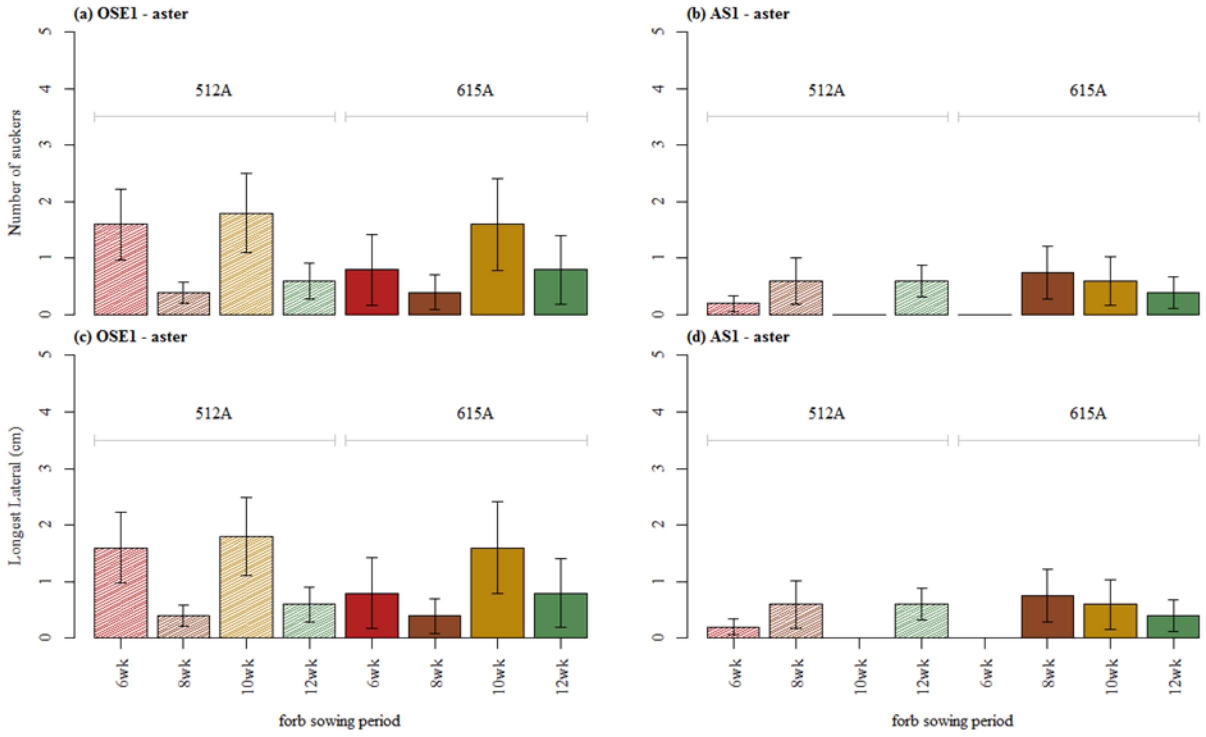


Figure 2-10: Percent cover of non-native, native, and grass species, as well as total cover versus aster cover for stock treatments grown with aster (n=21) results from the 2016 growing season. The non-linear regression equation (exponential) was determined as the one with the lowest AIC value. Panel (a) has the non-native species cover results ($y=20.49*(1-0.056)^x$), (b) the total cover results ($y=47.39*(1-0.04)^x$), (c) grass species cover results ($y=14.09*(1-0.041)^x$) and (d) native species cover results ($y=9.21*(1-0.02)^x$).

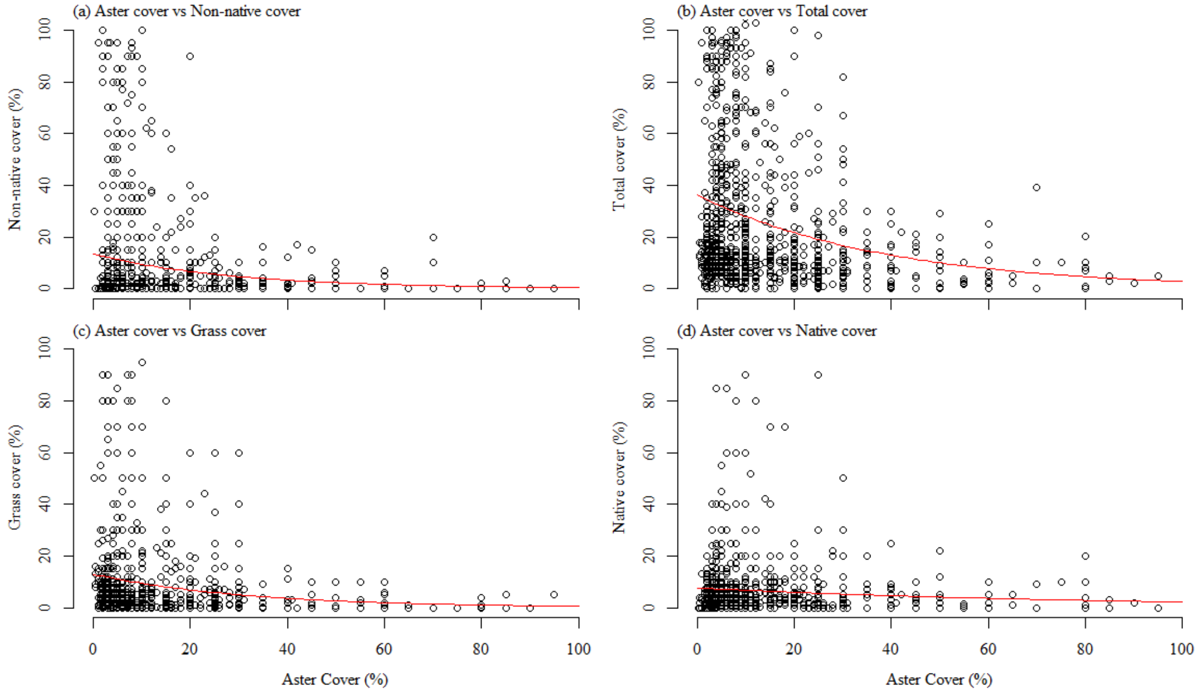
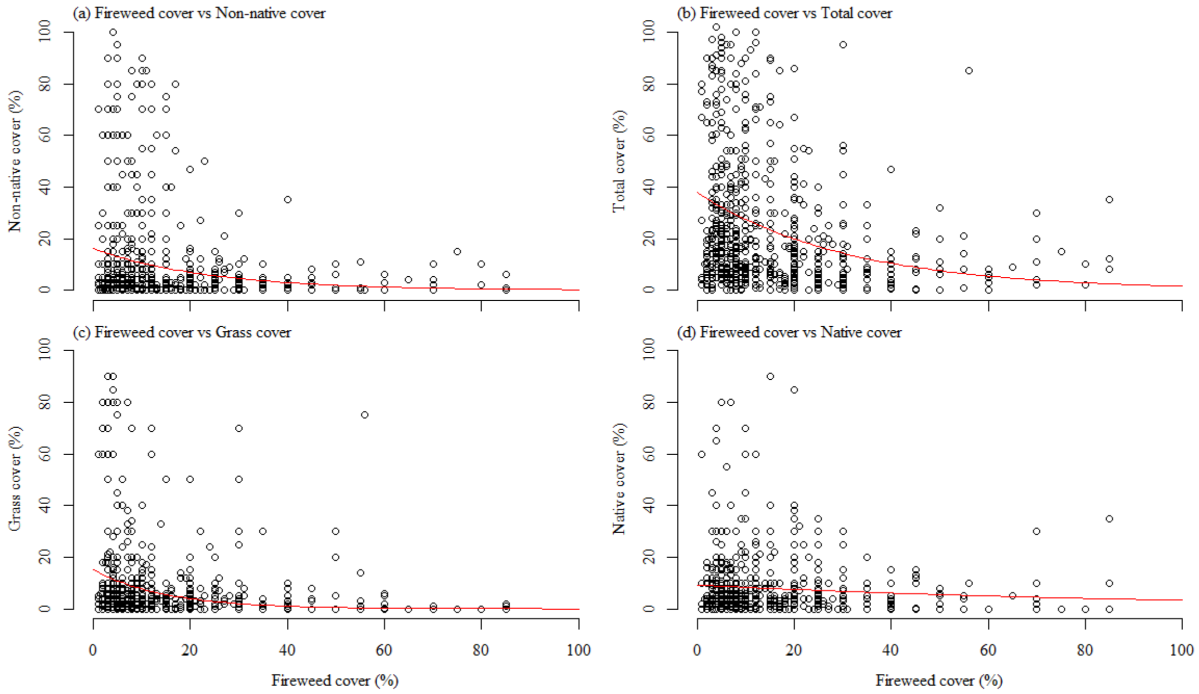


Figure 2-11: Percent cover of non-native, native, and grass species, as well as total cover versus fireweed cover for stock treatments grown with fireweed (n=21) results from the 2016 growing season. The non-linear regression equation (exponential) was determined as the one with the lowest AIC value. Panel (a) has the non-native species cover results ($y=19.74*(1-0.055)^{x}$), (b) the total cover results ($y=39.31*(1-0.034)^{x}$), (c) grass species cover results ($y=14.28*(1-0.06)^{x}$) and (d) native species cover results ($y=8.79*(1-0.009)^{x}$).



Chapter 3 - Restoring soil function in disturbed landscapes: Hitchhiking native forbs with white spruce

3.1. Introduction

Land reclamation in Alberta has historically been based on restoring "equivalent land capability", which has been measured with presence and health of desired vegetation (ESRD, 2013). Government and industry have indicated a desire to shift towards building resilient ecosystems (Pyper et al., 2013), but resiliency is not currently measured (Poscente & Charette, 2012). Areas affected by wildfire recover without human assistance and that natural resiliency should be the indicator of successful reclamation. Harvesting by forestry companies has achieved this resiliency (Kishchuk et al., 2015), but it is yet to be consistently observed in areas disturbed for oil and gas exploration. The 2010 Reclamation Criteria for Wellsite's and Associated Facilities for Forested Lands (ESRD, 2013) list requirements for landscape, soil, and vegetation parameters that will obtain equivalent land capability. Currently no guidelines outline any direct measures for determining if a reclaimed landscape is resilient.

Ecosystem functional similarity is based on the premise of measuring ecosystem parameters on disturbed sites, as well as reference or benchmark sites, and evaluating the similarity. With a resilient ecosystem being one that can recover from fire or other disturbance unassisted, a restored ecosystem that it functionally similar to a resilient site, will also be resilient (Markham & Essery, 2015; Kishchuk, 2015). While this is a simple ideology for determining resiliency of an ecosystem, it is not easily testable (Poscente & Charette, 2012). Limitations exist between which parameters are indicative of ecological function, how to test them, and if they are comparable between disturbances. The testing of belowground parameters for resilience is important as the aboveground community may not reflect the belowground community. This is reflected through the common use of substrates such as peat for excellent tree growth in horticultural situations, but the known fact that peat is not a replacement for a native forest soil in the long term.

Using traditional soil metrics to compare sites recovering from anthropogenic disturbances to those recovering from natural disturbances is confounded by the lack of soil disturbance. Wildfire can alter the soil chemically (Neary et al., 2005) and forestry harvesting has physical impacts (Block et al., 2002). Oil and gas exploration however involves physical movement of soil, and has impacts on physical (bulk density, aggregate stability) and chemical (nutrient availability, soil organic matter) properties (Osko & Glasgow, 2010; Wick et al., 2009; Abdul-Kareem & Mcrae, 1984).

Comparisons of soil microorganisms between sites is difficult, as the complexity and variability of soil creates challenges for quantifying the role of microorganisms in recovery from disturbance, and the underlying mechanisms remain poorly understood (Griffiths & Philipot, 2013). Research has demonstrated however, that we can assess the quality of soil by measuring characteristics of the microbial community (Harris, 2003). These measurements, which include community level physiological profiling (CLPP), microbial biomass carbon (MB-C), microbial biomass nitrogen (MB-N), soil respiration and microbial metabolic quotient (MQ); enable us to characterize microbial properties of soil and the effects of management practices aimed at restoring ecosystem resilience (Swallow et al., 2009; Hahn, 2012; Lewis et al., 2010).

Ecosystem functional similarity is an idea that has and has been used for comparison of disturbed sites to natural benchmarks in Alberta (Howell et al., 2016; Hogberg et al., 2017) and was previously used as part of an index for mine restoration in India (Mukhopadhyay et al., 2014) The input of ecological data into a multivariate statistical environment (PCA, NMDS) allows for the interpretation of dissimilarity between groups. This dissimilarity can be used to determine if restored environments are similar to benchmark or reference sites in terms of ecological function (expected gradient of Oil& Gas > Cut blocks > Wildfire), relative to the parameters entered into the ordination. There are limitations based upon the data entered, however continued development will allow for an understanding of critical parameters versus confounding parameters.

In the boreal forest of Alberta, wildfire is the natural disturbance regime, and forestry harvesting is prominent on the landscape (Natural Resources Canada, 2017). By utilizing the wildfire and harvested sites as benchmarks, we can ideally gauge the effects of soil removal and stockpiling on soil microbial properties via CLPP, MB-C, MB-N, soil respiration and MQ. This could provide valuable insight into the effects of industrial disturbance on the soil microbial population. The forest harvested sites show the effect of vegetation removal, with and without soil compaction in the same site (Serrouya & D'Eon, 2004), which is more similar to the effects of oil exploration/extraction than fire sites. Fire sites show the effect of vegetation removal on soil microbial properties but with no soil removal or compaction (Rowe, 1983). The fire sites do have changes to the microbial properties however. Fire is shown to reduce soil respiration, regardless of burn severity (Hammam et al., 2007). This is due to fire reducing the forest floor layer, and respiration following fire primarily coming from the mineral soil layer (Singh et al., 2008). As well the legacy effects of charcoal can have an effect on ecosystem carbon balance and ecosystem functioning (Pluchon et al., 2016). Evolutionary germination cues; such as the serotinous cones produced by pine trees show adaptation of ecosystems to fire (Greene et al., 1998). This brings to light the question of whether the microbial community may have adapted to fire as well, although the exact response of the microbial community is relatively unknown (Griffiths & Phillipot, 2013; Smith et al., 2008; Dooley & Treseder, 2012; Tas et al., 2014).

Current forestry operations are designed to mimic the effects of wildfire (Serrouya & D'Eon, 2004), and if the effect of industrial disturbance can be reclaimed to closely resemble that of the disturbance of forestry or fire, the likelihood of successful reclamation or restoration should increase. Measuring the heterotrophic microbial function allows for an assessment of below-ground organismal activity (Gartzia-Bengoetxea et al., 2016; Lalor et al., 2007), while measurement of soil respiration and microbial biomass C/N allows for additional characterization of microbial properties, which can be compared to the reference sites. From this comparison, we can gain an understanding if our sites are on a trajectory to a similar functioning and resilient ecosystem, and if hitchhiker planting has an effect.

Following wildfire and forestry harvesting, a flush of native vegetation is observed, which includes fireweed (*Chamerion angustifolium*) and showy aster (*Eurybia conspicua)* (Province of British Columbia, 1997). On reclamation sites however, a flush of invasive species is often observed (Bauman et al., 2015). This suggests a need for improving native forb establishment by hitchhiker planting (see chapter 2) on reclamation sites, which could increase the similarity in terms of ecosystem function to the reference sites.

The variability of microbial properties between seasons due to changes in soil temperature and moisture restricts its use as a directly measurable parameter in future restoration guidelines. Comparing functional properties in the laboratory will allow us to understand the changes in microbial properties between sites and treatments, while excluding the dynamic nature of microbial properties between seasons (Cruz-Martinez et al., 2012; Durán et al., 2014).

By comparing the stock types to the control, and benchmark sites, we can obtain a value of microbial properties relative to a state of recoverable disturbance, while also seeing if the hitchhiker planting has a positive effect. This relative value has potential to be used in future restoration guidelines (Howell et al., 2016; Hogberg, 2017); specifically if soil quality is addressed as an issue as microbial properties comparable to a reference site is a positive sign of a healthy soil environment.

The overall research question for this chapter is to determine if companion planting a native herbaceous species with a white spruce seedling improves microbial properties on recently reclaimed industrial disturbances. We used two points of reference for this evaluation: (1) white spruce seedlings grown without a companion plant and (2) white spruce seedlings located on similarly aged disturbances recovering from wildfire or forest harvesting. The two objectives for this research question are:

(1) Will hitchhiker planting have an effect on soil microbial properties?
(2) How do soil microbial properties on the study sites compare to sites recovering from forestry harvesting and wildfire?

3.2. Methodology

3.2.1. Site Selection

The reclamation sites were established in 2015. Two sites (OSE1 and OSE2) were former oil sands exploration wells while the other two sites (BP1 and AS1) were located on a former airstrip. Each site was laid out in a series of 7 lines. The lines were 2m apart, and each line had the 17 stock types (See Chapter 2 for stock type details) planted in a random order, each 2m apart. Each stock type in each line consisted of 3 replicates, planted 2m apart (Figure 2-2). Please refer to chapter 2 for a detailed breakdown of the stock types and control.

Site conditions were variable across the study (Table 2-1). The BP1 site was the topsoil storage site for the airstrip, and the soils were coarse textured and prepared with a McNabb RipPlow (McNabb et al., 2013) before planting. AS1 had finer textured soils and topsoil was spread with a D7 cat before planting. The OSE1 site had fine textured soil which was spread with a D7 cat before planting. The OSE2 site had coarse textured soil and was prepared with a McNabb RipPlow (McNabb et al., 2013) before planting. All sites are located within the boreal forest, which has a mean annual temperature of around 0°C with average rainfall of 480mm (Alberta Parks, 2017) and has a dominant vegetation over story of *Picea glauca*, *Picea mariana*, *Pinus banksiana*, *Pinus contorta*, *Poplus tremuloides*, *Populus balsimifera* and *Betula papyrifera* (Canadian Encyclopedia, 2017) situated on primarily Brunisolic and Luvisolic soils in the uplands, with organic soils in the lowlands (Soil Classification Working Group, 1998). Please refer to Chapters 1 & 2 for additional information on the study area.

Fifteen reference sites were chosen. Four sites were located within 2011 wildfire burns, three within 2015 wildfire burns and the other eight were located within forest harvested sites (Harvested winter 2014/15). The forest harvest sites were planted in the same season (2015) as the study sites.

