Prairie Restoration in Post-Extraction Sandpits: Plant Response to Arbuscular Mycorrhizal Inoculum, Biochar, and Municipal Compost

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INTRODUCTION:

Depleted aggregate sites are good candidates for prairie restoration projects due to their 'open' nature, sandy substrata, and adaptability to management scenarios. This potential has been recognized by TOARC and has led to the support of this research initiative. The results of this study can be directly translated into the industrial-scale restoration of tallgrass prairies in post-extraction areas. This final research report describes the results of a multi-year, large-scale prairie restoration project established on the Norfolk Sand Plain in southern Ontario.

This research tested the effect of soil supplements (municipal compost, biochar) and plant symbionts (commercially-available arbuscular mycorrhizal fungi [AMF]) on prairie plant growth and soil rehabilitation. These treatments are hypothesized to positively alter microbe-driven biogeochemical cycles, soil building processes, soil food webs, and plant-mycorrhizal symbioses. It is hypothesized that the combined use of soil amendments and mycorrhizal inoculation will be additive with respect to soil development and plant growth.

Research Objectives:

This research will significantly contribute to the scientific fields of ecological restoration, mycorrhizal ecology, and soil ecology. Project goals include:

1) describing plant-soil-microbe interactions;
2) understanding the ecological role of a commercial mycorrhizas when restoring native plants in degraded landscapes;
3) determining soil supplement influence on prairie plant growth and soil food webs;
4) understanding ecological soil development properties in amended post-extraction substrate.

The research will answer two practical questions related to industrial scale restoration:

1. Does mycorrhizal inoculation (a relatively inexpensive application) positively influence plant growth, thus adding value to the overall restoration scheme?
2. Does the addition of soil supplements (biochar & compost) in various proportions significantly and cost effectively accelerate soil restoration thus promoting plant growth and survival?

Background:

Ontario's tallgrass prairies are treeless habitats dominated by native grasses and wildflowers. These prairie ecosystems are typically limited to an area within fifty kilometers of Great Lakes shoreline in southern Ontario. Interspersed among deciduous Carolinian forests, prairie vegetation is restricted to the xeric (i.e. dry) conditions of well-drained sandy to sandy-loam soils (Faber-Langendoen and Maycock, 1992). One of the largest bands of remnant prairie vegetation in Ontario is found on the Norfolk Sand Plain [also the location of the research site]. Currently, these ecosystems are under removal pressure resulting from urban sprawl, agriculture, invasive species colonization, and fire suppression. Rodger (1998) estimates that Ontario's prairies occupy less than three percent of their original coverage.

Sand plain prairie habitat supports a high biodiversity of regionally unique plants, insects, and animals (Gartshore et al., 1987). Habitat loss has elevated the status of many grassland species to provincially endangered or rare. Surveys indicate that approximately 22% of Ontario's rare plant species are found in prairie ecosystems (Ontario Biodiversity Council, 2010). Furthermore, sand plain prairies are home to a large number of rare grassland birds and insects. As prairie remnants become more isolated, dispersal is restricted for less mobile organisms, minimizing the chance of colonizing new habitat. Increasing patch size and quantity through

prairie restoration in southern Ontario will help facilitate the survival of the ecosystem in addition to Species-at-Risk and increase biodiversity.

Ecological Restoration of Post-Extraction Aggregate Sites:

Habitat restoration succeeds when projects incorporate sound ecological concepts and practical land management experience. When developing a project that recreates target habitat, knowledge of a landscape's historical context, remnant patch ecology, and scientific research is imperative (Hobbs and Harris, 2001). When these elements are considered, a restoration project has a greater chance of reaching ecological fidelity (Higgs, 1997). High ecological fidelity is accomplished when: 1) target biodiversity approaches remnant ecosystems, 2) ecosystem service goals are restored (i.e. erosion control), and 3) the restored ecosystem has long-term durability / resilience.

Practitioners restoring grassland habitat in post-mine substrata must acknowledge that: 1) a negative abiotic threshold may restrict plant community development, 2) patches of remnant ecosystems on the landscape are typically isolated, and 3) a complex set of ecosystem-level interactions exist among plants-soils-microbes. A holistic restoration approach designed to recreate the abiotic and biotic components of reference ecosystems will ultimately increase ecosystem durability. Addressing each of these challenges:

1) Post-extraction aggregate sites have inherent edaphic (i.e. soil) characteristics that limit the spontaneous development of high diversity plant communities (Wali, 1999; Prach and Hobbs, 2008). Although Ontario's prairie vegetation is adapted to xeric soil conditions, the steep abiotic and biotic thresholds of sandpit substrata are difficult to supplant (Suding et al., 2004). If left unassisted, soil development trajectories favorable to diverse plant communities can range from decades to hundreds of years in post-mine substrate (Bradshaw, 1997). Rapid spontaneous

ecosystem development without intervention is possible under restricted, moderately disturbed circumstances (Prach and Hobbs, 2008). Severely disturbed sites such as post-mine sand pits often require technical reclamation to overcome the persistent stable state. Cost effective interventions that accelerate soil development and increase plant community durability include incorporating carbon based soil amendments, beneficial symbionts, or fertilizers.

2) Natural recolonization of target plant communities is hampered by the lack of plant species in the regional pool. Řehounková and Prach (2008) found that spontaneous plant community regeneration is possible after 25 years following gravel-sand pit activity. In this study, successional development of target communities was contingent upon soil moisture and the presence of adjacent (semi)natural vegetation. In southern Ontario, patches of remnant grassland vegetation are disjointed, restricting plant immigration rates. The incorporation of locally sourced plant material is an essential component to native plant restoration in southern Ontario's aggregate sites.

3) Plants are the foundation of terrestrial food webs, link aboveground /belowground biogeochemical cycles, and create ecological niches for organisms. Plant primary production is contingent upon an array of biotic factors (i.e. pathogens, grazers, symbionts) and abiotic factors (i.e. soil chemistry, hydrology, nutrient availability). These ecosystem components ultimately mediate plant community composition (Wardle et al., 2004), determining restored ecosystem durability. Associated with the rhizosphere, belowground symbiotic microorganisms, such as arbuscular mycorrhizas (Klironomos, 2002; Maherali and Klironomos, 2007) and nitrogen-fixing bacteria (van der Heijden et al., 2008), are key soil functional groups that play a pivotal role in determining the outcome of a restoration project. A successful restoration project in severely degraded substrata must consider the substrate's biogeochemical components. After evaluating

the plant-soil-microbial components (or lack thereof), technical reclamation can reintroduce biotic / abiotic components necessary for ecosystem durability.

Assessing Soil Development via Soil Food Webs:

Ecosystem productivity and fertility are characterized by organic matter (OM) inputs, plant community primary production, and microbial energy pathways (Wardle et al., 2004). Soil development is contingent upon interactions of soil biota with OM decomposition and primary production inputs (Wardle, 1999; Holtkamp et al., 2008). OM decomposition, orchestrated by basal trophic levels (i.e. bacteria and fungi), dictates the release and retention of soil nutrients. In turn, bacterial and fungal populations are grazed upon by soil invertebrates such as nematodes, mites, and springtails. Soil fauna are typically subdivided in the functional groups of fungivores, bacteriovores, and top predators based on feeding preferences. Food source availability determines the population size of soil fauna functional groups within a system. Re-establishing a complex, high biomass detrital food web is an essential component of recovering soil systems.

Fungal:bacterial ratios have been used as surrogates to describe the trajectory of recovering soil (Harris, 2009). Assessing the relative biomass ratios of the main decomposer assemblages can elucidate the stage of soil development. Bacteria out compete fungal assemblages in ecosystems with poorly developed, low OM soils with easily accessible nutrients. Conversely, fungal assemblages dominate systems when organic matter sources increase in complexity and litter layers develop (van der Wal et al., 2006).

Restoration ecologists target a high fungal:bacterial biomass relationship to indicate successful grassland restoration (Bardgett and McAlister, 1999; Smith et al., 2003). Native grassland systems tend to be dominated by fungal decomposers due to the higher volume of complex organic matter and constant litter inputs. By producing of exploratory mycelium, fungal

assemblages increase decomposition efficiency and stabilize soils in recovering grasslands. Increasing fungal biomass and diversity also contributes to the higher plant community diversity and more favorable biogeochemical cycling for late successional plant species (van der Heijden et al., 2008).

Nematodes are small, roundworms found ubiquitously throughout the world's soil systems. Soil nematodes comprise multiple positions in the soil functional feeding group spectra ranging from bacteriovores, fungivores, to top predators (Bongers and Bongers, 1998). Soil nematodes alter soil nutrient cycles and influence organic matter decomposition (Ritz and Trudgill, 1999). Nematode community structure reveals information regarding the energetic state of a system, its prospects towards ecosystem development, and the creation of more complete biogeochemical models (Bongers, 1990). Soil nematodes are easily collected, respond rapidly to environmental change, and can be easily sorted into functional feeding groups. Thus, nematodes have been employed to evaluate mine land recovery in a variety of disturbance scenarios (Biederman et al., 2008; Háněl, 2008; Courtney et al., 2011).

Other components of the soil food web were evaluated by functional feeding groups. There organisms included the collembola and soil mites (i.e. microarthropods). The microarthropods feed on detrital residues and graze on associated fungal and bacterial assemblages. These organisms play a role in the breakdown and processing of plant litter in the soil and soil mixing (Frouz et al., 2006). Predatory mites are a top domain predatory that would require a functional detrital food web to support a thriving population. The diversity of these functional groups can indicate the recovery status of developing soils (Frouz, 2013).