Wildfire sites were initially selected by determining suitable areas based on historical fire data using GIS. Suitable areas were cross referenced with imagery to locate upland areas for site scouting. Scouting was used to confirm that the wildfire sites were a comparable ecosite (d on the edatopic grid) and within 1.5km of an access road. In the 2015 wildfires, sites were established where there was sufficient white spruce density pre-fire to allow for natural regeneration (minimum 50% white spruce cover) and in the 2011 fires, sites were established where there was sufficient white spruce regeneration (minimum 5 seedlings from different mother trees).

Harvested sites were initially selected by determining suitable areas based on forest inventory maps. Suitable sites were selected within 200km of the reclamation sites, planted with white spruce , on comparable ecosites (d on the edatopic grid), and within 1.5km of an access road.

3.2.2. Soil monitoring

In May of 2016 Decagon Devices EC-5 soil moisture and temperature probes were installed at each of the 4 reclamation sites and 15 separate reference sites. Probes were installed by digging a 10cm deep and 120 cm long trench, and then placing the probes horizontally into the side of the trench at a depth of 10cm into the mineral soil layer, 30cm apart from each other. The order of the probes from the logger was kept consistent, and went moisture-temperature-moisture. The probes were retrieved in August of 2016, at the time of vegetation surveys, and the data offloaded for analysis.

3.2.3. Soil sampling

Soil from three stock types was sampled for analysis. The control was sampled, as well as the 10 week sow date, 615A plugs for both fireweed and showy aster. The 10 week 615A plugs were chosen as they showed the strongest forb development after accounting for unimpeded white spruce growth. Additionally three “Bulk Soil” samples were obtained from areas between planted stock types where planted vegetation was absent (n=3). From each reclamation site,7 soil samples per stock type were collected, with the replicate sample coinciding with those randomly selected for destructive harvest. This allowed for an overall n=7 per treatment, and n=28 when the reclamation sites are combined together.

At each of the forest harvest sites we identified 20 potential sampling points and randomly selected 3 of each for sampling. This allowed for n=3 for each site, and n=24 when combined. A suitable sampling point at a forest harvest site was a white spruce seedling, of comparable height and health to the seedlings onsite and was growing in a microsite that was comparable to the reclamation sites. At each of the 2011 wildfire sites, we identified 3 white spruce that had naturally established for sampling. This allowed for n=3 for each site, and n=12 if the sites are combined. At each of the 2015 wildfire sites, we identified 3 white spruce that had been killed by fire, and sampled among their roots. This allowed for n=3 for each site, and n=9 if the sites are combined.

Each soil sample was composed of approximately 200g collected from the rhizosphere (before destructive harvest occurred on the reclamation sites). The samples were obtained with a 2cm by 10cm push probe. Only mineral soil was sampled, and the LFH was removed on reference sites before sampling. All samples were placed in sealed plastic soil bags and frozen at -4°C until analysis.

3.2.4. EC/pH

The soils were allowed to thaw to room temperature (20°C) before analysis. Once the soil was thawed, 5g of soil was placed in 20ml of water and shaken for 30min. The mixture was then vacuum filtered through 5nm filter paper. The extracts were collected and pH and EC measured with Mettler Toledo Five Easy pH and electrical conductivity meters (Mettler Toledo Inc, Canada).

3.2.5. Soil Incubation

Soil samples were thawed to 4°C before processing. Approximately 150 g of soil was placed in a 500 mL glass mason jar for incubation, brought up to approximately 60% of field capacity as measured on a pressure plate apparatus and sealed. The jars were placed in an incubator at a temperature of 25°C. Every 48 hours the jars were aerated for 5 minutes and the moisture level brought back to 60% of field capacity. The soils were incubated for 14 days before testing.

3.2.6. Community Level Physiological Profiles

Community level physiological profiling (CLPP) was used to determine soil heterotrophic microbial community function by measuring respiration with differing carbon substrates. Forty-eight custom deepwell plates were used with 15 carbon substrates (See Appendix A) and one blank (Deionized H_20). The substrates and control were measured in triplicate. Soil was then transferred into the custom deepwells via a loading plate. The deepwells were then sealed with a custom rubber gasket that has 48 tiny holes, one in the center of each well. On top of the gasket a 48 well indicator plate was pressed on. The indicator plate contained a mix of agar and phenotheline indicator solution. The deepwell, gasket and indicator plate were clamped together to ensure an airtight seal. They were then placed in the incubator, at 25°C, for 6 hours. After 6 hours the apparatus was removed from the incubator, and the indicator plate was taken to the plate reader (Biotek® Synergy HT Microplate Reader) where % CO_2 was recorded as a measure of respiration for each of the substrates. The after 6 hour reading was compared to a baseline reading taken for each plate prior to the incubation, and we were able to calculate our final numbers ($\mu gCO_2\text{-}C \cdot g^{-1} \cdot hr^{-1}$) based on the methodology in the MicroResp™ Technical Manual (2015).

3.2.7. Soil Respiration

Following incubation, a LI-COR LI-8100A Automated Soil CO_2 Flux system with multiplexor was used for soil respiration measurements. The machine was preprogrammed with the chamber dimensions, pre-purge time (30 sec), observation time (90 sec) post-purge time (45 sec) and data output options so that the readings were made instantly when the soils were removed from the incubator. The CO_2 gas fluxes were measured by tracking the change in concentration of CO_2 within the closed chamber over time.

3.2.8. Microbial Biomass Carbon and Nitrogen

Following incubation, 5g of sample was combined with 40ml of K_2SO_4 in a plastic centrifuge tube. This was repeated for each sample in a glass test tube with 0.5ml of chloroform added. Two additional control samples were created, with and without chloroform, and no soil added. The tubes were shaken for four hour before being vacuum filtered with 5nm filter paper into clean centrifuge tubes (no chloroform samples) and clean boston rounds (chloroform added samples). All of the extracts were then bubbled for 20min to remove the chloroform before being transferred to clean centrifuge tubes. This process was an adaptation of the rapid direct chloroform extraction method adapted by Gregorich et al. (1990). The extracts were frozen for preservation before being analyzed for non-purgable organic carbon (NPOC) and total dissolved nitrogen (TDN) using a Shimadzu TOC-V Total Organic Carbon Analyzer (Shimadzu Corporation, Japan). Following analysis the methodology in Voroney et al. (2008) was used to obtain final numbers from the difference between the results for the chloroform and non-chloroform samples.

3.2.9. Plant available Nutrients

Resin exchange membranes were used for their unique ability to simulate biological ion “sinks” (Qian & Schoenau, 2002). They were placed in a subset of jars before incubation. The membranes were placed vertically in the soil, and were completely covered. Following the two week incubation the membranes were removed, and all soil particles rinsed off with deionized water. The membranes were then placed in sealed plastic bags with 50 ml of 0.5 M HCl, and shaken for 1 hour. The membranes were removed, and the effluent was analyzed for N using a

Shimadzu TOC-V Total Organic Carbon Analyzer (Shimadzu Corporation, Japan) and P, K, S, Ca, Mg, Mn, Cu, Fe, Zn, Na, Pb and B using an inductively coupled plasma-optical emission spectrometer (ICP-OES) (Thermo iCAP6300 duo, Thermo Fisher Corp., North America).

3.2.10. Statistical analysis

A linear mixed-effects analysis of variance (ANOVA) was performed using “lme4” package in R (R Core Team, 2015) to analyze the fixed effect of site on each of the environmental and microbial parameters (excluding CLPP). Assumptions of ANOVA were checked with diagnostic plots prior to the analysis for all sets of data. Model-estimated β coefficients of fixed effects were derived using “effects” package. The post hoc comparison of treatment means was compared using Tukey post-hoc tests with a significant difference determined by a $p\text{-value}<0.05$ (Tukey, 1949).

Non-metric multidimensional scaling (NMDS) with PCORD V6.0 software (McCune & Mefford, 2011) was used to view differences between stock types and sites for CLPP data using the Bray-Curtis dissimilarity matrix in ordination space (Bray & Curtis, 1957). Assumptions of NMDS were checked with diagnostic plots prior to the analysis for all sets of data. A general relativization was applied before analysis on the “slow-and-thorough” setting, and statistical significance was determined using the multiple response permutations procedure (MRPP) (McCune & Mefford, 2011) For examining ecological functional similarity, the process was adapted from Howell et al. (2016), where T values were used to determine dissimilarity between groups (sites) and A values represent within group (site) homogeneity with p-values confirming significance ($p\text{-value}<0.05$) (McCune & Mefford, 2011).

3.3. Results

3.3.1. Site Physical and Chemical Characteristics

Electrical conductivity was not significantly different among the reference sites, with all sites having very low values (relative to guidelines, ESRD 2013). The reclamation sites were significantly different from each other (Table 3-1), with the highest EC on OSE1, followed by AS1, BP1, and finally OSE2, which were in range with the reference sites.

Results for pH were very consistent across the reclamation and reference sites (Table 3-1), with few significant differences. The soils on AS1 and OSE2 were considered neutral (pH 6-8) while OSE1, BP1 and the reference sites were slightly acidic (pH <6, Table 3-1).

The different stock types showed no differences in plant available nutrients (Appendix A), so they were homogenized by site. Plant available nutrients were similar between all study and reference sites, however some statistical significant differences did exist (Tables 3-2 & 3-3). Calcium (Ca) was significantly higher on reclamation sites compared to reference sites. AS1 and OSE2 had significantly higher Copper (Cu) than all other sites (Table 3-3). Sulphur (S), total inorganic nitrogen (TIN), and iron (Fe) were also significantly higher on AS1 (Tables 3-2 & 3-3). Potassium (K) was significantly higher on the 2011 fire sites compared to the rest (Table 3-2). Magnesium (Mg) had significant differences, but no trends across sites (Table 3-2). Manganese (Mn) was significantly higher on the reference and OSE1 sites, compared to the other reclamation sites (Table 3-3).

3.3.2. Microbial Biomass Carbon, Nitrogen, Respiration and Metabolic Quotient

Microbial biomass carbon (MB-C), microbial biomass nitrogen (MB-N) soil respiration and metabolic quotient (MQ) were compared between the aster, fireweed and control stock types sampled, as well with the bulk soil samples individually on each reclamation site (Table 3-4).

The only significant differences between them were for MB-C and MB-N on BP1. For both parameters the aster stock type was significantly higher than the rest, with for MB-N the bulk soil sample also being significantly lower than all others (Table 3-4).

Results for MB-C, MB-N, soil respiration and MQ were combined by stock type for all the reclamation sites to incorporate the variability, and then compared to the reference sites (Table 3-5). No significantly different results were observed for any of the parameters.

Following the conclusion of lack of variability among the stock types, all stock types were combined on each reclamation site, to allow for an assessment of reclamation site microbial properties as compared to the reference sites. MB-C was variable across both reclamation and reference sites. The highest was on OSE1 (significantly higher), with the airstrip sites (AS1 and BP1) and cut block sites falling in the middle, and the fires and OSE2 being the lowest (Table 3-6). When looking at MB-N, soil respiration and MQ, slight variation occurred, but no significant differences were noted (Table 3-6). A similar trend to MB-C was observed for MB-N with OSE1 again being the highest; however the lowest was observed on the 2011 fires for MB-N. Soil respiration showed AS1 and the 2015 fires being substantially higher than all other sites, with no apparent trends. When looking at the microbial metabolic quotient (MQ) the sites with higher MB-C had a lower MQ, with the exception of AS1, due to its increased soil respiration (Table 3-6). No trend was apparent though within the study sites, while in the reference sites the fires once again separated themselves from the cut blocks, albeit not significantly.

3.3.3. Community Level Physiological Profile

Microbial function did not show significant differences between any of the stock types, on any of the sites, with the exception of the aster treatment on the BP1 and OSE1 sites (Table 3-7). When all stock types were combined by site, there were significant differences between reclamation sites in terms of microbial function (Table 3-8). The BP1 and OSE2 were significantly different from OSE1 and AS1, as well as each other (Figure 3-1). When the reference sites were analyzed the same way, there were also significant differences between them. Both fire sites were significantly different from cut blocks and each other (Table 3-9, Figure 3-2).

Significant differences were observed between the study and reclamation sites when all were combined together (Table 3-10, Figure 3-3). The sites separated out with BP1 on one end, leading to OSE2 and AS1, which was closer to OSE1, both cutblocks and the 2015 fires and finally the 2011 fires separated themselves out. In order to visualize the relative differences between sites, reclamation and reference sites were compared in a pictograph based on T-values from MRPP analysis (Figure 3-4). The OSE1 site was the closest reclamation site to reference sites in terms of microbial properties. There is no apparent order amongst the reclamation sites when compared to the reference sites.

All microbial parameters (CLPP, MBC, MBN, soil respiration and MQ) along with the plant available nutrient data were analyzed in the main ordination matrix (Figure 3-5). The only significant differences observed was the OSE2 site separating itself from OSE1, BP1 and the 2011 Fires (Table 3-11).

Microbial function was analyzed along with plant available nutrients in the main ordination matrix (Figure 3-6). The only sites not significantly different from each other were CB2 and both fires, along with OSE1 not being significantly different than CB2 and only just significantly different (p-vale=0.053) from the 2015 fire (Table 3-12).

3.4. Discussion

3.4.1. Site Characteristics

Site chemical characteristics appear to be influencing observations in the microbial measurements, but the exact influence is inconclusive. Soil moisture and temperature according to the data loggers was consistent across all sites, with no patterns present. Electrical conductivity did vary, with the two fine textured reclamation sites being significantly higher than all other sites, however all sites fell into the “good” rating (EC<2dS/m) for topsoil according to the Alberta Salt Contamination Assessment & Remediation Guidelines (AEP, 2001), which indicates no negative effects on plant growth (with some agricultural exceptions). The same sites that had higher EC, had higher pH which actually fell within the Alberta Tier 1 Soil and Groundwater Remediation Guidelines (pH = 6 - 8.5) (AEP, 2016) with the remaining sites actually falling below the lower threshold and being slightly acidic. This however is typical of forest soils in Alberta (Alberta Agriculture and Forestry, 2017), and does not affect plant growth of common boreal species (Zhang, 2015). Soil pH is a driver of microbial community structure (Fierer & Jackson, 2006) and higher (or very low) pH can have a negative effect on microbial populations (Dimitriu et al., 2010; Högberg et al., 2007). This suggests that pH should be closely monitored in restoration projects, however; the difference in pH on our reclamation sites does not appear to be causing an effect on the microbial community, as no correlating trends were noted among the reclamation sites in the CLPP results. Plant available nutrient data by itself showed no trends that would be indicative of our expected disturbance gradient (Oil& Gas > Cut blocks > Wildfire); however, patterns were present based on site conditions. Some results, such as the increased TIN on AS1 can be explained by invasive sweet clover, a nitrogen fixing legume, while differences in S were likely caused by increased subsoil close to the surface, which can have highly variable S contents in Alberta (AER, 2015). The only difference of note is the Mn results. All study sites were lower in Mn when compared to the reference sites. Additionally the reclamation site with the highest results for Mn (OSE1) was also the most similar in terms of microbial function. Mn is garnering attention in the soil science community as an essential micronutrient due to its critical role in litter decomposition rates (Berg et al., 2015; Keiluweit et al., 2015). When the CLPP data was ordinated with the nutrient data, OSE1 was not significantly different from half of the reference sites, and was again the most similar of the reclamation sites to the reference sites. While no formal conclusions can be interpreted from the limited sampling, it does suggest an interesting avenue to explore in future research.

3.4.2. Comparisons between Stocktypes

A consistent result for all the parameters measured was that stock type did not consistently have any significant effect, with the exception of aster results for MB-C, MB-N and CLPP on BP1; and CLPP on OSE1 (Tables 3-4 & 3-7). It is interesting to note that all differences in microbial parameters involved aster as the forb species, however the limited significant results does not allow a definitive answer on if aster is influencing microbial properties. It is likely that the secondary vegetation community is not currently measurably affecting the microbial community in terms of function, but community assemblage could vary (Hannam et al., 2006). The primary vegetation was the same at each stock type sampling point (white spruce) which could be why function does not consistently vary. However, samples taken from soil without any vegetation on each site showed no difference from the stock types in terms of microbial function (and for all other microbial parameters excluding MB-C and MB-N on BP1) (Table 3-4) which suggests there was no vegetative effect on soil microbial function. It is likely too early for that effect to show up, as the plants are still in the early stage of colonization (Province of British Columbia, 1997; Royer & Dickinson, 2007; Phillips, 2012). The differences that have been observed on a site level are likely caused by varying site conditions (Dimitriu et al., 2010; Howell et al., 2016; Griffiths & Phillipot, 2013; Bach et al., 2008). Differences that were observed showed general groupings around soil properties and disturbance type; which indicates that initial site characteristics and differences caused by disturbance type (ie. charcoal input) may play an important role in microbial function early on by altering microbial habitat, growth rates and sorptive abilities (Pietikainen et al., 2000; Zackrisson et al., 1996).

3.4.3. Comparisons between Sites

Microbial function, as measured by CLPP, as an indicator of ecosystem resilience using ordination space and T values provided by MRPP analysis in terms of ecological functional similarity holds merit even though hitchhiker planting may not have an effect. The heavily disturbed reclamation sites separated themselves from the fire sites, which we know are resilient ecosystems (Markham & Essery, 2015). The cut blocks fell in between the reclamation and wildfire sites, which is expected as the effect of wildfire is what forestry disturbances are trying to emulate (Serrouya & D'Eon, 2004); since it is the natural disturbance regime in the boreal forest (Larsen, 1996). This disturbance gradient (Oil& Gas > Cut blocks > Wildfire) correlates with the expected resiliency of these natural disturbances based on previous research. (Hannam et al., 2006; Serrouya & D'Eon, 2004; Markham & Essery, 2015; Klischuk et al., 2015). Continued monitoring may show an effect of hitchhiker planting on microbial function, and may push reclamation sites closer to reference sites (according to T values) in terms of ecological functional similarity. Regardless of hitchhiker effect, we have showed evidence that CLPP is an effective measure of microbial function, and is appropriate for use in evaluating the ecological functional similarity of disturbed sites to reference sites or benchmarks (Howell et al., 2016; Hogberg et al., 2017; Lalor et al., 2007; Markham & Essery, 2015; Klischuk et al., 2015). For all additional microbial parameters (MB-C, MB-N, soil respiration, MQ), no apparent trend was observed that could be correlated to expected site resilience, as was indicated by the CLPP results. Observed individually reference fires had the lowest results for MB-C which was congruent with results from another study in the same reference area by Smith et al., (2008) which indicated it was likely the heat of the fire killed a substantial portion of the microbial biomass. Similar results were observed when looking at microbial biomass nitrogen, where the fires had one of the highest and one of the lowest results, however there were no significant differences, and all could be considered low, which is consistent with other findings that indicated MB-N is quite variable with disturbance (Smith et al. 2008). The highest results for both MB-C and MB-N were observed on the OSE1 site. This site had the most vegetation cover, however wAs dominated by undesirable species, primarily sweet clover. Soil respiration and metabolic quotient varied between sites, but nothing was statistically significant. The lack of significant differences is likely due to the extremely high variability in the data (S.E.>=result). This lack of significant results was evident when all microbial parameters, along with the plant available nutrient data were combined in the main matrix. The only significant differences observed were the separation of OSE2 from three other sites. The lack of significant results, or comparability to when the CLPP data was analyzed on its own, or with just the nutrient data, is likely due to the large amount variability in the results for the rest of the microbial parameters. This large variation is indicative of the issues of trying to simplify something as complex as the soil microbial community to easily measurable parameters.

3.5. Conclusion

No conclusive hitchhiker effect was seen in the end, which was likely due to the vegetation community not having had sufficient time to impact the microbial community. CLPP however is supported as an effective method for determining ecosystem resilience based on soil microbial function data as part of ecological functional similarity. Site conditions and characteristics were an important factor in soil microbial function, with disturbance type having a notable effect, as expected. The potential role of manganese warrants further investigation.

Tables

Table 3-1: Reclamation and reference site conditions based on 2016 data. Numbers represent the average of all stock types on a site. For soil temperature and moisture the numbers are an average of the entire growing season of May to August inclusive. For all other parameters, statistical significance is indicated by the superscript letter, which indicates a significant difference (p-value<0.05) according to Tukey's post-hoc significance test (n=24 for reclamation, n=18 for reference). Results are presented with the standard error.

Site Characteristics	Soil Moisture ($m^3 \cdot m^{3\ -1}$)	Soil Temperature (°C)	Electrical Conductivity ($dS \cdot m^{-1}$)	pH
BP1	0.247	13.837	0.823 ± 0.093^{b}	$5.18 \pm 0.13^{c,d}$
AS1	0.363	13.291	1.085 ± 0.093^{b}	7.09 ± 0.13^{a}
OSE2	0.231	14.061	0.464 ± 0.101^{c}	6.38 ± 0.14^{b}
OSE1	0.255	12.957	1.703 ± 0.093^{a}	5.58 ± 0.13^{c}
CB2	0.272	13.213	0.452 ± 0.086^{c}	4.79 ± 0.12^{d}
CB1	0.384	13.502	0.391 ± 0.107^{c}	4.76 ± 0.16^{d}
2011 Fire	0.256	11.869	0.255 ± 0.093^{c}	5.67 ± 0.13^{c}
2015 Fire	0.292	11.648	0.423 ± 0.086^{c}	4.71 ± 0.17^{d}

Table 3-2: Reclamation and reference site plant available macro nutrient results from the 2016 sampling event. Numbers represent the average of all stock types on a site. Statistical significance is indicated by the superscript letter, which indicates a significant difference (p-value<0.05) according to Tukey's post-hoc significance test (n=12). Results are presented with the standard error.

Plant Available Nutrients	Ca (μg·10cm2·14 days)	K (μg·10cm^2·14 days)	Mg (μg·10cm2·14 days)	TIN (μg·10cm2·14 days)	P (μg·10cm2·14 days)	S (μg·10cm2·14 days)
BP1	$3.01 \pm 0.16^{b,c}$	$0.085 \pm 0.056^{b,c}$	$0.546 \pm 0.041^{b,c,d}$	1.02 ± 48.5^{b}	$2.3\text{x}10^{-3} \pm 5\text{x}10^{-4\ a,b}$	0.102 ± 0.07^{b}
AS1	3.28 ± 0.16^{b}	0.057 ± 0.056^{c}	$0.535 \pm 0.041^{b,c,d}$	10.63 ± 48.5^{a}	$2.5\text{x}10^{-3} \pm 5\text{x}10^{-4\ a,b}$	1.063 ± 0.07^{a}
OSE2	5.01 ± 0.16^{a}	$0.09 \pm 0.056^{b,c}$	$0.647 \pm 0.041^{a,b,c}$	2.37 ± 48.5^{b}	$3.5\text{x}10^{-3} \pm 5\text{x}10^{-4\ a}$	0.238 ± 0.07^{b}
OSE1	3.17 ± 0.16^{b}	$0.094 \pm 0.056^{b,c}$	$0.528 \pm 0.041^{c,d}$	1.42 ± 48.5^{b}	$1.1\text{x}10^{-3} \pm 5\text{x}10^{-4\ b}$	0.143 ± 0.07^{b}
CB2	3.26 ± 0.16^{b}	$0.102 \pm 0.056^{b,c}$	$0.716 \pm 0.041^{a,b}$	3.70 ± 48.5^{b}	$1.4\text{x}10^{-3} \pm 5\text{x}10^{-4\ b}$	0.368 ± 0.07^{b}
CB1	1.66 ± 0.21^{d}	$0.373 \pm 0.073^{a,b}$	0.767 ± 0.053^{a}	0.44 ± 62.6^{b}	$9\text{x}10^{-4} \pm 6\text{x}10^{-4\ b}$	0.044 ± 0.09^{b}
2011 Fire	$2.34 \pm 0.15^{c,d}$	0.619 ± 0.052^{a}	$0.412 \pm 0.038^{d,e}$	0.83 ± 44.2^{b}	$3.6\text{x}10^{-3} \pm 4\text{x}10^{-4\ a}$	0.083 ± 0.07^{b}
2015 Fire	1.58 ± 0.21^{d}	$0.094 \pm 0.073^{b,c}$	0.303 ± 0.053^{e}	0.62 ± 62.6^{b}	$3\text{x}10^{-4} \pm 6\text{x}10^{-4\ b}$	0.062 ± 0.09^{b}

Table 3-3: Reclamation and reference site plant available micro nutrient results from the 2016 sampling event. Numbers represent the average of all stock types on a site. Statistical significance is indicated by the superscript letter, which indicates a significant difference (p-value<0.05) according to Tukey's post-hoc significance test (n=12). Results are presented with the standard error.

Plant Available Nutrients	B (µg· 10cm2· 14 days)	Cu (µg· 10cm2· 14 days)	Fe (µg· 10cm2· 14 days)	Mn (µg· 10cm2· 14 days)	Na (µg· 10cm2· 14 days)	Pb (µg· 10cm2· 14 days)	Zn (µg· 10cm2· 14 days)
BP1	6.6x10^{-4}±2x10$^{-4\ b}$	1.3x10-3±2x10$^{-4\ b,c}$	0.095±0.014^{a,b}	0.012±0.014^{c}	1.22±0.17^{a,b}	6x10^{-4}±1x10$^{-4\ b,c}$	2.2x10^{-3}±7x10$^{-4\ a}$
AS1	7.3x10^{-4}±2x10$^{-4\ b}$	2.410x-3±2x10$^{-4\ a}$	0.128±0.014^{a}	0.013±0.014^{c}	0.81±0.17^{a,b,c}	7x10^{-4}±1x10$^{-4\ b}$	4.4x10^{-3}±7x10$^{-4\ a}$
OSE2	1.81x10^{-3}±2x10$^{-4\ a}$	1.7x10-3±2x10$^{-4\ a,b}$	0.093±0.014^{a,b,c}	0.011±0.014^{c}	1.42±0.17^{a}	1.2x10^{-3}±1x10$^{-4\ a}$	4.5x10^{-3}±7x10$^{-4\ a}$
OSE1	3.2x10-4±2x10$^{-4\ b}$	4x10-4±2x10$^{-4\ d}$	0.032±0.0.014^{c}	0.046±0.014^{b,c}	0.12±0.17^{d}	3x10^{-4}±1x10$^{-4\ c,d}$	4x10^{-3}±7x10$^{-4\ a}$
CB2	1.6x10^{-4}±2x10$^{-4\ b}$	7x10-4±2x10$^{-4\ c,d}$	0.066±0.014^{a,b,c}	0.071±0.014^{b,c}	0.10±0.17^{d}	3x10^{-4}±1x10$^{-4\ c,d}$	4.2x10^{-3}±7x10$^{-4\ a}$
CB1	9x10^{-5}±3x10$^{-4\ b}$	4x10-4±2x10$^{-4\ d}$	0.063±0.018^{a,b,c}	0.09±0.018^{a,b}	0.49±0.21^{b,c,d}	3x10^{-4}±1x10$^{-4\ c,d}$	4.2x10^{-3}±9x10$^{-4\ a}$
2011 Fire	1.7x10^{-4}±2x10$^{-4\ b}$	2x10-4±2x10$^{-4\ d}$	0.055±0.013^{b,c}	0.137±0.013^{a}	0.33±0.15^{c,d}	3x10^{-4}±9 x10-$^{5\ c,d}$	4.3x10^{-3}±0.7x10$^{-4\ a}$
2015 Fire	8x10^{-5}±3x10$^{-4\ b}$	2x10-4±2x10$^{-4\ d}$	0.036±0.018^{b,c}	0.058±0.018^{b,c}	0.09±0.21^{d}	1x10^{-4}±1x10$^{-4\ d}$	2x10^{-3}±9x10$^{-4\ a}$

Table 3-4: Reclamation and reference site microbial conditions from the 2016 sampling event. Numbers represent the average of all stock types on a site. For all parameters, statistical significance is indicated by the superscript letter, which indicates a significant difference (p-value<0.05) according to Tukey's post-hoc significance test (n=24 for reference sites and n=18). Results are presented with the standard error.

Site Microbial Characteristics	Microbial Biomass Carbon (g·kg^{-1})	Microbial Biomass Nitrogen (g·kg^{-1})	Soil Respiration (ppm)	Metabolic Quotient
BP1	34.36±11.32^{a,b}	5.43±1.25^{b}	8.09±6.18^{a}	0.220±0.33^{a}
AS1	68.8±9.8^{b}	6.36±1.08^{b}	22.82±5.35^{a}	0.691±0.29^{a}
OSE2	16.68±11.32^{a}	3.77±1.25^{b}	7.74±6.18^{a}	1.21±0.33^{a}
OSE1	88.35±13.87^{b}	13.01±1.53^{a}	9.91±7.57^{a}	0.096±0.41^{a}
CB2	57.21±9.08^{a,b}	4.85±1.00^{b}	3.41±4.96^{a}	0.064±0.27^{a}
CB1	44.37±11.32^{a,b}	5.11±1.25^{b}	5.45±6.18^{a}	0.140±0.33^{a}
2011 Fire	33.68±12.01^{a,b}	3.23±1.33^{b}	5.44±6.56^{a}	0.207±0.35^{a}
2015 Fire	33.14±13.87^{a,b}	5.16±1.53^{b}	26.95±7.57^{a}	1.271±0.41^{a}

Table 3-5: Microbial function (as determined by CLPP results) within the reclamation sites from the 2016 sampling event. Each site is represented by a panel. Four different stock types are represented for each site. A 'Y' means the two stock types are significantly different from each other, and 'N' means they are not. Significant difference is determined by a p-value<0.05 (n=7 for treatments, n=3 for bulk soil) according to the results of a multiple response permutations procedure.

BP1	Aster	Fireweed	Control	Bulk Soil	**AS1**	Aster	Fireweed	Control	Bulk Soil
Aster	■	▨	▨	▨	Aster	■	▨	▨	▨
Fireweed	N	■	▨	▨	Fireweed	N	■	▨	▨
Control	Y	N	■	▨	Control	N	N	■	▨
Bulk Soil	N	N	N	■	Bulk Soil	N	N	N	■
OSE2	Aster	Fireweed	Control	Bulk Soil	**OSE1**	Aster	Fireweed	Control	Bulk Soil
Aster	■	▨	▨	▨	Aster	■	▨	▨	▨
Fireweed	N	■	▨	▨	Fireweed	N	■	▨	▨
Control	N	N	■	▨	Control	Y	N	■	▨
Bulk Soil	N	N	N	■	Bulk Soil	N	N	N	■

Table 3-6: MRPP results for Figure 3-1. Microbial function (as determined by CLPP) between reclamation sites. Significant difference is determined by a p-value<0.05 (n=25). Stress = 8.64616.

Groups Compared			T	A	P
OSE2	vs	OSE1	-3.509	0.0515	0.0089
OSe2	vs	BP1	-9.938	0.1319	$8.5x10^{-6}$
OSE2	vs	AS1	-2.294	0.0323	0.036
OSE1	vs	BP1	-13.771	0.205	$3.6x10^{-7}$
OSE1	vs	AS1	-1.831	0.0245	0.0583
BP1	vs	AS1	-12.06	0.1584	$8.5x10^{-7}$

Table 3-7: MRPP results for Figure 3-2. Microbial Function (as determined by CLPP results) between reference sites. Significant difference is determined by a p-value<0.05 (n=18). Stress = 9.57747.

Groups Compared			T	A	P
CB1	vs	2015 Fires	0.548	-0.00957	0.663
CB1	vs	CB2	-0.486	0.00591	0.257
CB1	vs	2011 Fires	-2.131	0.02912	0.039
2015 Fires	vs	CB2	-0.399	0.00533	0.284
2015 Fires	vs	2011 Fires	-0.826	0.01169	0.178
CB2	vs	2011 Fires	-4.022	0.04334	0.004

Table 3-8: MRPP results for Figure 3-3. Microbial function (as determined by CLPP results) between all sites. Significant difference is determined by a p-value<0.05 (n=24 for reclamation sites, n=18 for reference sites). Stress = 10.45852.

Groups Compared			T	A	P
OSE2	vs	OSE1	-4.301	0.055	0.002
OSE2	vs	BP1	-9.676	0.115	<0.001
OSE2	vs	CB1	-3.549	0.051	0.006
OSE2	vs	2015 Fires	-5.489	0.087	<0.001
OSE2	vs	CB2	-7.654	0.081	<0.001
OSE2	vs	2011 Fires	-8.008	0.1	<0.001
OSE2	vs	AS1	-2.388	0.029	0.027
OSE1	vs	BP1	-3.969	0.046	0.003
OSE1	vs	CB1	-1.434	0.019	0.089
OSE1	vs	2015 Fires	-2.984	0.042	0.011
OSE1	vs	CB2	-3.032	0.032	0.013
OSE1	vs	2011 Fires	-7.605	0.088	<0.001
OSE1	vs	AS1	-5.215	0.06	<0.001
BP1	vs	CB1	-8.855	0.115	<0.001
BP1	vs	2015 Fires	-10.409	0.115	<0.001
BP1	vs	CB2	-10.77	0.119	<0.001
BP1	vs	2011 Fires	-15.987	0.194	<0.001
BP1	vs	AS1	-7.925	0.082	<0.001
CB1	vs	2015 Fires	0.548	-0.01	0.663
CB1	vs	CB2	-0.486	0.006	0.257
CB1	vs	2011 Fires	-2.131	0.029	0.039
CB1	vs	AS1	-4.601	0.057	0.001
2015 Fires	vs	CB2	-0.399	0.005	0.284
2015 Fires	vs	2011 Fires	-0.826	0.012	0.178
2015 Fires	vs	AS1	-7.136	0.096	<0.001
CB2	vs	2011 Fires	-4.022	0.043	0.004
CB2	vs	AS1	-8.274	0.081	<0.001
2011 Fires	vs	AS1	-6.009	0.068	<0.001

Table 3-9: MRPP results for Figure 3-5. Microbial function (as determined by CLPP results), MB-C, MB-N, soil respiration, MQ and plant available nutrients between all sites. Significant difference is determined by a p-value<0.05 (n=24 for reclamation sites, n=18 for reference sites). Stress = 7.58013.

Groups Compared			T	A	P
OSE2	vs	OSE1	-3.24	0.0581	0.0163
OSE2	vs	BP1	-2.235	0.0373	0.0407
OSE2	vs	CB1	-1.292	0.0217	0.0975
OSE2	vs	2015 Fires	-1.675	0.0327	0.0682
OSE2	vs	CB2	-1.094	0.0216	0.1217
OSE2	vs	2011 Fires	-3.16	0.0497	0.0178
OSE2	vs	AS1	-1.622	0.0256	0.0722
OSE1	vs	BP1	-0.627	0.0101	0.1778
OSE1	vs	CB1	-0.727	0.0121	0.1621
OSE1	vs	2015 Fires	0.201	-0.0039	0.4126
OSE1	vs	CB2	-1.215	0.0239	0.1032
OSE1	vs	2011 Fires	0.219	-0.0034	0.4211
OSE1	vs	AS1	-1.941	0.0312	0.0533
BP1	vs	CB1	-0.789	0.0116	0.1524
BP1	vs	2015 Fires	-0.341	0.006	0.2356
BP1	vs	CB2	-0.961	0.0167	0.1313
BP1	vs	2011 Fires	-1.487	0.0215	0.0801
BP1	vs	AS1	-1.808	0.0267	0.0602
CB1	vs	2015 Fires	-0.48	0.0085	0.2063
CB1	vs	CB2	-0.996	0.0183	0.128
CB1	vs	2011 Fires	-0.86	0.0126	0.1416
CB1	vs	AS1	-1.314	0.0198	0.0945
2015 Fires	vs	CB2	-0.171	0.0036	0.2831
2015 Fires	vs	2011 Fires	-0.251	0.0042	0.258
2015 Fires	vs	AS1	-0.714	0.0123	0.1663
CB2	vs	2011 Fires	-1.168	0.0198	0.1078
CB2	vs	AS1	0.339	-0.0057	0.4974
2011 Fires	vs	AS1	-1.301	0.0185	0.0952

Table 3-10: MRPP results for Figure 3-6. Microbial function (as determined by CLPP results) and plant available nutrients between all sites. Significant difference is determined by a p-value<0.05 (n=24 for reclamation sites, n=18 for reference sites). Stress = 12.89549.