When evaluating components of the detrital food web in post-mine habitat, land managers should be aware of the depauperate conditions within these systems. Post-mine

sandpits have lowered soil fauna biomass due to the recently exposed, stressful environment of sand substrate. Selecting the appropriate soil amendment carefully is essential. The addition of inorganic fertilizers favors opportunistic species such as bacterial assemblages (Smith et al., 2003) and weedy plants (Major et al., 2005) in nutrient poor soils.

Complex organic matter amendments, such as compost, shift fungal:bacterial ratios towards fungal dominated systems (Biederman and Whisenant, 2009; Biederman, 2013) and increase populations of nematodes communities (Steel et al., 2012, 2013). Such amendments also increase soil aggregation, creates favorable nutrient cycling, and increases soil water holding capacity (Termorshuizen et al., 2004). Increasing fungal:bacterial biomass ratios, and subsequent associated soil fauna, lead to more stable plant communities.

Technical Reclamation – Components of the Research Project:

Native, Locally Sourced Plants:

Plants with analogous life history adaptations to abiotic and biotic influences are defined as functional groups. Functional group diversity is an important driver of plant community productivity (Tilman and Downing, 1994; Tilman et al., 2001). Remnant grasslands are composed of four main functional groups: (1) cool season [C_3] grasses, (2) warm season [C_4] grasses, (3) composite wildflowers, and (4) nitrogen [N]-fixing legumes. Cool season grasses provide spring/fall plant cover and herbivore fodder. Highly productive warm season grasses are major structural component of the grassland landscape. Warm season grasses are drought resistant, provide herbivore fodder, and create habitat for grassland animals and invertebrates. Composite wildflowers are integral in colonizing bare soil patches (especially after grazing or fire disturbances), supporting pollinator populations, and drivers overall plant community diversity. Plants in the legume family (Fabaceae) form a symbiotic relationship with N-fixing

bacteria. N-fixing bacteria are found within legume root nodules, and convert biologically unavailable atmospheric N_2 gas into forms of nitrogen useable by plants. In exchange for usable N, the plant delivers a source of food in the form of carbohydrates. N-rich legumes can contribute to the total N pool of soils during growth and after senescence (Peoples et al., 1995).

Restoration projects using locally collected, high-diversity seed mixtures maximize plant richness and rate of vegetative establishment (Piper and Pimm, 2002). Locally-sourced plants are adapted to regional growing conditions. Comparing local vs. commercial seed sources, locally sourced plant material ranges from neutral (Carter and Blair, 2013) to positive (Prach et al., 2013) effects on plant establishment rates and total cover. In addition, plant nurseries that source local plants have expertise of endemic plant species, regional climatic / edaphic conditions, and local biodiversity challenges.

Soil Amendments (Biochar and Compost):

Biochar is a relatively new soil amendment used for the management of agricultural soils and mine land rehabilitation (Blackwell et al., 2009). Biochar is created from heating organic matter at high temperatures ($500^oC – 700^oC$) in an anoxic kiln. The remaining charcoal (a.k.a. biochar) is a carbon-rich, porous substance that increases cation exchange capacity (Liang et al., 2006), adsorbs soil nutrients(Xu et al., 2013), decreases acidity (Novak et al., 2009), and increases water retention when added to soils. Biochar is resistant to microbial breakdown, remaining in the soil profile for 100+ years (Blackwell et al., 2009). Biochar's use as a soil amendment in mine land restoration scenarios is limited. As a relatively new technology, costs are being reduced increasing the feasibility of biochar in large-scale restoration projects.

Initial biochar research has shown positive plant growth benefits in degraded, nutrient poor soils (Glaser et al., 2002; Lehmann, 2007). A meta-analysis by Jeffery et al. (2011)

indicates that biochar significantly increased agricultural crop biomass, especially in acidic, coarse textured soils. This study hypothesized the liming and water retention properties of the biochar resulted in increased plant mass. A paucity of research has been conducted on the benefit of biochar as a soil amendment when growing prairie plants. One recent study indicates increased primary production of grassland plant biomass after the addition of biochar (Adams et al., 2013).

To date, mechanisms of biochar's effect on soil microbial communities and soil fauna are poorly understood (Lehmann et al., 2011). Biochar is anticipated to stimulate microbial / soil fauna biomass due to its positive soil conditioning properties. Warnock et al.'s (2007) concepts and mechanisms paper suggest a stimulation of arbuscular mycorrhizal association with plants (Warnock et al., 2007).

Compost is a traditional soil conditioner used to stimulate plant growth and improve soil properties in agricultural fields and mine land restoration. Compost is created by mixing and aerating organic matter, thus controlling the decomposition process. As a soil amendment, compost increases soil OM fractions, increases soil water holding capacity, and supplies plants with slow release nutrients (Termorshuizen et al., 2004). The addition of compost to integrated sandpit ecosystems is a cheap and effective way to benefit long-term plant growth and soil food web health. The wide availability of compost due to community recycling programs makes this an ideal substrate for use in the restoration of native tallgrass prairie habitat. In addition to increased soil fertility, compost soil amendments can create a natural mulch layer that reduces topsoil erosion thus enhancing soil stability.

When used synergistically, **compost and biochar** are anticipated to be an effective land management strategy. Biochar, when used alone, has the capacity to reduce the available

nutrients in the soil solution. Biochar's highly charged negative surface attracts ions in the soil solution, thus making them unavailable to plants and other soil organisms. Concurrent compost addition can charge the negative surfaces of biochar, creating a slow-release fertilizer in highly leached sand pit substrate. Research shows that using both compost and biochar together, either by mixing or soaking a compost tea, shows the highest amendment effectiveness (Blackwell et al., 2009).

Arbuscular mycorrhizas:

Arbuscular mycorrhizal fungi (AMF) are ubiquitous symbionts with the majority of plant species. This ancient symbiosis is over 400 million years old and is hypothesized to be a key component in the colonization of land plants. In exchange for photosynthetically produced plant sugars, arbuscular mycorrhizas scavenge the soil for nutrients, contribute to soil stability, and protect plants from pathogens (Smith and Read, 2008).

Arbuscular mycorrhizas vary in morphological (Hart and Reader, 2002) and functional ecological traits (Van Der Heijden and Scheublin, 2007). Chagnon et al. (2013) proposed a functional AMF trait-based approach using Grime's Competition-Stress-Ruderal (C-S-R) plant model to understand the ecology of mycorrhizas. When applied to AMF, the C-S-R model uses a mycocentric perspective to categorize AMF species by competitive, stress-tolerant, or ruderal life history traits. The model emphasizes the complexity and specificity of fungal / plant pairing in an applied restoration context.

Arbuscular mycorrhizal inoculum has been used as technical reclamation tool over the past thirty years in a variety of restoration scenarios. Biotechnological propagation of AMF is now available to mass produce fungal inoculum, thus making it a land management option for industrialized scale plant restoration projects (Ijdo et al., 2011). Commercial mycorrhizal

inoculum may ultimately benefit plant growth during initial establishment and survival of plant species and habitats were substrate is severely degraded.

Post-mine areas are stressful environments devoid of beneficial soil microbes such as arbuscular mycorrhizas. Even if topsoil is stockpiled and retained, mining activities have been shown to degrade the efficacy of pre-mine populations of arbuscular mycorrhizas (Stahl et al., 1988). There are many restoration scenarios indicating a positive plant response to AMF inoculum addition in post-mine areas (Pattinson et al., 2004; Wu et al., 2009; Madejón et al., 2010). Plant response to AMF inoculum may be dependent on the combination of mycorrhizal isolate, plant species, and soil environment (Johnson et al., 1997; Klironomos, 2003). For example, research indicates that arbuscular mycorrhizas isolated from mine lands outperform non-mine land AMF communities in terms of plant production (Taheri and Bever, 2010). A balance must be struck because the collection, cultivation, and ultimate propagation AMF species may prove challenging overtime.

METHODS:

Research Site Establishment:

The Nature Conservancy of Canada (NCC) has granted us permission to conduct this research on their land holdings near Port Rowan, Ontario. The St. Williams, Ontario area is within the historic range of tallgrass prairie ecosystems in southern Ontario. The experimental site is set-up on a recently active sand pit (established summer 2010). The research team conducted two field trials at the restoration site: a plant plug trial (*Exp. #1*) and a seed addition trial (*Exp. #2*). These experiments tested the efficacy of two planting strategies (See Photo #1).

Experiment #1 – Plant Plugs Trial:

The plant plug experiment was constructed during the spring 2010. One metric ton (T) [1,000 kg] of biochar, 1.5T of compost, and 8,640 plant plugs (8 grassland plant taxa) were utilized. Plants were grown as plugs (April 2010) at Pterophylla / St. Williams Nursery & Ecology Centre, St. Williams, Ontario, Canada. Pterophylla is a commercial scale nursery located within 2 km of the restoration project. The nursery uses locally sourced prairie plant material that is collected in the vicinity of the restoration project. At the time of plug sowing in April 2010, a commercial AMF inoculum (*Rhizophagus irregularis*) was added to 50% of the plant plug containers at the recommended application rate.