Groups Compared			T	A	P
OSE2	vs	OSE1	-5.775	0.081	<0.001
OSE2	vs	BP1	-9.089	0.12	<0.001
OSE2	vs	CB1	-2.698	0.04	0.022
OSE2	vs	2015 Fires	-6.747	0.101	<0.001
OSE2	vs	CB2	-7.947	0.13	<0.001
OSE2	vs	2011 Fires	-5.847	0.068	<0.001
OSE2	vs	AS1	-6.755	0.094	<0.001
OSE1	vs	BP1	-2.466	0.03	0.028
OSE1	vs	CB1	-6.268	0.078	<0.001
OSE1	vs	2015 Fires	-1.846	0.025	0.053
OSE1	vs	CB2	-3.619	0.051	0.045
OSE1	vs	2011 Fires	-2.175	0.022	0.037
OSE1	vs	AS1	-5.909	0.076	<0.001
BP1	vs	CB1	-8.011	0.093	<0.001
BP1	vs	2015 Fires	-7.5	0.099	<0.001
BP1	vs	CB2	-9.161	0.135	<0.001
BP1	vs	2011 Fires	-7.688	0.08	<0.001
BP1	vs	AS1	-11.497	0.15	<0.001
CB1	vs	2015 Fires	-6.202	0.083	<0.001
CB1	vs	CB2	-7.491	0.11	<0.001
CB1	vs	2011 Fires	-5.271	0.058	0.002
CB1	vs	AS1	-7.335	0.092	<0.001
2015 Fires	vs	CB2	0.461	-0.008	0.625
2015 Fires	vs	2011 Fires	-1.07	0.011	0.136
2015 Fires	vs	AS1	-3.287	0.046	0.009
CB2	vs	2011 Fires	-1.026	0.012	0.144
CB2	vs	AS1	-3.494	0.052	0.007
2011 Fires	vs	AS1	-3.147	0.034	0.012

Figures

Figure 3-1: Microbial properties by stock type on each reclamation site from the 2016 sampling event. For all parameters, statistical significance is indicated by the superscript letter, which indicates a significant difference (p-value<0.05) according to Tukey's post-hoc significance test. Results are presented with the standard error. Error bars represent the standard error of the mean (n=7 for treatments, n=3 for bulk soil results).

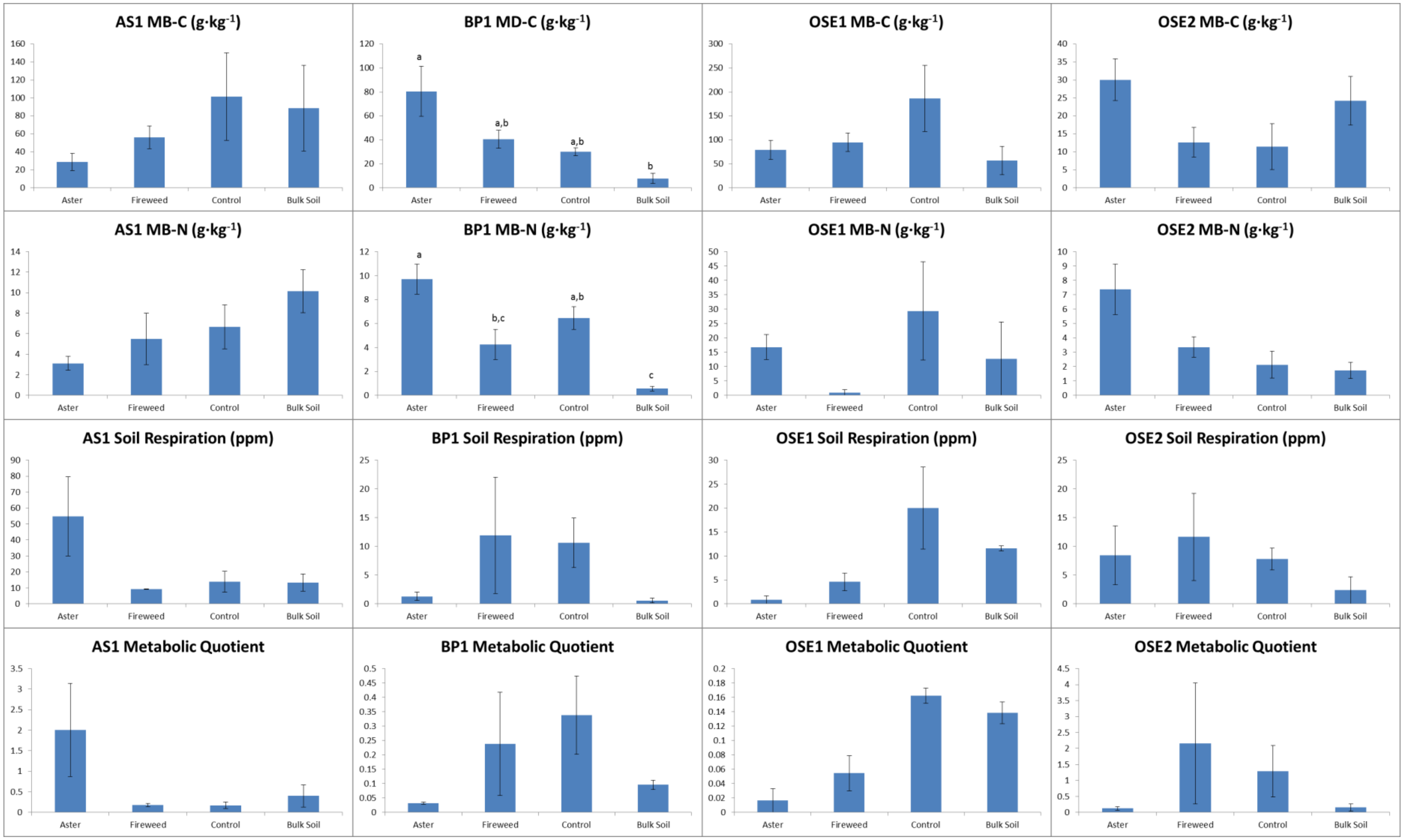


Figure 3-2: Hitchhiker stock type and reference site microbial properties from the 0016 sampling event. For all parameters, statistical significance is indicated by the superscript letter, which indicates a significant difference (p-value<0.05) according to Tukey's post-hoc significance test. Results are presented with the standard error. Error bars represent the standard error of the mean (n=21 for treatments, n=18 for reference sites, n=12 for bulk soil results).

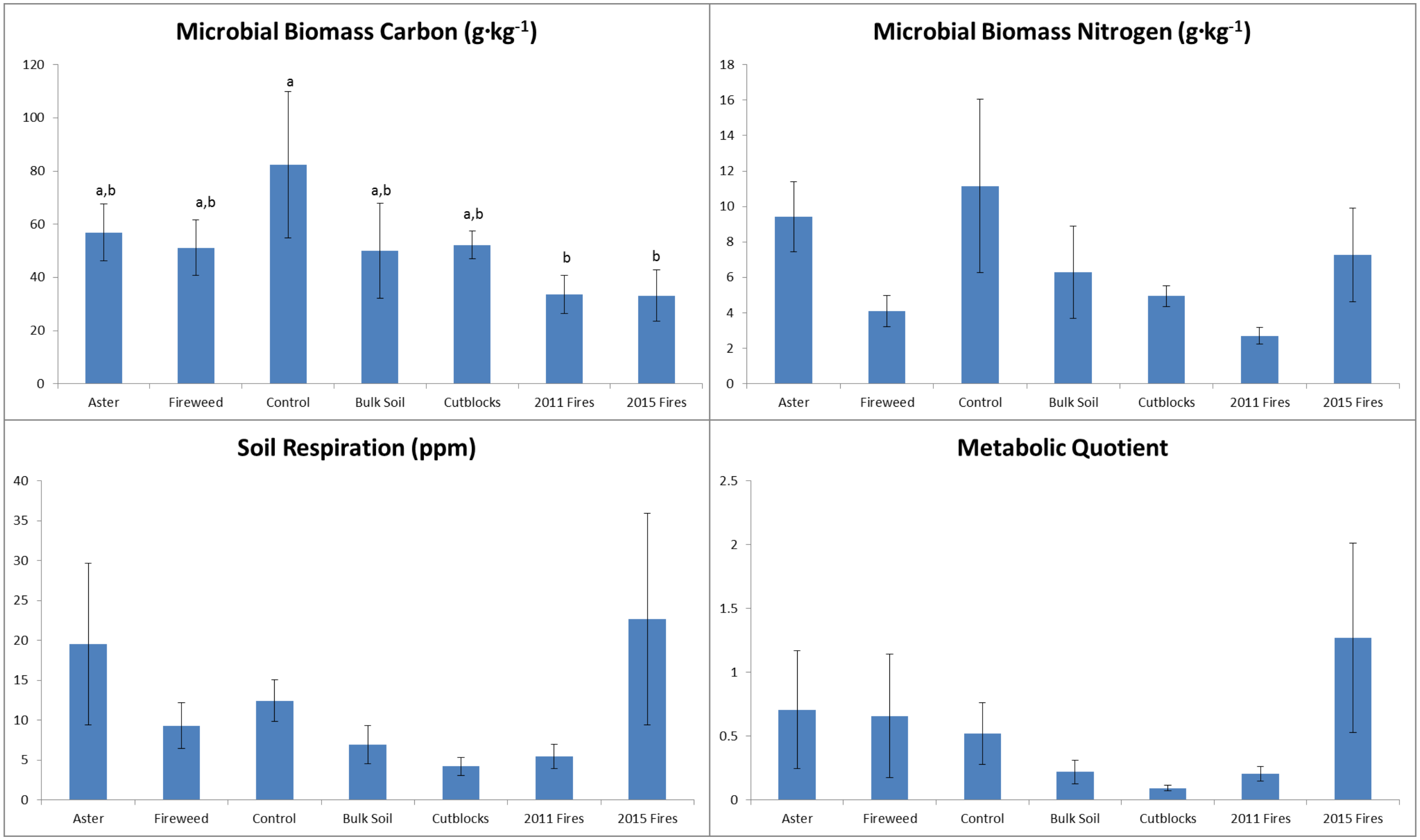


Figure 3-3: Microbial function on reclamation sites from the 2016 sampling event. The NMDS ordination shows the four study sites, with the stock types on each site combined for analysis. Percent variation of each axis is listed on the axis. Statistical information (including MRPP values) is listed in Table 3-8.

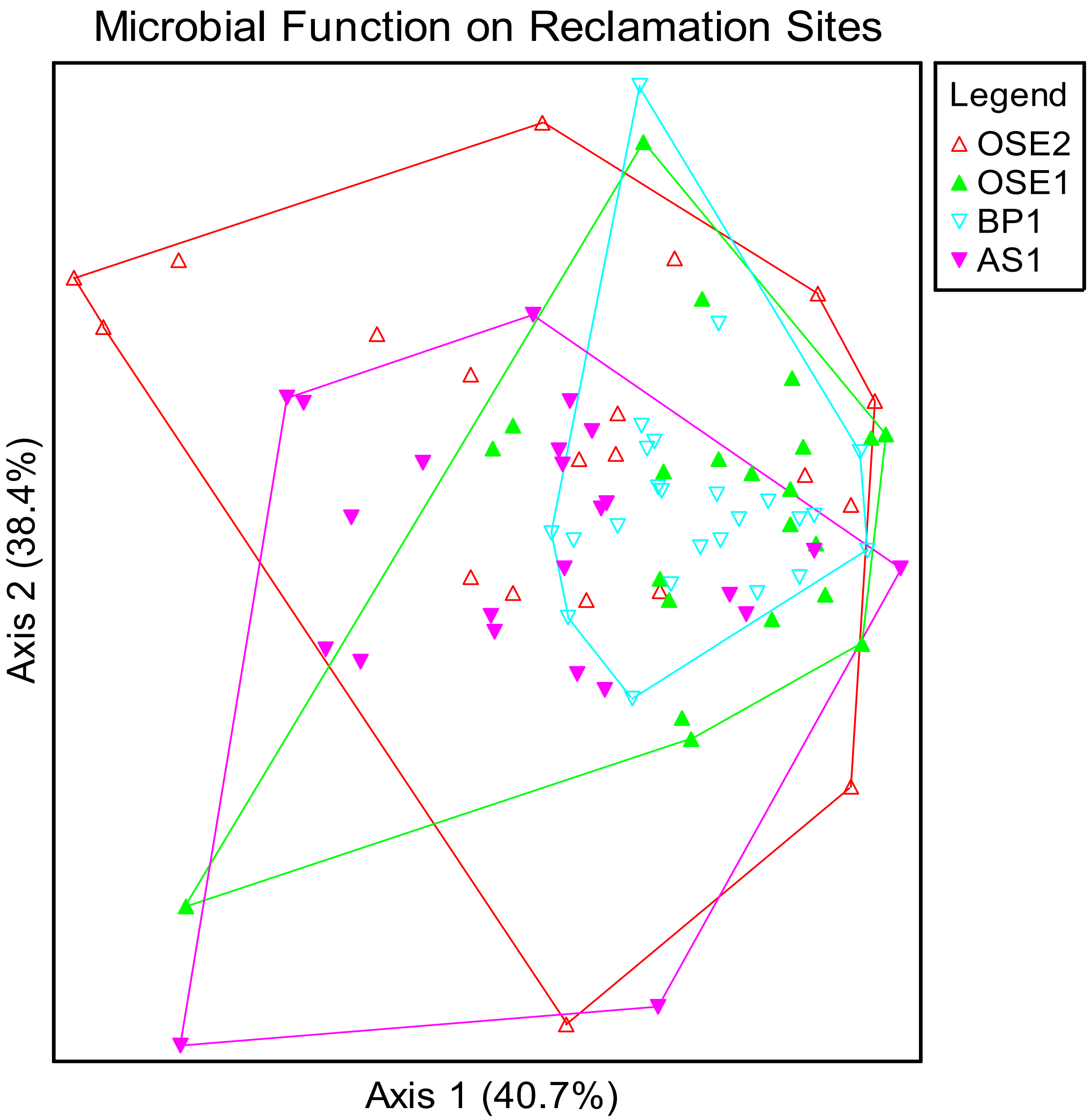


Figure 3-4: Microbial function on reference sites from the 2016 sampling event. The NMDS ordination shows the four reference site types, with the stock types on each site combined for analysis. Percent variation of each axis is listed on the axis. Statistical information (including MRPP values) is listed in Table 3-9.

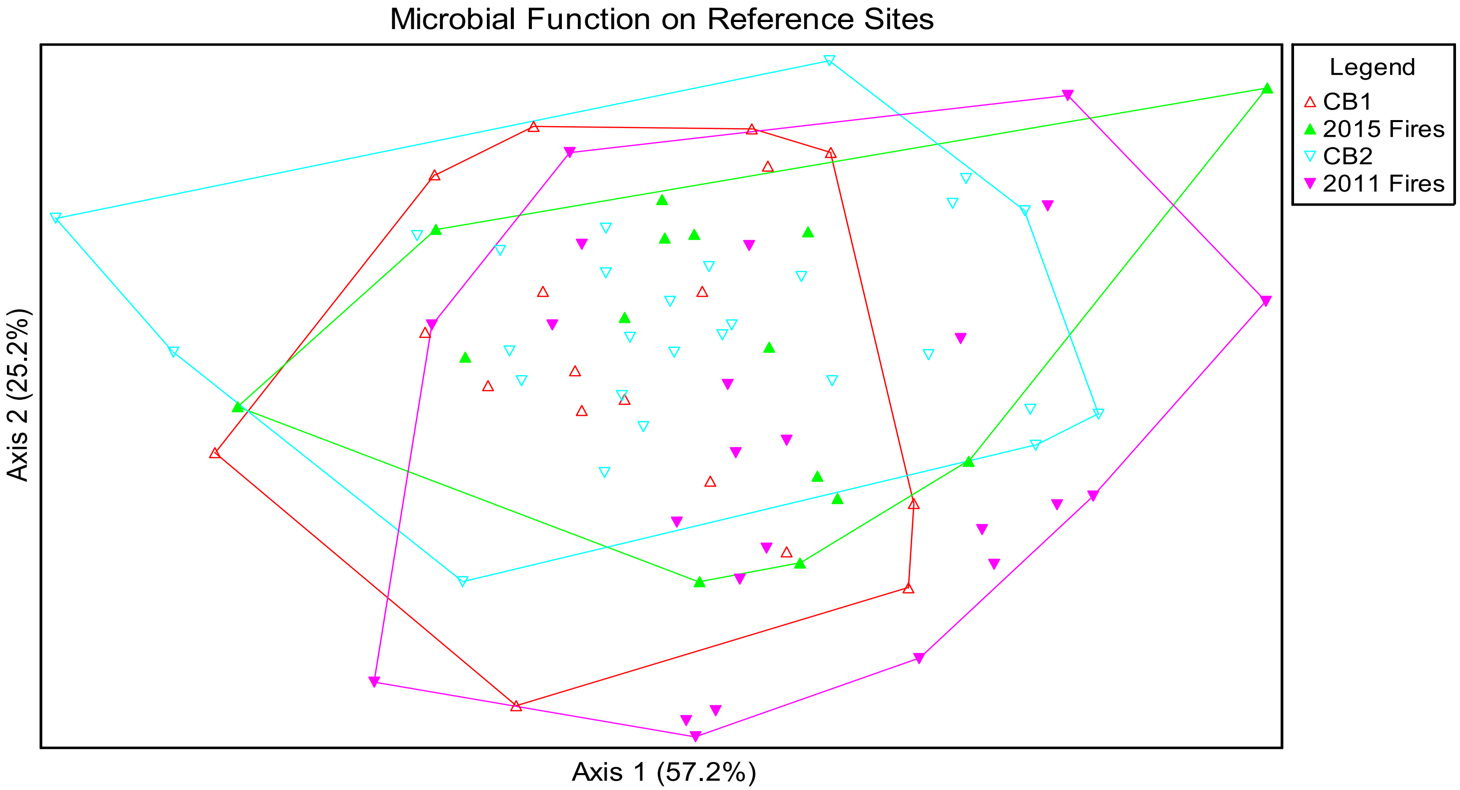


Figure 3-5: Microbial function on reclamation and reference sites from the 2016 sampling event. The NMDS ordination shows the four reference and four reclamation sites, with the stock types on each site combined for analysis. Percent variation of each axis is listed on the axis. Statistical information (including MRPP values) is listed in Table 3-10.

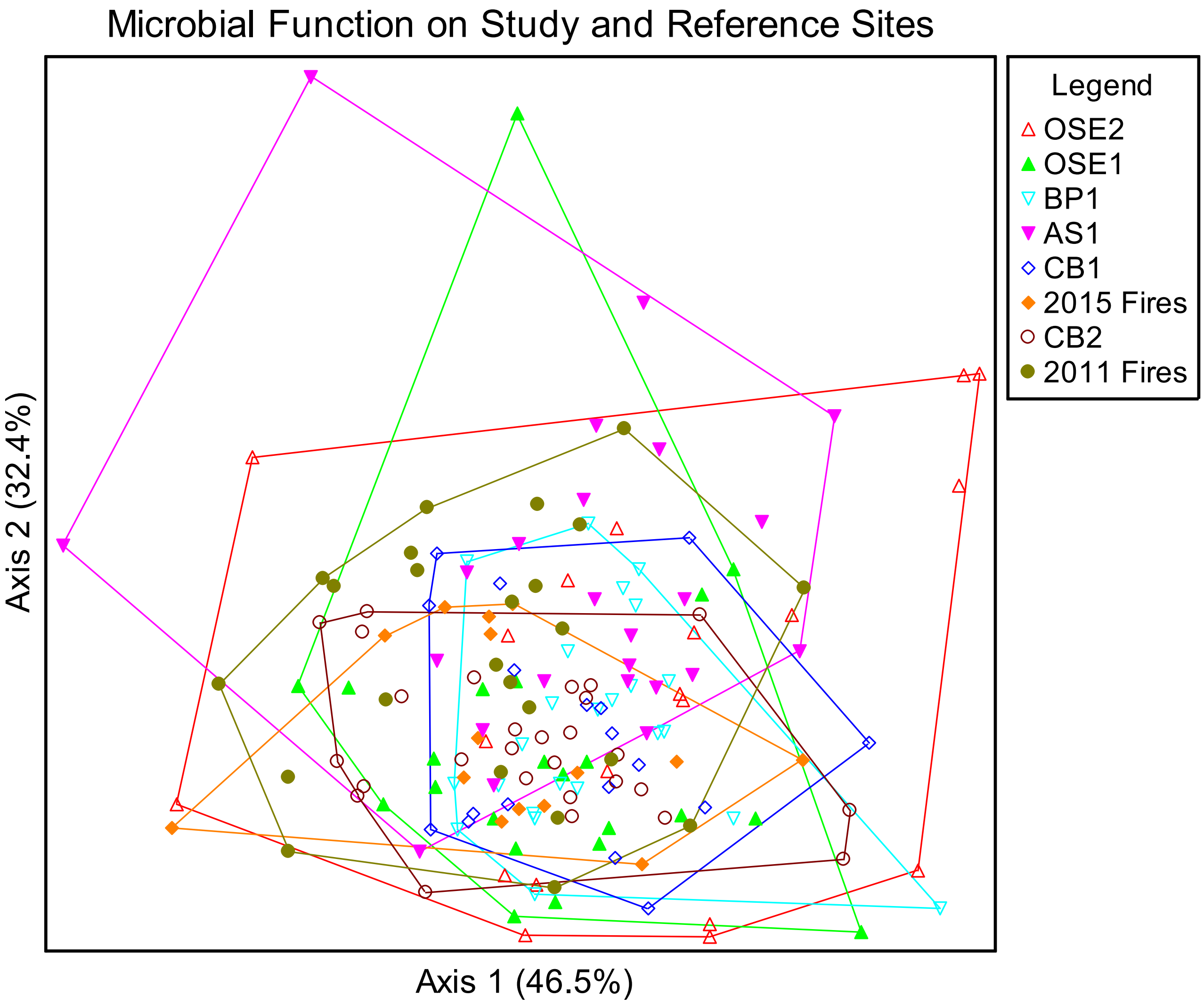


Figure 3-6: Pictograph representation of the data displayed in Figure 3-3. Orientation of the points is based off of the T values. Overlapping points are not significantly different from each other. Points that are separated are significantly different (p-value<0.05). Please refer to Table 3-10 for exact values.

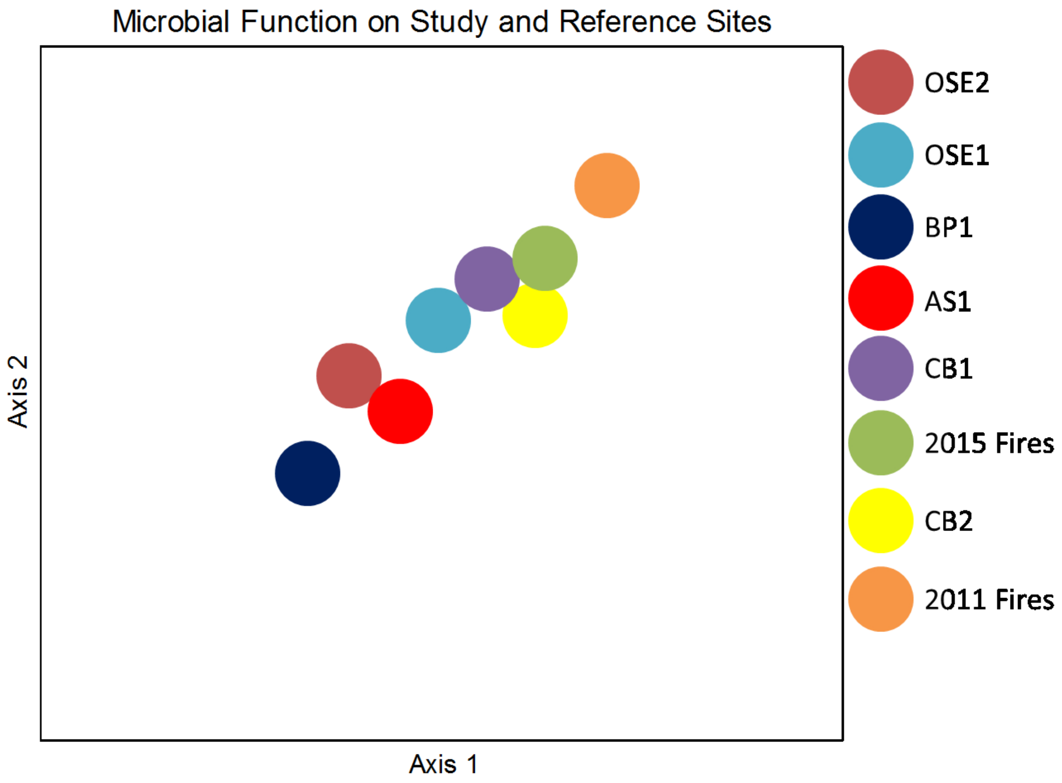
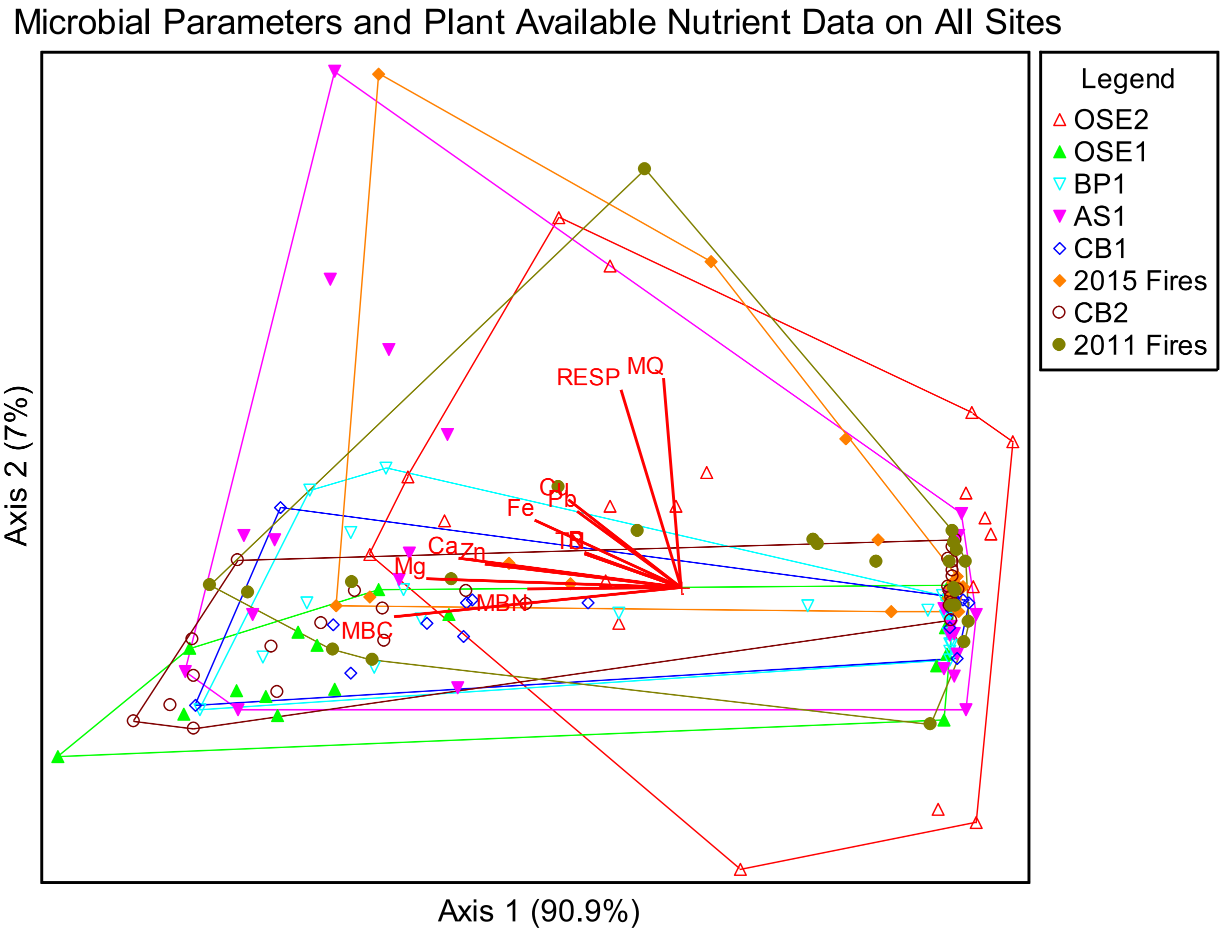
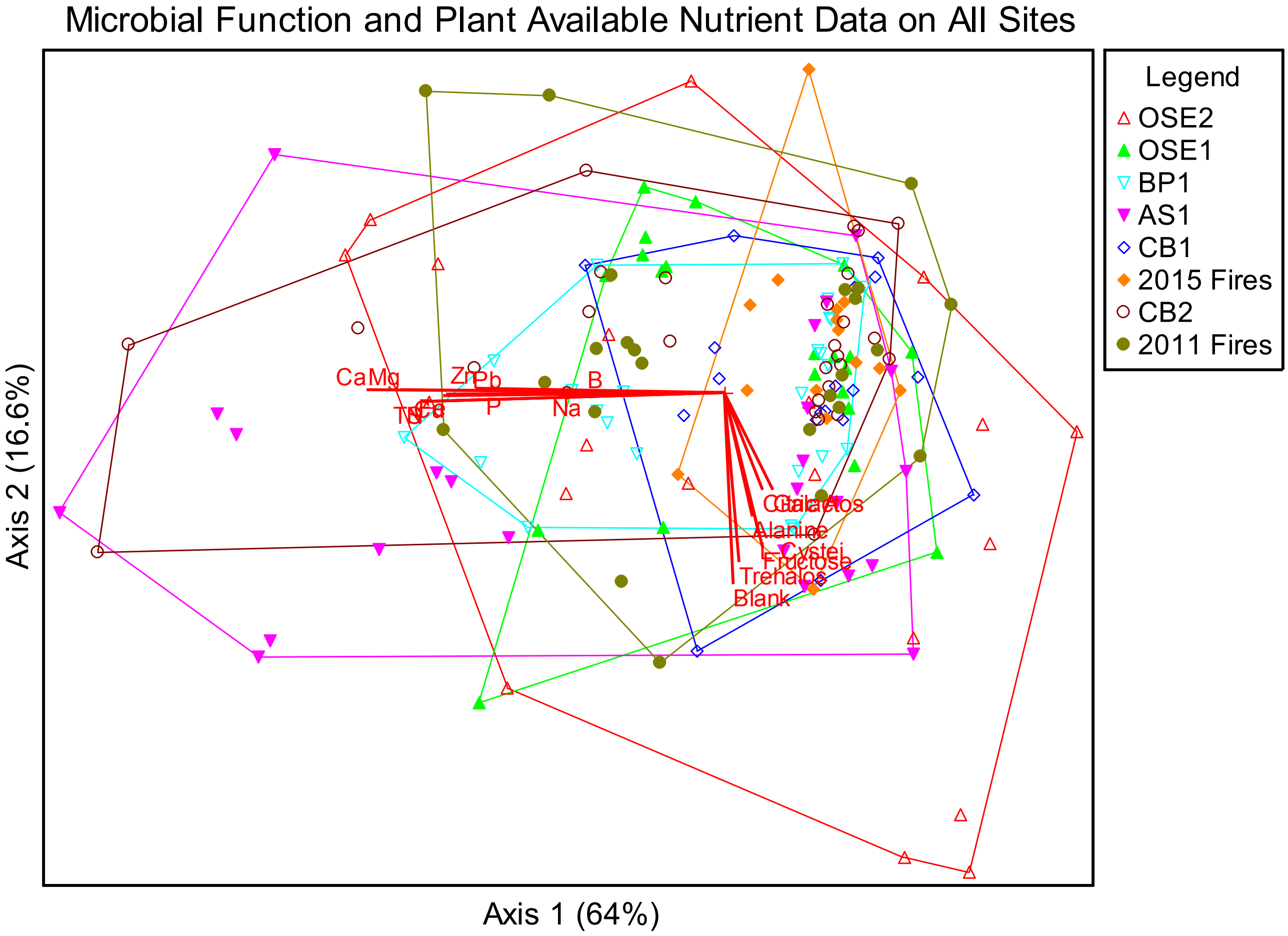


Figure 3-7: Microbial function, MBC, MBN, soil respiration, MQ and plant available nutrient data on reclamation and reference sites from the 2016 sampling event. The NMDS ordination shows the four reference and four reclamation sites, with the stock types on each site combined for analysis. Percent variation of each axis is listed on the axis. Statistical information (including MRPP values) is listed in Table 3-11.



Microbial Parameters and Plant Available Nutrient Data on All Sites

Figure 3-8: Microbial function and plant available nutrient data on reclamation and reference sites from the 2016 sampling event. The NMDS ordination shows the four reference and four reclamation sites, with the stock types on each site combined for analysis. Percent variation of each axis is listed on the axis. Statistical information (including MRPP values) is listed in Table 3-12.



Chapter 4 – Conclusion

4.1. Summary

The objective of the first half of the study was to determine if a viable hitchhiker stock type could be produced, and observe its aboveground effects. A total of sixteen different stock types varying by species stock size and sow date were created, and compared to a benchmark, initially in the greenhouse, and then across four different sites varying in soil texture and site preparation techniques. Our data showed that a stock type was able to be produced that had a comparable spruce in terms of total height, increment length and root collar diameter; and also increased target forb cover. The ideal stock type was the later sow dates (10-12 week) in preferably the larger (615A) stock size. Competition was observed between the hitchhiker forb and the spruce in the earlier sow dates and smaller stock size, which was to be expected based on previous research which demonstrates the competitive effect of forbs (especially fireweed (*Chamerion angustifolium*)) on white spruce (*Picea glauca*) (Province of British Columbia, 1997; Hangs et al., 2002; Bianco, 1990). Fireweed was more aggressive than aster (*Eurybia conspicua)*, which has positives for rapid site colonization, but management implications in careful stock type selection. In some cases the spruce was significantly taller than the benchmark, however this was due to the spruce utilizing the larger stock size in less competitive conditions (later sow date, less competitive forb). No effect on survival of the spruce was observed, however sow date did impact forb survival, with earlier sow dates being more favorable for the forb species. Trends indicate that expansion of the forbs will have an effect on reducing undesirable and non-native species cover over time.

In the second half of the study it was investigated if the inclusion of native forbs in restoration had an effect on the soil microbial community, and how the soil and its microbial community compared to other sites recovering from wildfire and forestry disturbance – deemed reference sites. There was no noticeable effect of hitchhiker planting on the soil microbial community outside of some instances where our aster treatments were significantly different from our control treatments for microbial function, but this was confounded by them not being significantly different from our bulk soil samples. Additionally the aster treatment was significantly different from the bulk soil samples for MB-C and MB-N, but not the control samples. Next the differences between the restoration and reference sites were documented, with the attempt to confirm a “disturbance gradient”. Soil microbial function as determined by the community level physiological profiles was analyzed with a comparison in ordination space using NMDS to observe functional similarity, and indicated a disturbance gradient was present that separated restoration sites, from the cut blocks, and from the fires in that order. This was expected based on research by Klischuk et al. (2015) which indicated forestry operations are comparable to the natural wildfire disturbance regime, a known resilient ecosystem (Markham & Essery, 2015); something that is yet to be quantified on oil and gas reclamation. The other microbial parameters measured did not follow the disturbance gradient trend. Plant available nutrient data showed specifics of site conditions, but no trends that followed the disturbance gradient approach with the exception of the manganese results, which were significantly higher on the reference sites. The manganese results are interesting as Mn content has been shown to be a critical component of litter decomposition rates (Berg et al., 2015; Keiluweit et al., 2015); higher concentrations are an indicator of mature soils (Mellert & Ewald, 2014), and it has been considered a potentially limiting nutrient in restoration (Rowland, 2008). The most consistent result was that the OSE1 site was the closest reclamation site to the reference sites in terms of plant available nutrients (driven by Mn) and all microbial parameters. This similarity of OSE1 to the reference sites was potentially a result of it being the least disturbed study site (short disturbance time, not surface ripped) and the abundance of native plant species that have appeared on site through natural ingress and the native seed bank. Additionally, on all sites phosphorus was extremely low, which has been shown to lead to a decline in soil microbial activity (Lagerström et al., 2009) however, the results could also be due to an issue in analysis as the results are uncharacteristic for the region.