The plant species selected for this project meet the following criteria: 1) core plant species that are a common in Ontario prairies, 2) tolerant of sandy soils, 3) tolerant of dry to dry-mesic moisture regimes, and 4) endemic to the study site area. The native prairie plant species grown are as follows:

- C_3 Grasses: Prairie Brome (*Bromus kalmia*)
  Canada Wild Rye (*Elymus canadensis*)
- C_4 Grasses: Switchgrass (*Panicum virgatum*)
  Big Blue Stem (*Andropogon gerardii*)
- N-Fixing Forbs: Showy Tick Trefoil (*Desmodium canadense*),
  Round-headed Bushclover (*Lespedeza capitata*)
- Composites: Ontario Blazing Star (*Liatris cylindracea*)
  Smooth Blue Aster (*Symphyotrichum laeve*)

Experimental plots were established in June 2010. Each plot was established by using a fully-crossed factorial design. Factors were biochar (BC) / compost (CP) application rates at metric tons per hectare and plant plug inoculation:

Amendment Application	AMF Inoculum
0.0 T/ha 5 T/ha BC 10 T/ha BC 20 T/ha CP 20 T/ha CP + 5 T/ha BC 20 T/ha CP + 10 T/ha BC	*Rhizophagus irregularis* Addition / No Addition

Each 10.2 m^2 plot was replicated (n=10). Thirty plots without plant plugs were established as non-vegetated controls. A total of 150 plots were set-up in a fully randomized order. A one meter buffer zone separates each hexagonal plot to minimize plant interactions.

Plant plugs grown with/without AMF in the greenhouse were transplanted to the field in June 2010. Randomly sorted and pre-mapped, a total of seventy-two (72) native prairie plant plugs were sown (June 2010) into each field plot (plug spacing = 33cm). Each plot has an identical spatial relationship in the 120 plots. Only two plug "misplants" were noted during vegetative censuses. This phase of the tallgrass prairie restoration project was monitored for a total of three field seasons (2010 – 2012).

Experiment #2 – Seed Application Trial:

Exp. #2, adjacent to *Exp. #1*, used a fully-crossed experimental design. *Exp. #2* tested the effect of amendment application rate and *R. irregularis* inoculum on native seed establishment and growth. One metric ton of biochar, one metric ton of compost, and seeds of eight grassland species are utilized in *Exp. #2*. Each amendment combination was replicated twice for a total of seventy-two 10.2 m^2 plots. Soil amendments were added to *Exp. #2* in August 2010. Fully-crossed soil amendment application rates are described in the following chart. For example, 0T/ha BC was combined with a 0.0T/ha CP to establish one plot. Next, 0 T/ha BC was combined with 2.5 T/ha CP to established another plot. This systematic assignment continued until as possible combinations of BC and CP application rates were associated.

Biochar Application Rate	Compost Application Rate
0.0 T/ha	0.0 T/ha
2.5 T/ha	2.5 T/ha
5.0 T/ha	5.0 T/ha
10.0 T/ha	10.0 T/ha
20.0 T/ ha	20.0 T/ ha
40.0 T/ha	40.0 T/ha

To minimize overwinter seed mortality and undesired seed movement, native plant seeds and mycorrhizal inoculum were applied to *Exp.* #2 in May 2011. After distributing the seed, a seed roller was used to press the seed into the sand pit floor. Mycorrhizal inoculum was added to one set of the amendment application rates via a liquid medium containing spores. Seeds and mycorrhizal inoculum were applied at standard rates for recommended for tallgrass prairie restoration projects.

Arbuscular Mycorrhizal Inoculation:

AMF colonization of roots was quantified for greenhouse grown plant plugs (June 2010) and field plots (September 2011 / 2012) for *Exp. #1*. To evaluate inoculum presence in the plant plug roots, ten control and ten inoculated plugs from each plant species were randomly selected in June 2010. Plant roots were gently washed with water, cut into 1 cm pieces and preserved in 50% ethanol until analysis. To determine percent colonization, roots were dyed with a fungal specific stain. Stained roots were counted systematically under a microscope using the gridline intersect method (McGonigle et al., 1990). Photo #2 is an example of stained fungi in roots.

To evaluate AMF inoculum presence roots growing in the field, soil cores were systematically collected and pooled at the plot level in September 2011 / September 2012 near designated plant plug locations. Soil cores were washed, roots removed and cut into 1cm pieces, and preserved in 50% ethanol until microscopic analysis. Approximately 1,500 soil cores were collected from the site during each field season.

Plant Growth Dynamics:

An important aspect of this project is to accurately measure plant growth. Ideally, plant biomass should be tracked over several years to best understand the plant community growth patterns. Furthermore, large-scale destructive harvests would negatively influence long-term data collection procedures. We developed innovative methods to accurately determine plant biomass that minimized plant destruction within the plots. Three biomass assessment techniques were used for this experiment:

Technique #1 - Aboveground Photographs

Plant cover can be used to estimate the growth of the plant community. A photographic technique was implemented to estimate the percent cover of plant growth for each plot. This simple, non-destructive technique was used repeatedly throughout the experiment to track plant growth patterns.

To accomplish this, an apparatus was constructed to take overhead pictures in each plot (Photo #3). Photos are analyzed for green pixel coverage to estimate the cover of photosynthetic (active) tissues using the computer program, SamplePoint (Booth and Cox, 2008). Percent cover measurements are based on the classification of 100 pixels per standardized photograph taken for *Exp. #1* and *Exp. #2*. Photographs were taken at three sampling points for *Exp. #1* and *Exp. #2*: September 2011, June 2012, and September 2012. Plot-level percent cover data for *Exp. #2* is presented in a 3-D graph (Graph #3).

Technique #2 – Plant Plug Survivorship

Plant survivorship was estimated for *Exp. #1*. Since the plant plug experiment was spatially mapped, plant plug survivorship can be tracked. Thirty-six (36) plant plug locations in the center of each plot were analyzed for new growth each growing season. Plant survivorship

was determined for aboveground plant structures only. Survival of a plant plug was estimated by the presence of new, photosynthetically active leaf tissue for that growing season. Survivorship data was collected for September 2010 / 2011/ 2012.

Technique # 3 - Plant Biomass Estimation for Individual Plant Plugs

We developed a statistical technique from the organic chemistry literature to non-destructively estimate plant biomass. Partial least squares regression (PLSR) is a multivariate statistical method that uses multiple collinear predictor variables to accurately predict a response variable. This method incorporates a variable selection statistical method named BIC, Bayesian Information Criterion. BIC selects the best predictor variables to estimate the response variable.

Related to this project, a subset of the plots had to be destructively harvested to create a PLSR standard curve. A suite of measurements, such as plant height, basal diameter, leaf number, stem height, were collected for each plant species in the project (36 replicates). These measurements are the predictor variables. Each plug location was harvested after predictor variable data was collected via double sampling methodology. Once harvested, aboveground biomass was dried at 60^{o}C in a forced air drying oven and weighed to determine plant mass. For each species, the best predictor variables for the PLSR standard curves were selected via BIC model selection. The selected predictor variables were used to non-destructively measure plant plugs in the field. Approximately 3,900 individuals were measured during the each field season.

Soil Food Web Analysis

Sixteen soil cores from each plot were collected and pooled in September 2012 from *Exp #1*. At the time of collection, soil samples were stored in a cooler on ice until final storage at 4^{o}C. The soil corer was cleaned of substrate with a clean cloth and water between each sampling. Soil food web trophic groups were analyzed as follows:

Bacterial and fungal abundance

Bacterial and fungal abundance was estimated by differential fluorescent staining (DFS) following an adapted protocol by Klironomos et al. (1996). For fungal counts, 200 mL of soil was suspended with 1 mL of DFS stain for 1 hour. Once stained, the suspension was filtered through nitrocellulose filter paper using a 50% ethanol wash. Filters were then mounted on microscope slides for visual inspection under UV light (620 nm). Active cellular material was visually highlighted with red fluorescence under UV light. For bacterial abundance, smears were established from soil dilutions and stained with DFS for 1 hour. Filters were rinsed with 50% ethanol wash and slides mounted for visual inspection using UV microscopy.

Fungal and bacterial biomass was calculated using computer imaging software. Fungal biomass was estimated using the measured hyphal length and published estimates of hyphal diameter (1.65 μm)(Kjøller and Struwe, 1982), density (0.33 g cm^{-3}) (van Veen and Paul, 1979), and C content (45%)(Swift et al., 1979). Bacterial biomass was estimated with the conversion factor of 6.4×10^{-14} gC $cell^{-1}$(Hunt and Fogel, 1983).

Nematode abundance

The number of nematodes was determined using the same wet sieve sucrose centrifuge approach for extracting arbuscular mycorrhizal spores, as described in Klironomos et al. (1993). As a brief summary, soil samples were suspended in water and passed through a series of mesh sieves decreasing in pore size (1.0 mm - 45 μm). After rinsing with water, the material retained in the 45 μm sieve was suspended on top of a 60% sucrose solution and centrifuged for 20 minutes. Nematodes were collected via a pipet at the sucrose–water interface. Nematodes were manually counted under a microscope and sorted into functional feeding groups.

Mite and Collembola Abundance

A high efficiency canister-type soil arthropod extractor (Lussenhop, 1971) was used to extract mites and collembola onto dishes containing picric acid as described in Klironomos et al. (1996). Each microarthropod was manually counted and sorted to obtain abundances per plot.

Statistical Analyses

Mycorrhizal colonization and soil food web data were analyzed using generalized linear models (GLM). The error structure of count and proportion data is non-normal and tends to follow a Poisson or negative binomial distribution. The data is analyzed by fitting the appropriate link function to the dataset. GLMs eliminate the need to statistically transform the dataset to approximate a normal distribution. For each analysis, the full combination of experimental factors (i.e. soil amendment, AMF inoculation) and interaction terms are entered into the GLM and compared to a null model. If the full model significantly explains more of the data than the null, then the experimental factors are explaining the dependent variable in some capacity. Using maximum likelihood estimates, Bayesian Information Criterion (BIC) estimates evaluate iterations of each model, successively removing insignificant interaction terms and experimental factors. The most parsimonious model selected by the lowest BIC estimate was employed and the data visualized. Data was analyzed using the glm.nb package associated with the statistical language program R.