Overall the study showed that hitchhiker planting is a viable option for improving native forb establishment on restoration sites, provided due care is paid to stock size, timing and forb selection. A viable spruce, with excellent survival can be produced,; along with adequate forb survival and growth accompanying it. This re-vegetation method can allow for simultaneous spruce and forb establishment on disturbed sites, an industry first. The effect of hitchhiker planting on the soil microbial community was not obviously apparent after two years, however it was confirmed that CLPP it is effective measurement for use in comparing disturbed sites to natural benchmarks.

4.2. Research adaptations

While hitchhiker planting is new to the realm of boreal forest restoration, it is a proven technique in agriculture (Curran et al., 1993; Davis, 1962) and variations have been used in restoration previously (Garnett, 2003; Nelson, 2005). The results of this thesis is the culmination of the first known large scale field deployment of this idea. Difficulties in broadcast seeding of native forbs in restoration has been previously observed (Schoonmaker et al., 2014; deBortoli, 2017; Nelson et al., 1969; Koniak, 1983), and while drill seeding can be effective (Ott et al., 2016) it is limited by access and site conditions. Hitchhiker planting allows for forb survival and establishment levels similar to direct placement of seedlings (Smreci & Gould, 2015), without incurring additional costs when tree planting is already the main revegetation method for woody species. The successful development of a viable stock type in the first half of the study will allow for simultaneous establishment of woody species and native forbs on a disturbed site. The effect of undesirable species on spruce development is well documented (Hoggs & Lieffers, 1991; Lieffers & Stadt, 1994; Pinno & Hawkes, 2015), and hitchhiker planting has the ability to reduce that competition through the physical occupation of space, as regression results indicate is beginning to occur.

Forb success was influenced by site conditions, particularly by site preparation. The ripped sites (OSE2 and BP1) showed greater forb growth and expansion compared to the non-ripped (OSE1 and AS1) sites. This aligns with research by Bauman et al. (2015) showing the positive effect of ripping soils on native forb establishment and reducing undesirable species cover. These results are likely a combination of the effects of the ripped soil being easier to expand through, and the reduced initial undesirable species cover which allows the hitchhiker forbs to expand without competition. Overall this exemplifies the importance of site preparation in restoration, regardless of revegetation method. After two years the stock type results are clear, however the trends involving cover, and specifically undesirable and non-native species cover, are still developing. Measurements in year's three to five should continue to show the effect of the hitchhiker forb.

With current guidelines based on easy to measure parameters (ESRD, 2013; AEP, 1993), the utilization of the idea of ecological functional similarity allows for the assessment of more difficult to measure and potentially more informative measures. Soil microbial function is measureable (Gartzia-Bengoetxea et al., 2016; Lalor et al., 2007) and comparable to reference sites using an ordination environment (Banning et al., 2012; Howell & MacKenzie, 2017); and the results of this study followed the expected disturbance gradient. This thesis research showed that soil microbial function followed the expected disturbance gradient, which correlated with existing research on resiliency of ecosystems along our disturbance gradient (Markham & Essery, 2015; Kishchuk, 2015); and indicates that soil microbial function may be an estimator of an ecosystems resiliency, which should be an important consideration in restoration. The other microbial parameters measured (MB-C, MB-C, soil respiration, metabolic quotient) did not follow the expected disturbance gradient trend, nor did any other site effects present themselves, besides the similarity of the OSE1 reclamation site to the reference sites. The OSE1 site was the least disturbed of the reclamation sites, both in initial industrial disturbance, and lack of surface ripping. It also was the site with the most natural ingress of fireweed and aster, and shared the similar level of Mn. While no conclusions can be drawn on exactly why OSE1 is the most similar, it is important to keep in mind that site treatments such as ripping that may have positive effects on aboveground growth, could have negative effects on the belowground community. Comparatively the similarity could be as simple as a missing micronutrient, or an anomaly of Mother Nature.

When the belowground part of the study was analyzed, the lack of apparent effect of the hitchhiker plant on the soil microbial community was not a complete surprise. Research by Habekost et al., (2008) and Claassens et al., (2008) has indicated that vegetation's effects on the soil microbial community in the field has considerable lag time; however it is well documented that the vegetation community can affect the soil microbial community (Lamb, 2011). Undesirable and invasive species can alter the soil microbial community to benefit them (Bever, 2010; Rodrigues et al., 2015), which underlies the importance of establishing native vegetation on a restoration site from the beginning. The traditional practice in restoration of planting a grass cover crop may have had belowground affects that contributed to the difficulty in removing the grass cover later on in the restoration process.

4.3. Future research

Improved measurement techniques could provide additional and more conclusive insight into the egression of the hitchhiker forbs over time. During root exposure and egression measurements for the fireweed stock types, it was determined that the forbs had egressed up to 3m from the initial plant, and additional aboveground plants had formed from suckering well outside the area assessed for cover with the quadrat method. The egression measurements were not completed on all sites due to time constraints, which could be a challenge for pursuing it as the sole measure in the future. DNA sequencing of plants has taken leaps forward in recent years, and provides the future possibility of a simple (however costly) measurement technique for determining which plants on a site belong to the original hitchhiker forb, versus natural ingress (Hollingsworth et al., 2016; Tuhkanen et al., 2013). This would allow for an assessment of a number of plants in X years relative to the original planting, and their associated cover.

The ecological functional similarity approach is a work in progress, and continued work is needed to develop it, and build up the database of reference sites. This study indicated that microbial function is a parameter that can be very informative in an ordination space environment, and that our selection of reference sites may be appropriate. Further research is needed for the assessment of which parameters will be useful in an end model for regulators for assessing restoration success, and which reference sites are appropriate for which disturbances.

While year two measurements were informative in terms of stock type performance, and site conditions, they were not as informative for questions of undesirable and non-native species cover, as well as the hitchhiker forb effect on belowground properties. Current trends indicate we should see a conclusive reduction in undesirable species cover in year’s three to five, while significant microbial effects of hitchhiker planting may not be observed in the duration of this study.

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Appendices

Greenhouse growing conditions

The greenhouse study was conducted at the greenhouse facility at NAIT Boreal Research Institute, Peace River, Alberta. White spruce seeds used for the study were collected from two different seed collection locations (57˚ 15' N, 117˚ 56' W and 57˚ 8' N, 117˚ 42' W) and were cold stratified for 28 days at 4°C prior to sowing. Each of the native forbs were collected from the same geographic region as the white spruce (fireweed from 56˚ 18' N, 116˚ 32' W and showy aster from 56˚ 18' N, 116˚ 33' W).

Nine stock types were evaluated in order to optimize companion nursery stock production of white spruce with one of two different native forbs. These stock types varied in terms of the plug volume (container size), as well as the time interval between sowing white spruce and herbaceous species, with white spruce always sown first. The experiment was performed in two different sizes of Styroblock® containers (Beaver Plastics, Acheson AB), designated as 512A (220 ml cavity volume) and 615A (336 ml cavity volume). All Styroblocks® contained a growing substrate of peat and perlite. White spruce was sown on March 27, 2014. Next, the Styroblocks® with white spruce seedlings for each Styroblocks® size, were divided into two sets of 400 plugs each. One set was designated for sowing fireweed and the other for sowing showy aster. Each set was then further divided into four different treatment groups of 100 plugs each. Each of the native forbs were sown along with growing white spruce seedlings 6, 8, 10 and 12 weeks after the white spruce was sown, resulting in four treatments each. Greenhouse temperature and relative humidity were 19-27 °C and 45-80% respectively with a 12 h/12 h photoperiod until August 15, 2014. From August 16 onward, the plants were subjected to the ambient photoperiod and temperature (Peace River, latitude 56° 14' N). On April 17 a starter fertilizer mixture (Plant-prod Ultimate Plant Starter 10-52-10) was mixed in a 1:200 ratio with water and added to all stock types. On April 23 all plugs were thinned to 1 white spruce per plug. From April 30 onwards, fertilization occurred with a medium fertilization blend (Table A-1) every 7-10 days, with the interval being lengthened to every 14 days from June 27 onwards. Watering was completed as required, with no significant dry-down periods, and watering occurring on average every 2 days to keep the Styroblocks$^{®}$ moist. Whenever a forb was sown, fertilization would occur immediately before sowing, and then not occur for two weeks while the forb was germinating, before regular fertilization would re-commence. All fertilization was stopped on August 26, at which time the greenhouse was cooled to help facilitate hardening. A control group (no herbaceous plant addition) of 100 seedlings of white spruce were grown in Styroblock® type 412A (125 ml in volume). These seedlings were grown without any forb treatment and used as control because reclamation practitioners generally use this Styroblock® type to grow white spruce seedlings. The Styroblocks® were randomly rotated every week to balance the effects of microenvironments found in the greenhouse space due to the different light intensities. All seedlings were boxed and stored frozen (-4 ˚C) until planting in May 2015.

Figure A-1: Spruce total height (cm) for stock treatments grown with fireweed on each site. Forb sowing period on the x axis refers to the time delay from sowing the spruce seed to sowing the forb seed. The 512A and 615A groupings refer to the two different stock sizes used. Error bars represent the 95% confidence interval of the mean (n=21). Significance is determined by Dunnett's test with a p-value<0.05. An asterisk indicates the stock type is significantly different from the control group, located on the left side of each panel. Panel (a) has results from the BP1 site, (b) OSE2, (c) AS1 and (d) OSE1.

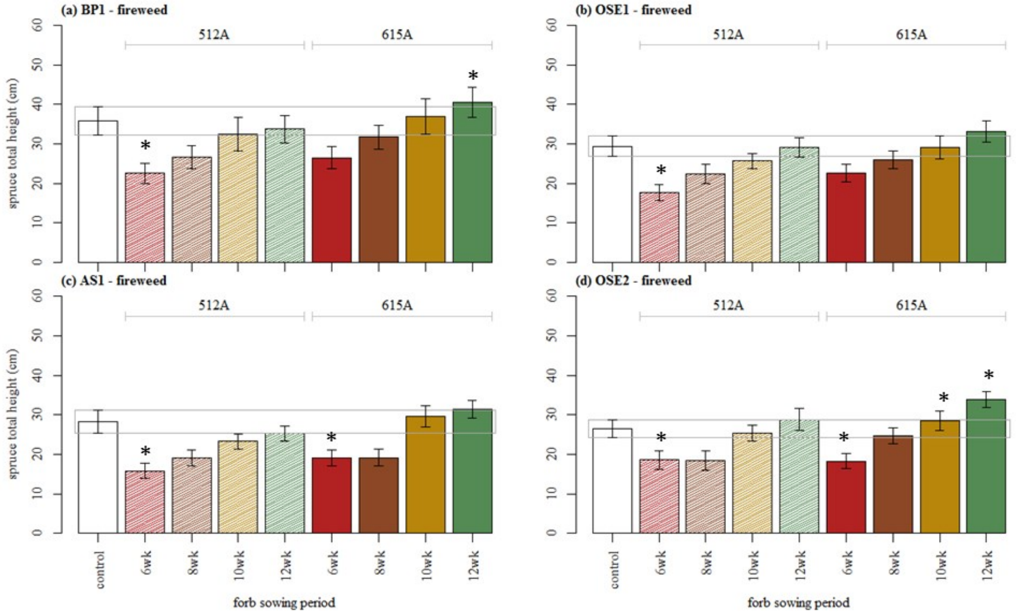

Figure A-2: Spruce total height (cm) for stock treatments grown with aster on each site. The 512A and 615A groupings refer to the two different stock sizes used. Error bars represent the 95% confidence interval of the mean (n=21). Significance is determined by Dunnett's test with a p-value<0.05. An asterisk indicates the stock type is significantly different from the control group, located on the left side of each panel. Panel (a) has results from the BP1 site, (b) OSE2, (c) AS1 and (d) OSE1.

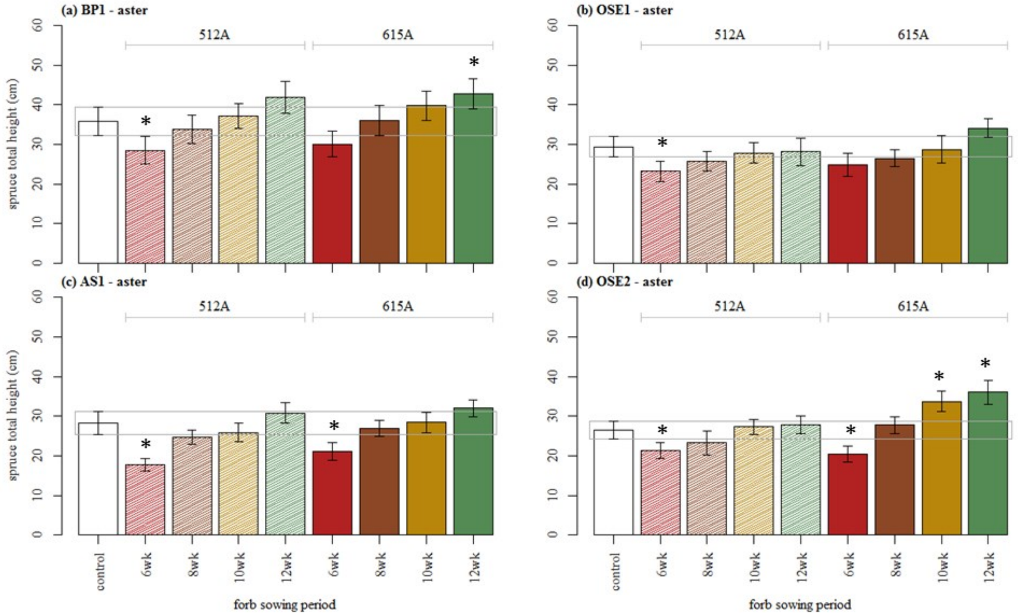

Figure A-3: Percent cover of non-native species for stock treatments grown with fireweed. The 512A and 615A groupings refer to the two different stock sizes used. Error bars represent the 95% confidence interval of the mean (n=21). Significance is determined by Dunnett's test with a p-value<0.05. An asterisk indicates the stock type is significantly different from the control group, located on the left side of each panel. Note the variation in axis scale required to observe stock type differences between sites. Panel (a) has results from the BP1 site, (b) OSE2, (c) AS1 and (d) OSE1.

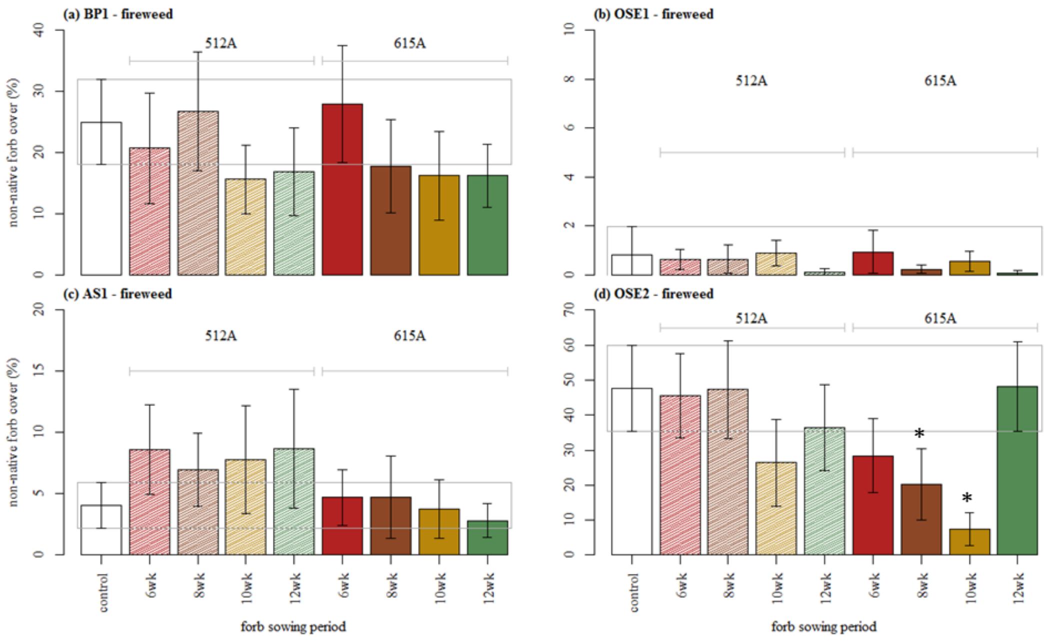

Figure A-4: Percent cover of non-native species for stock treatments grown with aster. The 512A and 615A groupings refer to the two different stock sizes used. Error bars represent the 95% confidence interval of the mean (n=21). Significance is determined by Dunnett's test with a p-value<0.05. An asterisk indicates the stock type is significantly different from the control group, located on the left side of each panel. Note the variation in axis scale required to observe stock type differences between sites. Panel (a) has results from the BP1 site, (b) OSE2, (c) AS1 and (d) OSE1.

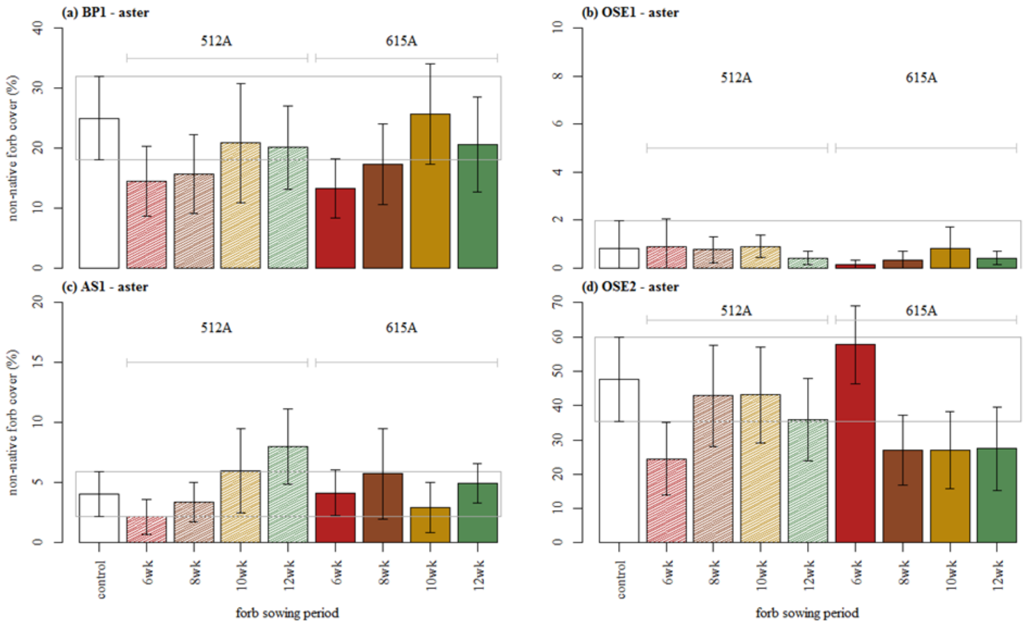

Figure A-5: Total herbaceous species cover for stock treatments grown with fireweed on each site. The 512A and 615A groupings refer to the two different stock sizes used. Error bars represent the 95% confidence interval of the mean (n=21). Significance is determined by Dunnett's test with a p-value<0.05. An asterisk indicates the stock type is significantly different from the control group, located on the left side of each panel. Note the variation in axis scale required to observe stock type differences between sites. Panel (a) has results from the BP1 site, (b) OSE2, (c) AS1 and (d) OSE1.

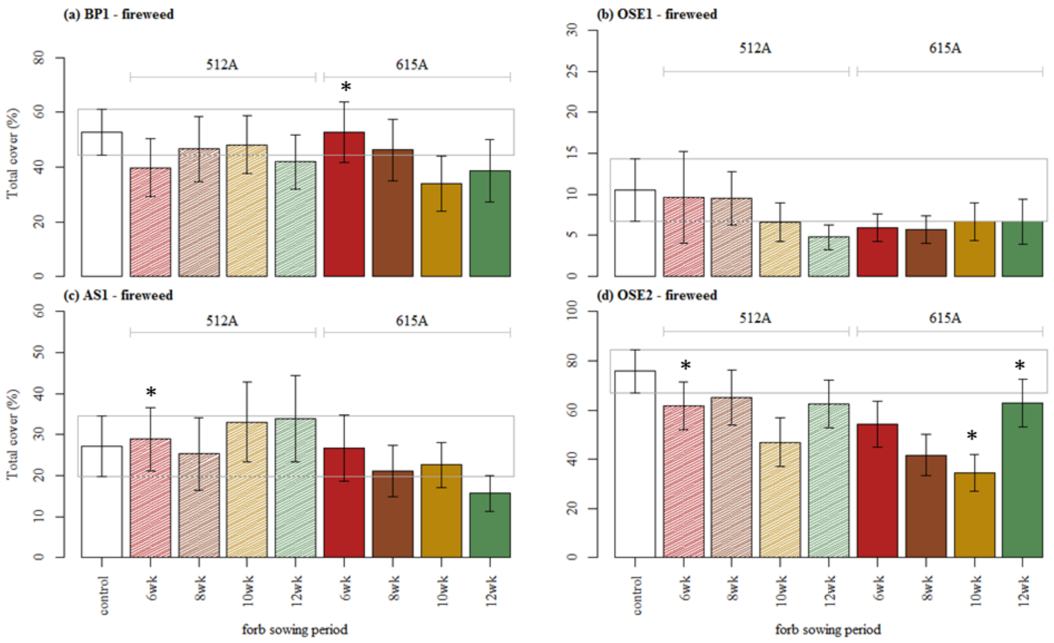

Figure A-6: Total herbaceous species cover for stock treatments grown with aster on each site. The 512A and 615A groupings refer to the two different stock sizes used. Error bars represent the 95% confidence interval of the mean (n=21). Significance is determined by Dunnett's test with a p-value<0.05. An asterisk indicates the stock type is significantly different from the control group, located on the left side of each panel. Note the variation in axis scale required to observe stock type differences between sites. Panel (a) has results from the BP1 site, (b) OSE2, (c) AS1 and (d) OSE1.

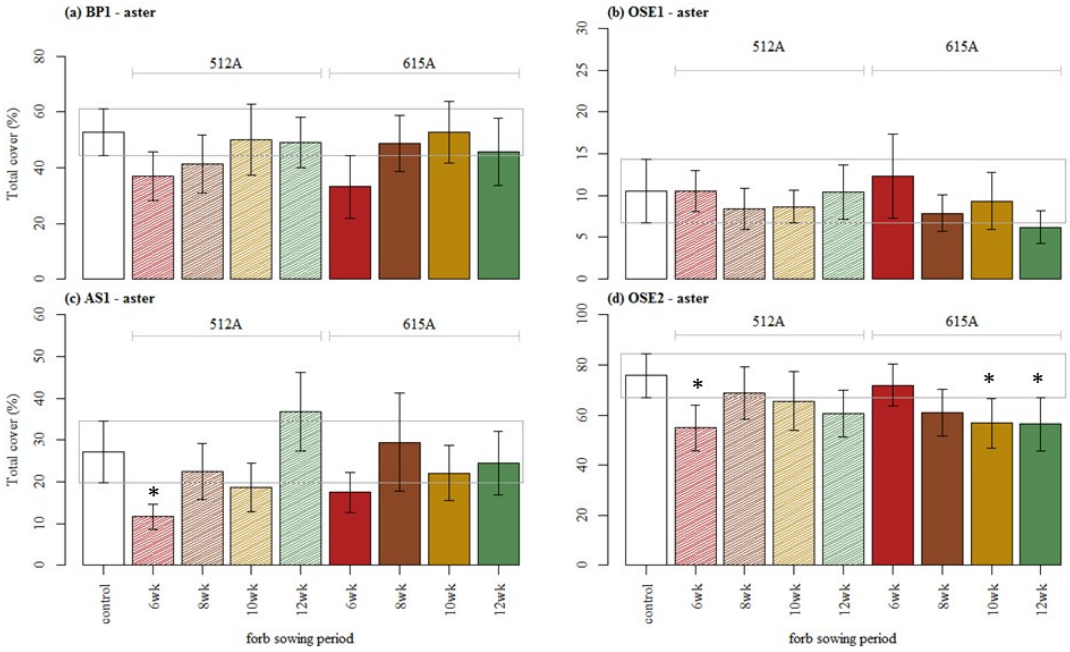

Figure A-7: Total percent cover of target forb (fireweed) of each site. The 512A and 615A groupings refer to the two different stock sizes used. Error bars represent the 95% confidence interval of the mean (n=21). Significance is determined by Dunnett's test with a p-value<0.05. An asterisk indicates the stock type is significantly different from the control group, located on the left side of each panel. Panel (a) has results from the BP1 site, (b) OSE2, (c) AS1 and (d) OSE1.

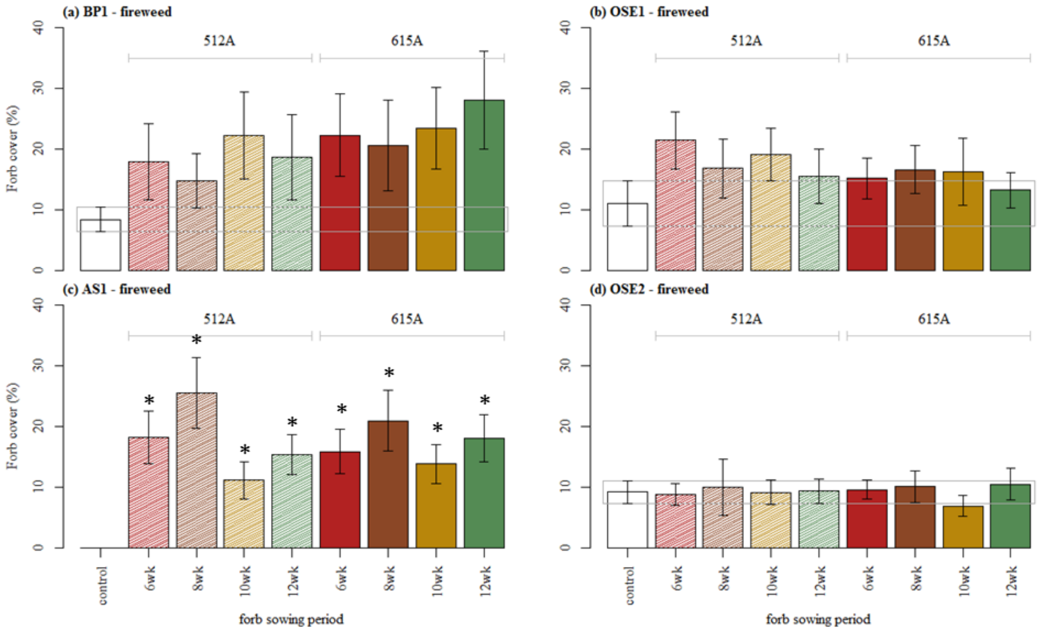

Figure A-8: Total percent cover of target forb (aster) on each site. The 512A and 615A groupings refer to the two different stock sizes used. Error bars represent the 95% confidence interval of the mean (n=21). Significance is determined by Dunnett's test with a p-value<0.05. An asterisk indicates the stock type is significantly different from the control group, located on the left side of each panel. Note the variation in axis scale required to observe stock type differences between sites. Panel (a) has results from the BP1 site, (b) OSE2, (c) AS1 and (d) OSE1.

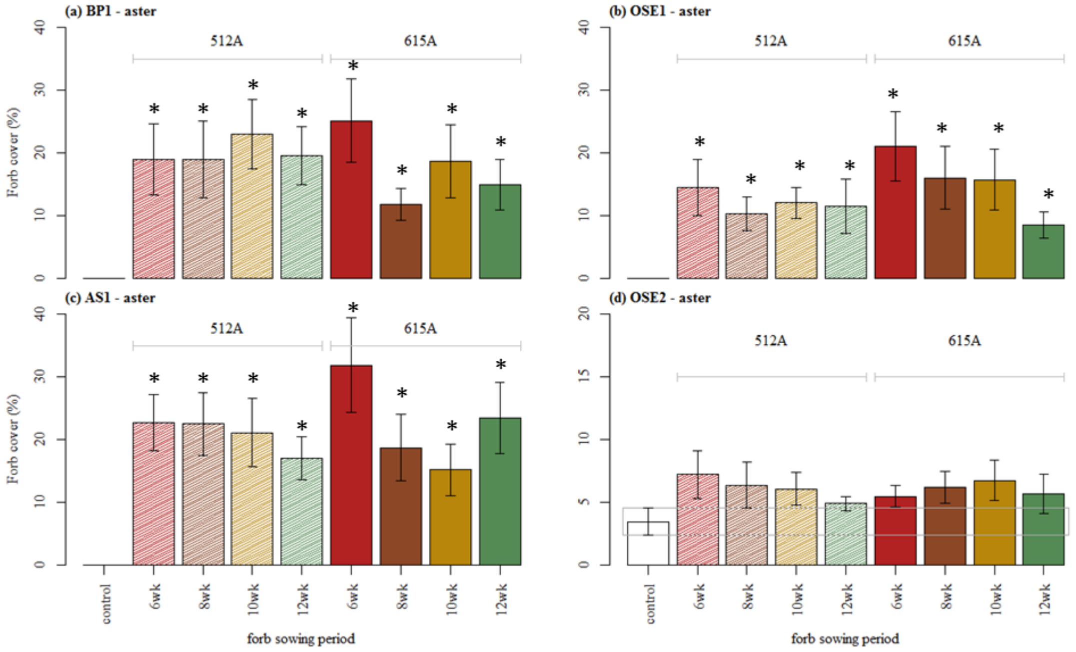

Figure A-9: Spruce root collar diameter (mm) for stock treatments grown with fireweed on each site. The 512A and 615A groupings refer to the two different stock sizes used. Error bars represent the 95% confidence interval of the mean (n=21). Significance is determined by Dunnett's test with a p-value<0.05. An asterisk indicates the stock type is significantly different from the control group, located on the left side of each panel. Panel (a) has results from the BP1 site, (b) OSE2, (c) AS1 and (d) OSE1.

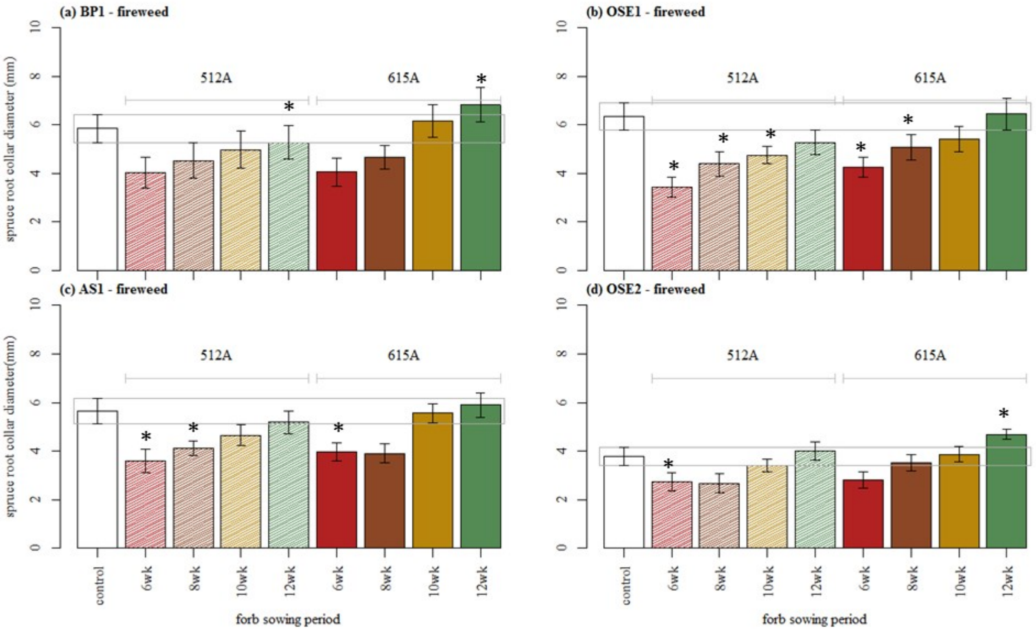

Figure A-10: Spruce root collar diameter (mm) for stock treatments grown with aster on each site. The 512A and 615A groupings refer to the two different stock sizes used. Error bars represent the 95% confidence interval of the mean (n=21). Significance is determined by Dunnett's test with a p-value<0.05. An asterisk indicates the stock type is significantly different from the control group, located on the left side of each panel. Panel (a) has results from the BP1 site, (b) OSE2, (c) AS1 and (d) OSE1.

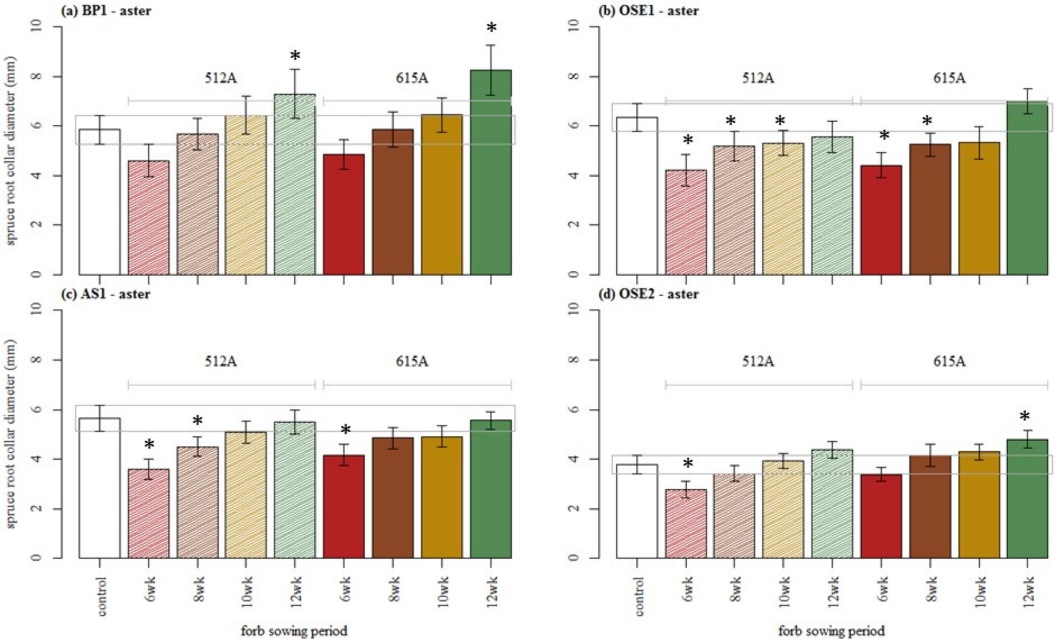

Figure A-11: Spruce growth increment (cm) for stock treatments grown with fireweed on each site. The 512A and 615A groupings refer to the two different stock sizes used. Error bars represent the 95% confidence interval of the mean (n=21). Significance is determined by Dunnett's test with a p-value<0.05. An asterisk indicates the stock type is significantly different from the control group, located on the left side of each panel. Note the variation in axis scale required to observe stock type differences between sites. Panel (a) has results from the BP1 site, (b) OSE2, (c) AS1 and (d) OSE1.

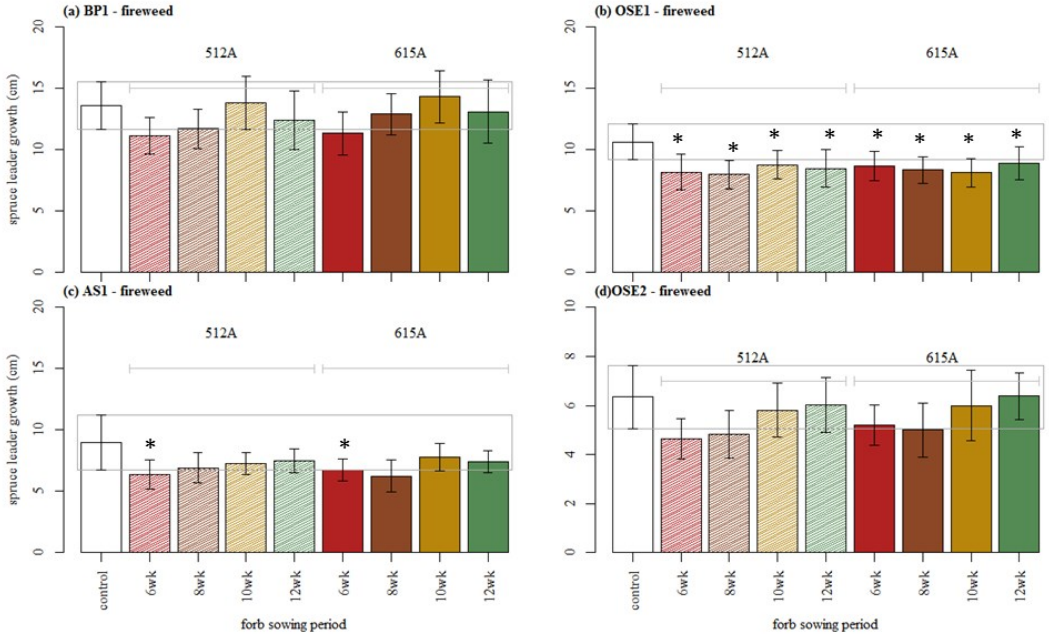

Figure A-12: Spruce growth increment (cm) for stock treatments grown with aster on each site. The 512A and 615A groupings refer to the two different stock sizes used. Error bars represent the 95% confidence interval of the mean (n=21). Significance is determined by Dunnett's test with a p-value<0.05. An asterisk indicates the stock type is significantly different from the control group, located on the left side of each panel. Note the variation in axis scale required to observe stock type differences between sites. Panel (a) has results from the BP1 site, (b) OSE2, (c) AS1 and (d) OSE1.