Tracking plant biomass estimates, linear mixed effects models were used to account for random variation associated with plots. Data transformations were employed when necessary to approximate an error structure resembling a normal distribution. The same methodology as above was used to select the most parsimonious model using BIC values. Linear mixed effects models were analyzed using the lme4 package associated with the statistical language program R.

Results and Discussion:

Slight variations in topography were detected at the field site creating a potential gradient of water availability. After a covariate analysis was performed, the relationship of topography to plant biomass was not significant. No topography corrections were implemented in these analyses.

Mycorrhizal Colonization:

The presence of mycorrhizal inoculum was detected in the roots of inoculated plant plugs in *Exp. #1*. Investigation of the greenhouse grown plant plugs indicates that all species are receptive to inoculation with *Rhizophagus irregularis* (Graph #2). Plant plug roots in the AMF inoculated treatment exhibited a significant increase in colonization compared to the non-inoculated controls. Non-inoculated plant plugs had low mean colonization rates [> 5%]. This result is expected due to the non-sterile growing conditions of the commercial greenhouse setting. The inoculated treatment was "super saturated" with *R. irregularis* resulting in higher colonization rates compared to controls [mean ranges: ~10% - 30%] (Graph #2). These results indicate that an AMF inoculum treatment was established for the *Exp. #1* field trial.

Total AMF structures were quantified in field roots and tracked for two growing seasons (Graph #3). AMF colonization rates for the mixed root samples indicate differences in the inoculated plots compared to non-inoculated plots after one and two growing seasons. Low colonization rates were noted in the non-inoculated treatments. *R. irregularis* inoculum persists in the field, nearly doubling rate of root colonization between the first and second growing season (Graph #3). In the second growing season, a significant increase in AMF colonization of roots in treatments where 10T/ha biochar + 20T/ha compost was added. The addition of the

biochar and compost in conjunction with AMF inoculum created more favorable conditions for the growth of mycorrhizal communities.

During the microscopic quantification of AMF colonization of roots, mycorrhizal colonization was categorized into morphological structures (i.e. arbuscules and vesicles). Arbuscules form inside the cortical cells of plant roots and serve as the site of nutrient exchange between the plant and fungus. Photo #2 is a visual representation of hyphae and arbuscules. Vesicles are AMF storage structures that contain lipids and cytoplasm. These long-term AMF structures form within cortical root tissue and can serve as fungal propagules within roots.

A significant increase in arbuscules between the first and second growing season is indicated in treatments adding both biochar and compost (Graph #4). An increase in arbuscules indicates that the symbiosis performing better in biochar/compost amended soils. The increased availability of nutrients and retention in these treatments are promoting the plant and fungal interactions. The addition of biochar or compost alone did not significantly affect the incidence rate of AMF arbuscules.

Soil amendment rates did not influence the formation of vesicles in the mixed root samples (Graph #5). AMF inoculum addition significantly increased the incidence rates of vesicles within the plant roots. Vesicle formation in both the inoculated and uninoculated treatments doubled between the first and second growing season. As vesicles can serve as fungal propagules, the fungal population within inoculated plots has a higher chance of long-term persistence compared to uninoculated plots.

Plant Plug Survivorship:

At the time of planting, all native plant plugs were alive. No significant difference in plant plug survivorship was detected between the inoculated and non-inoculated treatments. In

this analysis, inoculated and non-inoculated treatment survivorship data was pooled due to the lack of a significant difference. Total plant plug survivorship is high [mean ~ 90%] after one full growing season, regardless of soil amendment application (Graph #6). Mean total plant plug survivorship decreased by approximately 10% between the first and second growing season. The reduction in survivorship is similar across all soil amendment applications.

To investigate the source of plug mortality, plants were sub-divided into plant functional groups. C_4 grasses and nitrogen-fixing wildflowers had a consistently high survivorship across all growing seasons and treatments (Graph #7). This indicates that plants plugs in these functional groups are adapted to growth in sandy, post-mine substrates. The composites and C_3 grasses used in this project had a sharp decline in survivorship during the 2012 growing season (Graph #7). Although drought tolerant, these native species typically have a higher water requirement in comparison to the C_4 grasses and nitrogen-fixing plants. Reduced rainfall during the 2012 spring may have reduced plant recruitment and contributed to the decline in plug survivorship (Graph #1).

Plant Growth Dynamics in the Plant Plug Trial:

When restoring post-extraction sand pits, the plant plug option is less cost effective when compared to distributing native seed. However, if the post-mine aggregate site needs to be restored quickly and effectively, the results of *Exp. #1* indicate that sowing native plants plugs is a viable option. The installation of plant plugs produces more plant biomass than planting seed alone over a similar growing period (Photo #1). Plants grown from plugs are nurtured in a greenhouse setting, thus overcoming the initial stressfully soil conditions within post-mine substrates.

The installation of plant plugs reduced wind scouring on the research site. I have personally observed a reduction of laminar flow erosion by wind gusts after plug installation. Therefore, the installation of plant plugs may be a technical reclamation management tool to control wind erosion and accelerate soil stabilization at post-mine sites. Integrating plug installation with native seeded may be a viable hybrid technique to minimize seed loss and stabilize substrate at a restoration site.

The majority of the plants grown from plugs produced seed after one growing season. By the second growing season, most plants had a high seed set, indicating that our restoration plots are self-replicating and self-sustaining. Compared to natural site recolonization, sowing plant plugs is an effective strategy for rapid plant biomass development (Figure #1). When left to natural plant recolonization, control plots (i.e. no plant plugs) where sporadically colonized by weedy, ephemeral plants with low biomass. Control plots were interspersed with plots producing viable native seeds. Despite this, native seed recruitment was minimal in these control areas even with the addition of only soil amendments. Therefore, the use of native plant material is essential when restoring grassland habitat in post-mine aggregate sites. Further experiments need to be conducted to determine the most cost effective plant plug spacing while delivering the highest ecological benefit for the restoration project.

Although plant survivorship was generally high across all treatments in *Exp. #1*, these results do not indicate plant community growth and performance. Results indicate a positive trend in predicted total plant dry weight when compost or compost + biochar are added to post-mine substrate (Graph #8). This indicates the compost addition is a main driver translating into plant biomass. No significant AMF inoculum effect was detected on total plant dry weight (Graph #8). After one growing season, compost addition resulted in an increase in total plant

biomass compared to control. By the second growing season, no significant difference in total plant biomass was observed across soil amendment applications.

To understand species growth performance at the research site, Graphs #9 - #14 explore the total predicted mass of each species over two growing seasons. Note: Graphs #9 - #14 are represented on the same y-axis scale to portray relative biomass contributions for each species. In summary, each plant species responded differently to the suite of experimental treatments. All plant species, except for big blue stem, had a neutral to positive response to the compost or compost + biochar amendment addition. Plant species growing in biochar only treatments had a neutral to negative biomass response compared to control. Switchgrass and round-headed bush clover biomass was significantly greater in AMF inoculated compared to uninoculated plants. Big bluestem and showy tick trefoil biomass was significantly decreased after AMF inoculation.

Each plant species has a specific range of optimal conditions for growth and development. The environmental tolerance of each plant species to the biogeochemical conditions of altered post-mine substrates may favor one plant species, while being a detriment to another. Although individual species results may vary, the addition of 20 T/ha compost and 10T /ha biochar to post-mine substrate will optimally deliver conditions conducive to greater plant biomass. The increased plant community biomass is due to the slow release fertilizer effect, increased water retention, and favorable biogeochemical cycling from the synergistic combination of the amendments.

The varied plant biomass response of each species in the presence AMF inoculum accounts for the lack of a statistical effect in total plant mass. The use of commercial inoculum addition in post-mine sandpits in terms of plant biomass is inconclusive as shown by our research. Several factors may have led to inconclusive results: 1) Plants were inoculated under

commercial greenhouse conditions. Therefore, a low level of mycorrhizas was present in uninoculated plant plugs. Colonization of uninoculated plant plugs may have negated the effects of the AMF inoculum treatment. 2) The pairing of the commercial AMF inoculum may not have led to an optimized plant response. Inoculating plants with AMF isolated from sandy, post-mine habitats may increase plant biomass due to adaptation of the inoculum to post-mine substrate. 3) A higher AMF diversity in the commercial inoculum may illicit an increased plant mass response. Further research needs to be conducted regarding the most appropriate mycorrhizal inoculum to include in the restoration of tallgrass prairie species in abandoned aggregate sites.

Plant Growth Dynamics in the Seed Application Trial:

Percent native plant cover for the seed application trial is visualized in Graph #23. Although the graph is complex, trends indicate that native plant cover increases as compost rates increase in the presence of AMF inoculum. Biochar addition was most effective at low application rates when paired with high rates of compost addition. The addition of AMF inoculum, high-levels of compost (20 T/ha – 40 T/ha) and low levels of biochar (0 T/ha – 10 T/ha) will achieve optimal native plant growth conditions in post-extraction sand pits. Our results indicate that commercial inoculum is most effective when growing grassland plant from seed. Initial seedling establishment will benefit from increased nutrient acquisition supplied by AMF associations. AMF inoculum will help mitigate stressful environmental conditions of post-mine aggregate sites.

Soil Food Web Analysis in the Plant Plug Experiment:

Soil food web trophic levels were significantly altered by soil amendments in the experiment after two growing seasons. Bacterial biomass significantly increased in plots amended with compost and biochar (Graph #15). The addition of biochar alone significantly

decreased bacterial biomass. Due to a high cation exchange capacity, biochar only addition may have reduced the pool of available nutrients in the soil matrix. Thus, biochar addition only may further stress the post-mine systems. The addition of compost with biochar flushed the system with nutrients in the soil solution. Soil building benefits can be maximized when these amendments are used in tandem. Compost and compost + biochar addition significantly increased fungal biomass while biochar alone had no significant effect (Graph #16). The addition of complex organic material favors the promotion of fungal hyphae in the soil matrix. The promotion of fungal hyphae in mine substrates encourages soil stabilization and positive biogeochemical cycling. Overall, increasing the biomass of the basal microorganisms had a cascading effect on microarthropods and soil nematodes.

Substrates amended with biochar and compost resulted in increased fungal and bacterial biomass compared to control, thus significantly increasing the abundance of nematodes and microarthropods. Soil organism abundance displayed predictable patterns per soil supplement treatment based on the biomass of bacterial and fungal communities. Collembola abundance was highest in the treatments adding biochar and compost (Graph #17). Fungal and bacterial feeding nematode abundance increased based on the associated microorganism biomass (Graph #18 - #19). The highest increase in nematode abundance was exhibited treatments adding biochar and compost. Microbial feeding mite abundance significantly increased in treatments adding compost and biochar (Graph #21). Although more variable, the presence of predatory mites and nematodes were significantly increased in treatments adding compost and biochar (Graph #20, #22).

The largest stimulation of soil organisms occurred in plots adding compost and biochar. The rate of biochar addition (5T/ha – 10T/ha) in *Exp. #1* did not alter the structure of the higher

order soil organisms. The addition of compost alone had a moderate effect on the abundance of soil nematodes and microarthropods. When adding biochar alone, the reduction in bacterial biomass resulted in a reduction of bacterial feeding nematodes and microbial feeding mites. Soil development trajectories can be inference by microbial biomass and soil invertebrate abundance. Post-mine substrate adding low rates of biochar and compost can significantly alter soil food web structures belowground. Positively altering the organic matter and nutrient content of post-mine aggregate sites will result in more sustainable, functional soil matrices.

Conclusions:

Compared to natural site recolonization, sowing plant plugs is an effective strategy for rapid plant biomass development (Figure #1). When left to natural plant recolonization, control plots (i.e. no plant plugs) were sporadically colonized by weedy, ephemeral plants with low biomass. Control plots were interspersed among experimental plots producing viable native seeds. Despite this, native seed recruitment was minimal in these control areas even with the addition of only soil amendments. Therefore, the use of native plant material is essential when restoring grassland habitat in post-mine aggregate sites.

The results of this study indicated that the addition of municipal compost, biochar and mycorrhizal inoculum are simple land management tools that improve plant performance in post-extraction aggregate sites. In the plant plug experiment (*Exp. #1*), 20T/ha compost mixed with low rates of biochar (5T/ha – 10T/ha) had the highest positive effect on plant performance, AMF colonization of roots, and soil food web biomass and diversity. AMF inoculation, high rates of compost (20T/ha – 40 T/ha) and low rates of biochar (0T/ha – 10T/ha) resulted in optimized plant cover in the seed experiment. The conclusions from the plant plug and seed trials were consistent. The main driver of plant performance in this restoration project was the addition of

municipal compost and mycorrhizal inoculation (especially during the establishment of seed). Therefore, these amendments can reduce plant stress in post-extraction substrate where topsoil may be lacking.

The incorporation of biochar into quarry rehabilitation projects is not recommended at this time. The benefit of the biochar soil amendment does not outweigh its cost in the current market. Biochar's wide-scale availability has also not met the expectations promised by the biochar industry in southern Ontario. As availability increases and costs decrease over the next decade, incorporating low application rates of biochar is recommended (10T/ha) when administering compost to rehabilitate soils and facilitate plant growth.

Recommendations for Establishing Tallgrass Prairie:

Tallgrass prairie plants are a viable option to recreate natural habitat in aggregate pits. Many of Ontario's prairie plants are adapted to dry, well-drained soil conditions characteristic of aggregate pits. Altering substrates with easy to apply soil amendments and biological inoculants will positively influence plant growth in these systems.

Plant Species and Sourcing:

This restoration project used locally-collected seed mixtures which were adapted to regional growing conditions. Locally-sourced plant material has been shown to positively influence plant establishment rates and total plant cover. Be sure to choose a high diversity of plant material for your restoration project. A high diversity is considered to range from 10 – 30 plant species which includes a mixture of warm season grasses, cool season grasses, legumes (i.e. nitrogen-fixing plants), and wild flowers. Grasses will form the foundation of the habitat's structure. Incorporating a high diversity seed mixture will maximize vegetative establishment in the project.

When selecting a nursery for your restoration project, ensure that the company has a specialization in native plant material. Plant nurseries that source local vegetation have expertise on native plants, soil conditions, and restoration challenges. A nursery should have expertise about selecting plants adaptable to dry conditions of sand and gravel extraction sites. Providing the nursery with general site characteristics such as light availability, topography, hydrology, and site size is useful information in the plant selection process. With the environmental variables provided, the most appropriate plants can be selected for the pit restoration project in your area. For nurseries recommendations in your area, contact Tallgrass Ontario for a list of native plant suppliers (www.tallgrassontario.org). It is recommended that local native plant nurseries are contacted as they will be able to supply a quote and plant selection recommendations for the restoration project.

Arbuscular Mycorrhizal Inoculum:

The arbuscular mycorrhizal inoculum, *Rhizophagus irregularis*, is most effective during seed application. No significant effects of AMF inoculum were detected in the plant plug experiment (*Exp. #1*). The application of AMF inoculum as a seed coat at the time of sowing native plant seeds is recommended. *Rhizophagus irregularis* (a.k.a. *Glomus intraradices*) can be purchased as a seed coat powder from Myke® Pro (www.usemykepro.com) and applied at the rate suggested by the manufacturer. The inoculum recommended for agricultural crops, Myke® Pro PS3, would be the most effective AMF inoculum for grassland restoration in pits. A large list of inoculum suppliers can be found at http://usemykepro.com/store-locator-find_myke-pro/agriculture.aspx.

Compost:

Compost can be purchased locally at landscape supply locations across Ontario. The approximate cost of compost is $40 - $50 per metric ton plus delivery. Compost is typically generated from municipal waste collection streams. Compost should be incorporated directly into the upper 10cm of substrate at a rate of approximately 20T/ha – 30T/ha before sowing and plug planting.

Plant Plugs vs Seeds:

Approx. Cost to Establish One Hectare of Prairie Grasses	
Prairie Rehabilitation w/ Seed	
Seed Application / ha (no grading required)	$3,000
Miscellaneous Costs (Transportation, etc.)	$500
Subtotal	$3,500
Prairie Rehabilitation w/ Plugs	
Plug Cost ($1.00 each x 20,000 plants / ha [1 plant / 0.5 m^2]	$20,000
Miscellaneous Costs (Transportation, etc.)	$500
Subtotal	$22,750
Ecological Boosters	
AMF Inoculum (4kg inoculum = 5.3 ha coverage)	$400
Compost [$45 / metric ton x 20T/ha]	$900
***Please note that the cost per ha decreases as the rehabilitation area increases**	

The preceding table, ***Approx. Cost to Establish One Hectare of Prairie Grasses***, considers the projected materials cost of land rehabilitation in abandoned sand and gravel pits. Two viable options are available for prairie system rehabilitation: seed addition or plug addition. The decision to rehabilitate prairies with native plant seeds or plugs will be determined by desired speed of recovery and future maintenance considerations. Seeding the landscape incorporates drawback such as:

(1) slower and less successful plant establishment,

(2) possible increased time to achieve rehabilitation certification,

(3) increased site maintenance requirements (i.e. reseeding applications), and

(4) increased influence of weedy, invasive plant species (i.e. herbicide applications may be necessary).

Although the upfront cost of sowing native plant plugs and using ecological boosters in a rehabilitation project is initially more cost prohibitive, plant plugs and ecological boosters are projected to accelerate project recovery time and increase prairie plant competitiveness thus reducing future site maintenance. If the aggregate site needs to be restored quickly and effectively, the results of *Exp. #1* indicate that sowing native plants plugs is a viable option. Furthermore, plant growth and establishment is much greater in the plant plug trials as compared to the seed trial (Photo #1). Initiating plant plug growth in the greenhouse helps plants to overcome the harsh abiotic threshold found in post-mine substrate.

The majority of the plants grown from plugs were producing seed after one year of growth. By year two, most plants had a high seed set, indicating that our restoration plots are self-replicating and self-sustaining. The use of plant plugs can have dramatic growth results even after only one full growing season. Quick plant establishment is anticipated to accelerate soil stabilization by binding substrate with native plant roots and reducing laminar flow wind energy (i.e. reducing wind scouring). From personal observation, plant plug addition reduced surface erosion by wind energy.

Site Planning:

Planting plugs and seeds should be timed with the seasons. Seeds can be distributed in early spring (March – April) or mid-fall (October –November). Plug planting should coincide with the rainy season after the threat of frost (April – early May). Plant plugs will initially need the high rainfall levels to establish a rooting system. Plant plugs require a couple of months to

grow in the greenhouse. Contacting a nursery for seed source, growth timing, and material availability should be one of the initial steps in the planning process. The earlier in the process a nursery is contacted, the more efficient a restoration project will be accomplished.