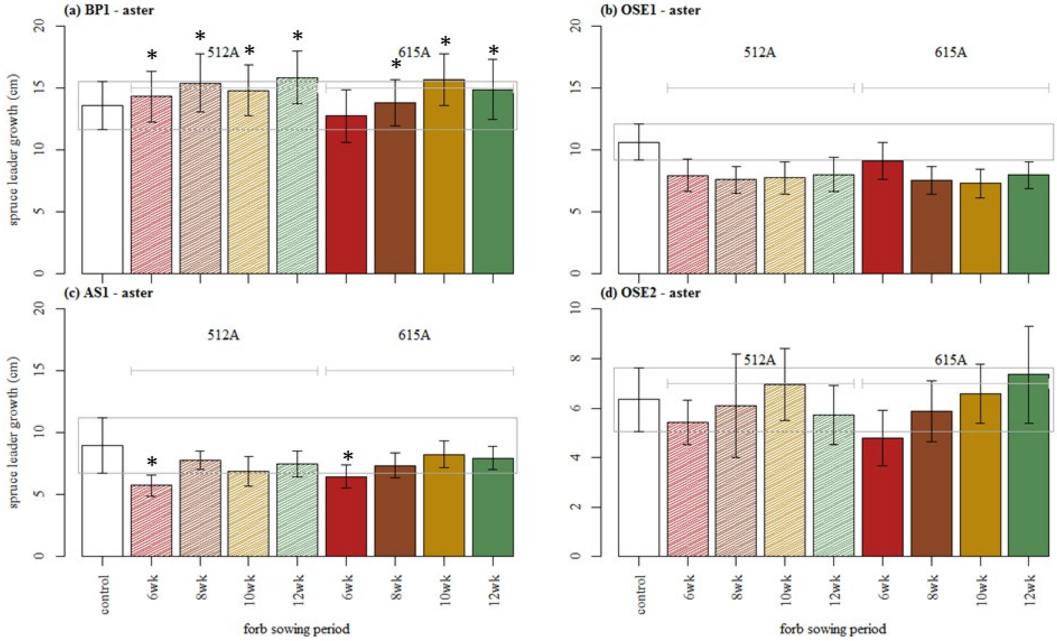

Figure A-13: Spruce aboveground biomass (g) for stock treatments grown with fireweed. The 512A and 615A groupings refer to the two different stock sizes used. Error bars represent the 95% confidence interval of the mean (n=7). Significance is determined by Dunnett's test with a p-value<0.05. An asterisk indicates the stock type is significantly different from the control group, located on the left side of each panel. Note the variation in axis scale required to observe stock type differences between sites. Panel (a) has results from the BP1 site, (b) OSE2, (c) AS1 and (d) OSE1.

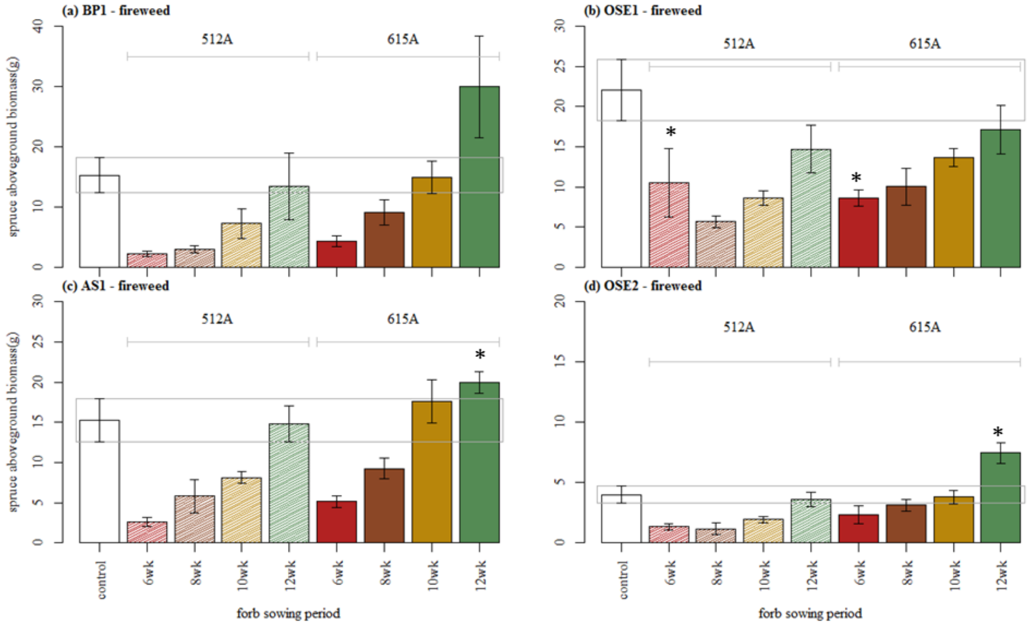

Figure A-14: Spruce belowground biomass (g) for stock treatments grown with fireweed. The 512A and 615A groupings refer to the two different stock sizes used. Error bars represent the 95% confidence interval of the mean (n=7). Significance is determined by Dunnett's test with a p-value<0.05. An asterisk indicates the stock type is significantly different from the control group, located on the left side of each panel. Panel (a) has results from the OSE2 site and (b) AS1.

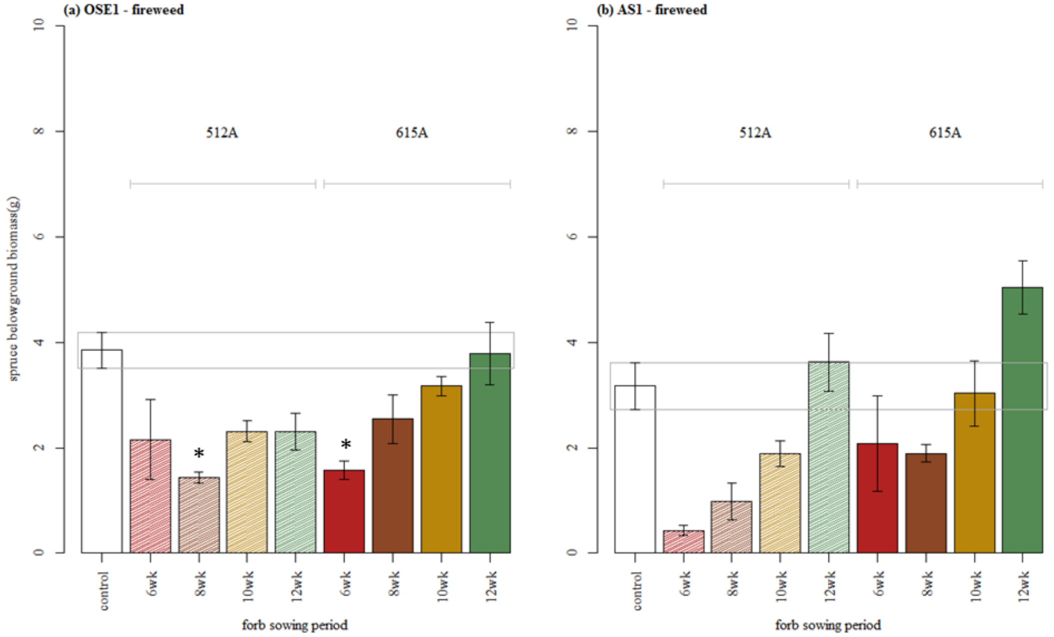

Figure A-15: Spruce aboveground biomass (g) for stock treatments grown with aster on each site. The 512A and 615A groupings refer to the two different stock sizes used. Error bars represent the 95% confidence interval of the mean (n=7). Significance is determined by Dunnett's test with a p-value<0.05. An asterisk indicates the stock type is significantly different from the control group, located on the left side of each panel. Note the variation in axis scale required to observe stock type differences between sites. Panel (a) has results from the BP1 site, (b) OSE2, (c) AS1 and (d) OSE1.

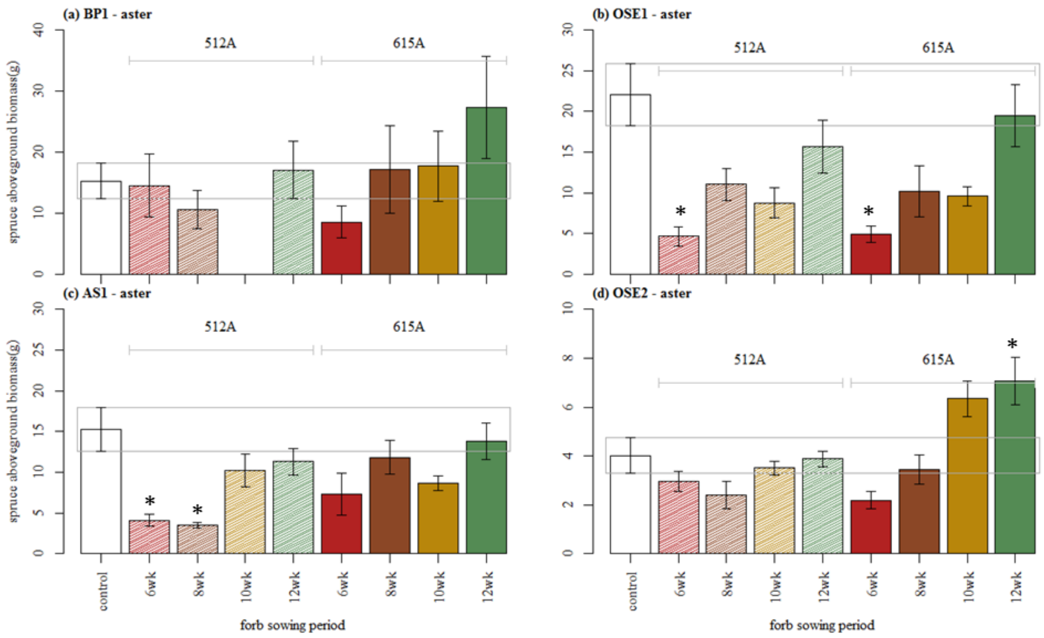

Figure A-16: Spruce belowground biomass (g) for stock treatments grown with aster on each site. The 512A and 615A groupings refer to the two different stock sizes used. Error bars represent the 95% confidence interval of the mean (n=7). Significance is determined by Dunnett's test with a p-value<0.05. An asterisk indicates the stock type is significantly different from the control group, located on the left side of each panel. Panel (a) has results from the OSE2 site and (b) AS1.

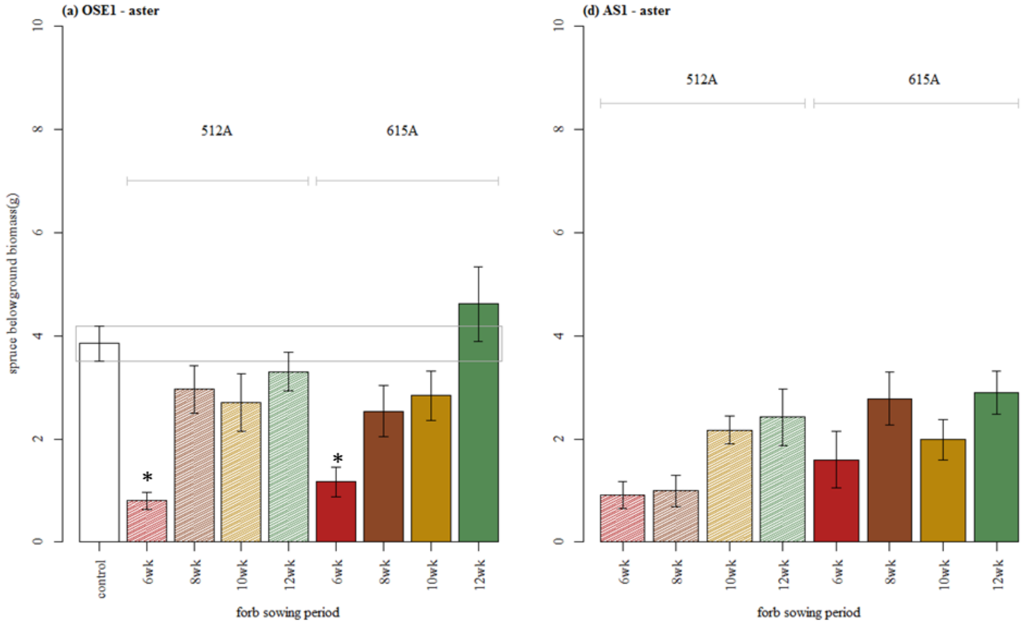

Table A-1: Medium Fertilizer Blend. List of components of the medium fertilizer blend in parts per million.

Component	Quantity (ppm)
NO_3-N	50.8
NH_4-N	15.6
N(t)	66.4
P	83.8
K	92.4
Ca	94.3
Mg	38.9
S	56.9
Fe	3.4
Mn	0.01
Zn	0.17
Cu	0.58
B	0.25
HCO_3	106
$CaCO_3$	87
Na	26.4

Table A-2: List of the 16 carbon substrates used for CLPP analysis.

Carbon Substrates:
Glucose
Galactose
Fructose
L-Cysteine-HCL
Citric Acid
Arginine
Arabinose
Alanine
Blank
Trehalose
Oxalic Acid
N-Acetyl glucosamine
Malic Acid
Lysine-HCL
Ketoglutaric Acid
Amino butyric acid

Table A-3: Reclamation and reference site plant available nutrients by stock type. Numbers represent the average for each type on a site. The stock types are listed as A=10week – 615A - Aster, F=10 week – 615A – Fireweed, C=Control and BS=Bulk Soil. Statistical significance is indicated by the superscript letter, which indicates a significant difference (p-value<0.05) according to Tukey's post-hoc significance test ($n=3$).

	Ca	Cu	Fe	K	Mg	Mn	Zn	Na	B	Pb	P	S	TN
AS1A	3.2899	0.0029	0.1393	0.0579	0.5238	0.0067	0.0052	0.9148	0.0007	0.0009	0.0033	1.1288	11.2881
AS1F	3.0454	0.0024	0.1139	0.0490	0.4861	0.0049	0.0042	0.7257	0.0006	0.0007	0.0017	0.9151	9.1513
AS1C	3.3977	0.0020	0.1465	0.0664	0.5622	0.0309	0.0039	0.8396	0.0009	0.0006	0.0025	1.0275	10.2754
AS1BS	3.6047	0.0021	0.0840	0.0459	0.6330	0.0053	0.0042	0.6269	0.0007	0.0006	0.0022	1.4192	14.1923
BP1A	3.1300	0.0016	0.0626	0.0918	0.5745	0.0112	0.0021	1.2176	0.0009	0.0005	0.0021	0.0920	0.9205
BP1F	2.6821	0.0011	0.0970	0.0922	0.5079	0.0163	0.0018	1.4548	0.0007	0.0005	0.0024	0.0959	0.9591
BP1C	3.1498	0.0016	0.0972	0.0728	0.5088	0.0101	0.0030	1.0434	0.0004	0.0010	0.0022	0.1296	1.2961
BP1BS	3.1922	0.0005	0.1813	0.0759	0.6882	0.0087	0.0011	1.0376	0.0008	0.0002	0.0027	0.0635	0.6348
OSE2A	4.7411	0.0014	0.0788	0.0697	0.6002	0.0044	0.0032	1.7000	0.0011	0.0011	0.0026	0.2273	2.2728
OSE2F	5.1724	0.0018	0.1026	0.1166	0.7072	0.0096	0.0073	1.7570	0.0030	0.0011	0.0036	0.2549	2.5492
OSE2C	5.2392	0.0022	0.1118	0.1075	0.6575	0.0143	0.0040	1.2629	0.0016	0.0017	0.0047	0.2816	2.8158
OSE2BS	4.6582	0.0010	0.0475	0.0213	0.5728	0.0210	0.0015	0.0736	0.0009	0.0005	0.0022	0.0833	0.8334
OSE1A	3.2394	0.0003	0.0295	0.0908	0.5286	0.0429	0.0035	0.0861	0.0002	0.0003	0.0013	0.1803	1.8031
OSE1F	2.8236	0.0002	0.0215	0.1239	0.5237	0.0433	0.0039	0.1513	0.0002	0.0002	0.0008	0.1475	1.4753
OSE1C	3.4401	0.0007	0.0412	0.0733	0.5303	0.0485	0.0046	0.0894	0.0006	0.0005	0.0013	0.1150	1.1498
OSE1BS	3.1462	0.0004	0.0384	0.0716	0.5336	0.0542	0.0042	0.2138	0.0001	0.0003	0.0004	0.0963	0.9627
F15	1.8244	0.0003	0.0242	0.0380	0.2255	0.0957	0.0024	0.0328	0.0000	0.0001	0.0005	0.0593	0.5931
F11	2.3354	0.0002	0.0552	0.6186	0.4121	0.1372	0.0043	0.3311	0.0002	0.0003	0.0036	0.0827	0.8274
CB1	1.6642	0.0004	0.0631	0.3726	0.7675	0.0902	0.0042	0.4894	0.0001	0.0003	0.0009	0.0442	0.4416
CB2	3.2610	0.0007	0.0657	0.1023	0.7164	0.0714	0.0042	0.1017	0.0002	0.0003	0.0014	0.3680	3.6796