Site Preparation:

When preparing the pit floor substrate for a grassland restoration project, the area should be roughly graded flat to allow for ease of planting. Once graded, compost can be tilled into the upper 10 cm of sandpit substrate before planting occurs. We recommend minimizing the time between compost incorporation and planting to reduce the colonization of unwanted weedy plants. Seeds and/or plant plugs can be sown by hand or with machinery depending upon the scale of the project. Ideally, seeds should be compacted with a seed roller to ensure solid contact with the pit floor. We do not recommend reincorporating long-term storage stock piles into the site. A high density of weedy plants will have developed on the stock-piled topsoil. If the stock-piled topsoil is recently excavated, topsoil may be re-incorporated into the pit floor substrate.

Restoration Project Conclusion:

Our goal was to optimize cost and effectively establish a tallgrass prairie ecosystem. It is suggested that integrating both planting approaches (i.e. plant plugs and seed) for the most effective ecosystem establishment. We recommend incorporating 20T/ha – 30T/ha of compost before planting and /or seeding the site. Incorporate plant plugs composed of legumes and warm season grasses at a rate of one plug per square meter. These plants have a high survivorship and growth success at the site, which will maximize the cost effectiveness of plant plugs. Sow a high diversity plant seed mixture containing warm season grasses, cool season grasses, legumes, and wildflowers among the plant plugs. This restoration strategy would complement the desired

outcome of rapid grass / herbaceous plant establishment with the cost effectiveness of using native seed mixtures.

For a complete treatment of grassland restoration in southern Ontario, please refer to:

PLANTING THE SEED: A GUIDE TO ESTABLISHING PRAIRIE AND MEADOW COMMUNITIES IN ONTARIO

Delaney, K., L. Rodgers, P.A. Woodliffe, G. Rhyndard, and P. Morris, 2000. Planting the Seed: A Guide to Establishing Prairie and Meadow Communities in Southern Ontario. Environment Canada.

Available online at:

http://www.csu.edu/cerc/researchreports/documents/PlantingTheSeedGuideEstablishingPrairieMeadowCommunities2004.pdf

List of Plant Suppliers Maintained by the Ontario Chapter of the Society for Ecological Restoration:

Available Online at: http://www.serontario.org/publications.htm

Resource for Grassland Ecosystems in Ontario: Tallgrass Ontario

Available Online at: http://www.tallgrassontario.org/index.html

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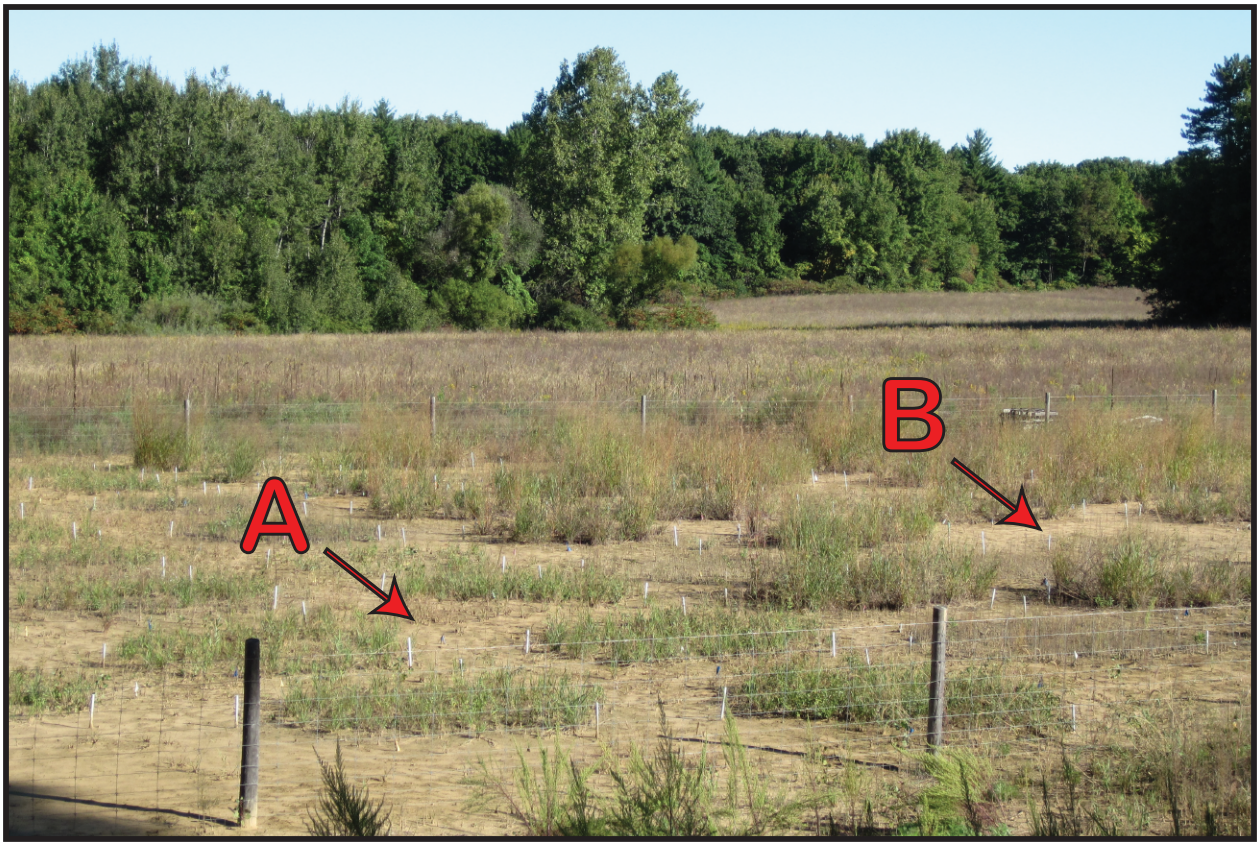


Photo #1: The seed experiment one year and five months after seed application (A). Plants are established and have sent deep roots into the sand substrate. Plant biomass is anticipated to dramatically increase during the third growing season. Compare and contrast the plot biomass from each experiment. Plots with plugs are indidated by (B). During a restoration, plant plugs will yield faster, more dramatic results. (Photo Taken: September 2012)

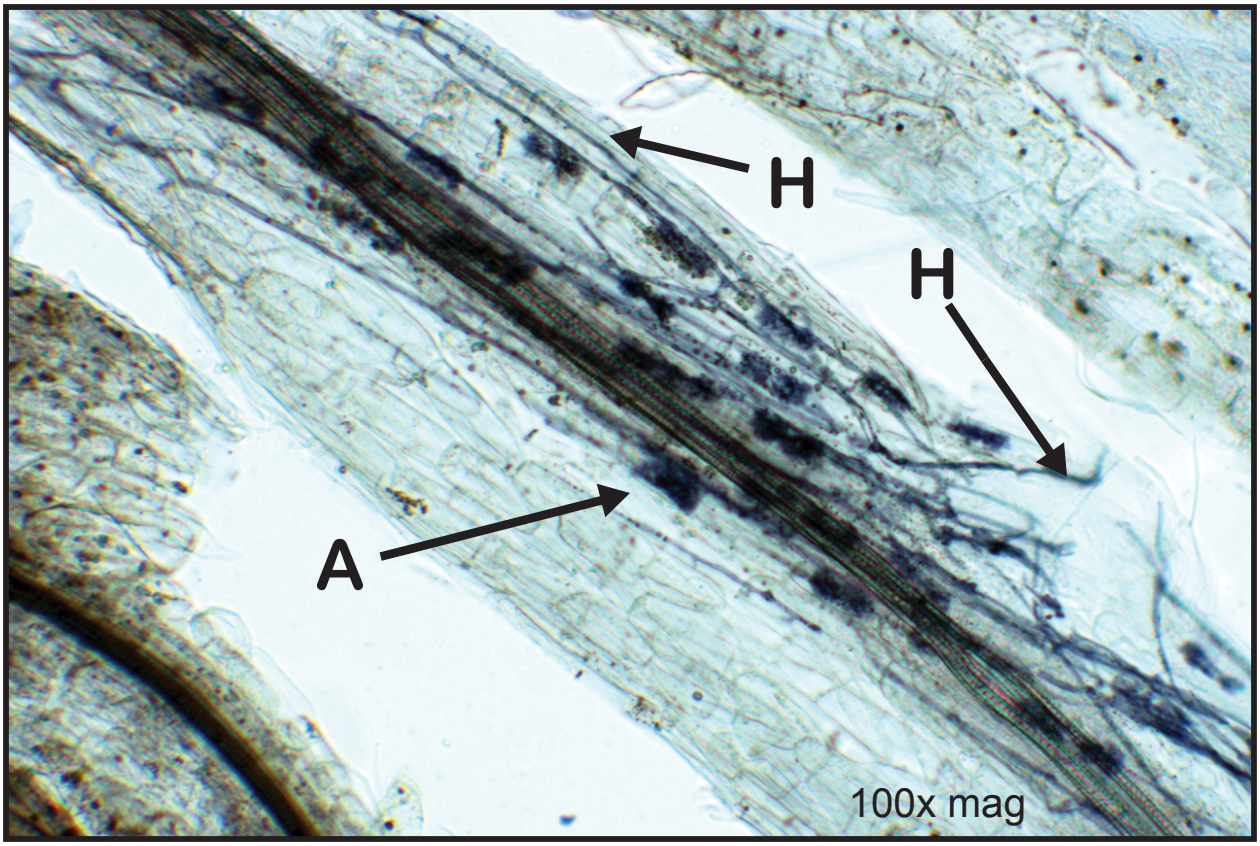


Photo #2: An example of roots colonized by arbuscular mycorrhizas visualized with a microscope (Magnification = 100x). Mycorrhizas are visualized with a fungal specific ink and vinegar stain. *Rhizophagus irregularis* (the experimental inoculum) is pictured here growing in the roots of *Plantago lanceolata*. The dark blue patches are arbuscules (A). Arbuscules, growing within the plant's root cells, are the site for chemical exchange between the plant and the fungus. The dark blue lines are hyphae (H). Hyphae (main body of the fungus) are tubular structures that connect arbuscules and explore the soil for nutrients. (Photo Taken: February 2013, Credit: Brian Ohsowski)

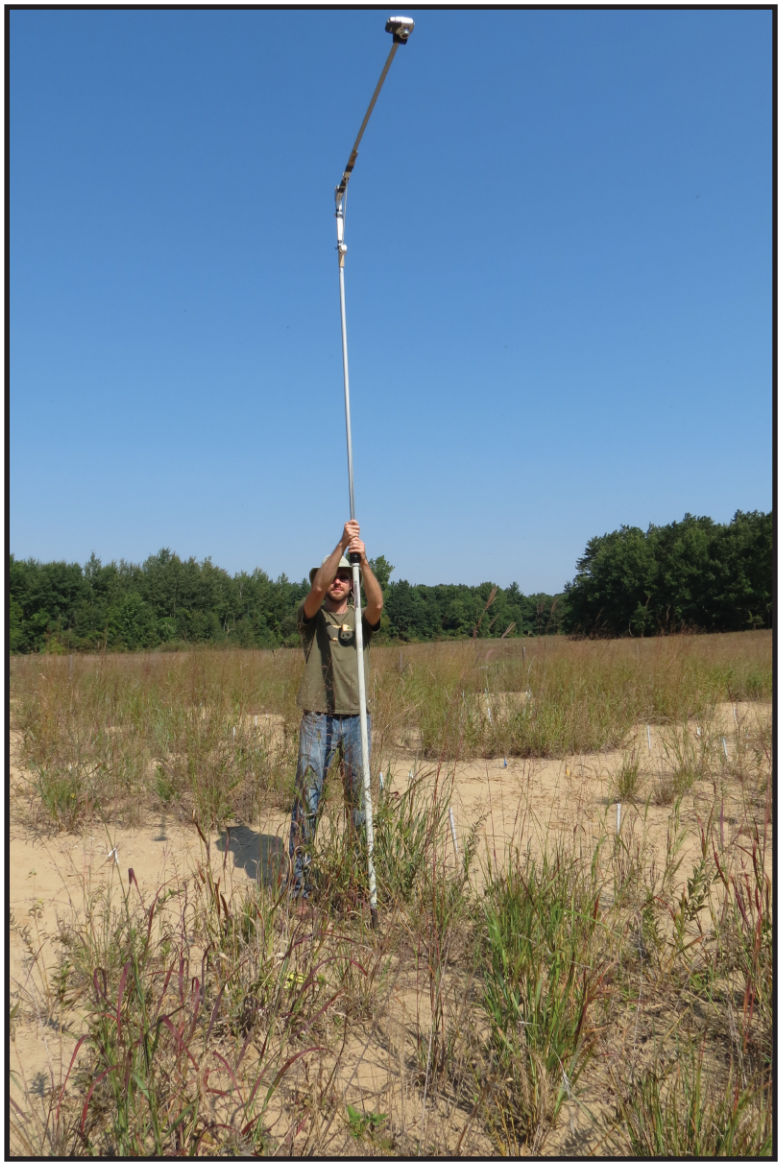
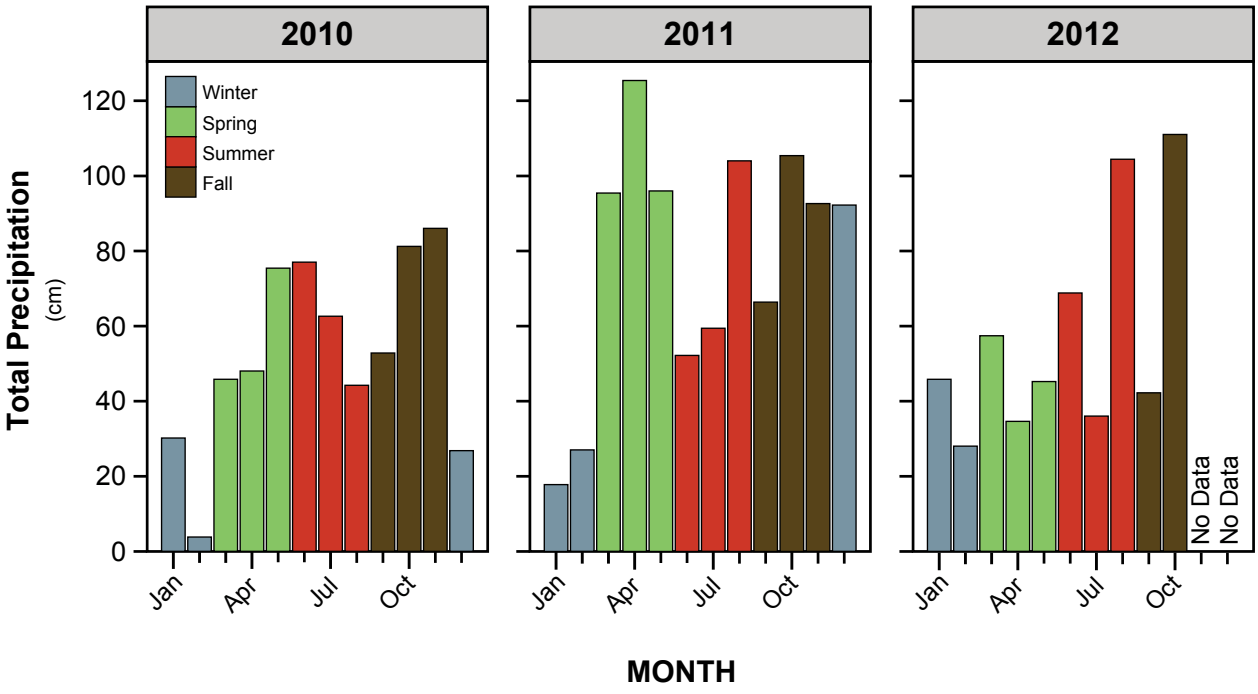
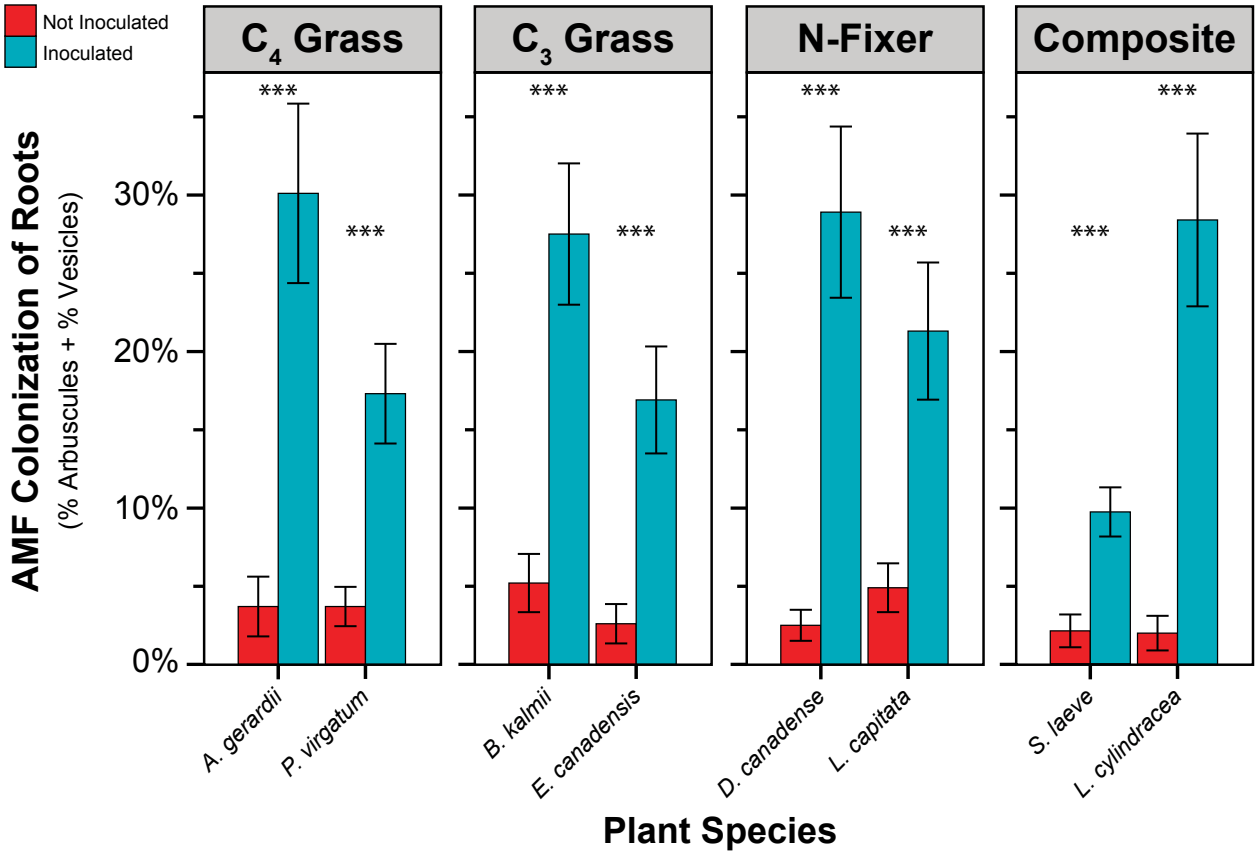


Photo #3: Collecting photographic data to analyze percent plant cover. We used innovative approaches to reduce the need to destructively harvest this long-term research site. (Photo Taken: September 2012, Credit: Brian Ohsowski)

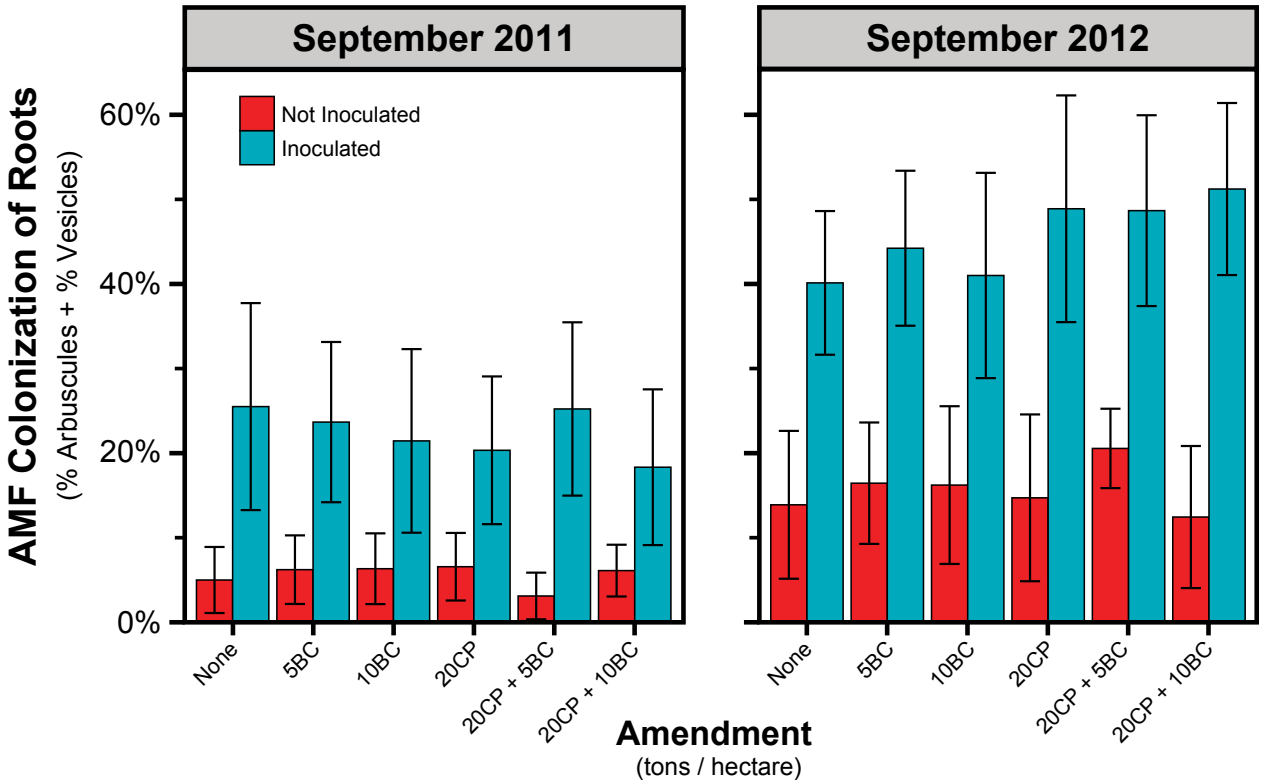


Graph #1: Sum of monthly precipitation (units = cm) collected at the Bird Studies Canada weather station in Port Rowan, Ontario. This weather station is approximately 6 km south of the grassland restoration research site. Note the periods of high rainfall during the spring of 2011 compared to spring 2010 and spring 2012.



Graph #2: Total AMF colonization of greenhouse grown plant plug roots. Total colonization of inoculated vs. inoculated plugs was compared separately for each plant species. Data is binned by plant functional group. Statistical significance determined by generalized linear models. Error bars +/- 1 standard deviation; p-value (***) < 0.000. Each treatment level is replicated ten times (n=10).

Plant Species: C_4 Grasses (*Andropogon gerardii, Panicum virgatum*), C_3 Grasses (*Elymus canadensis, Bromus kalmii*), nitrogen-fixing legumes (*Lespedeza capitata, Desmodium canadense*), composite flowers (*Liatris cylindracea, Symphyotrichum laeve*).

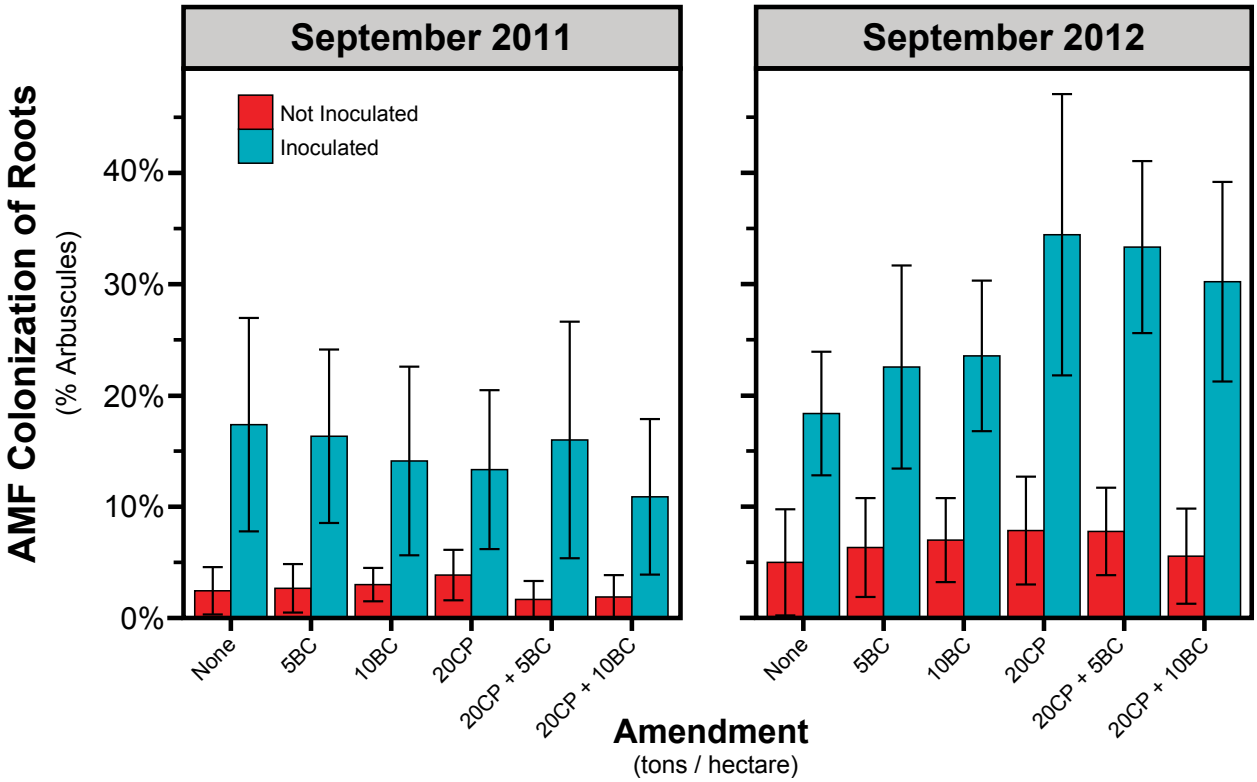


Treatment	Coeff. Est.	Coeff. Std Error	Pr(>|z|)	sig.
Main Effects				
(Intercept)	1.609	0.195	< 2e-16	***
5BC	0.219	0.267	0.413	n.s.
10BC	0.236	0.266	0.375	n.s.
20CP	0.273	0.282	0.333	n.s.
20CP + 5BC	-0.475	0.299	0.112	n.s.
20CP + 10BC	0.201	0.268	0.453	n.s.
Season	1.022	0.248	0.000	***
AMF	1.629	0.246	0.000	***
Significant Interactions				
20CP + 5BC x Season	0.867	0.366	0.018	*
Season x AMF	-0.568	0.323	0.079	(.)
20CP + 10BC x AMF x Season	0.885	0.450	0.049	*

Significance codes: *** < 0.001 | ** < 0.01 | * < 0.05 | . < 0.1 | n.s. > 0.1

Note: Significantly different intercepts with negative values in parentheses

Graph #3: Total AMF colonization of a mixed community of roots collected from Exp #1. The left panel represents the first full growing season. Statistical significance based the most parsimonious model selected via model comparison (lowest AIC value) using a negative binomial distributed generalized linear mixed effects model. Coefficients estimates are relative to the model intercept (no amendment, no amf inoculation, first growing season). Error bars +/- 1 standard deviation. Each treatment level is replicated nine times (n=9). Abbreviations: none = no amendment, 5BC = 5 T/ha biochar, 10BC = 10 T/ha biochar, 20CP = 20 T/ha compost, 20CP + 5 BC = 20 T/ha compost + 5 T/ha biochar, 20CP + 10 BC = 20 T/ha compost + 10 T/ha biochar.

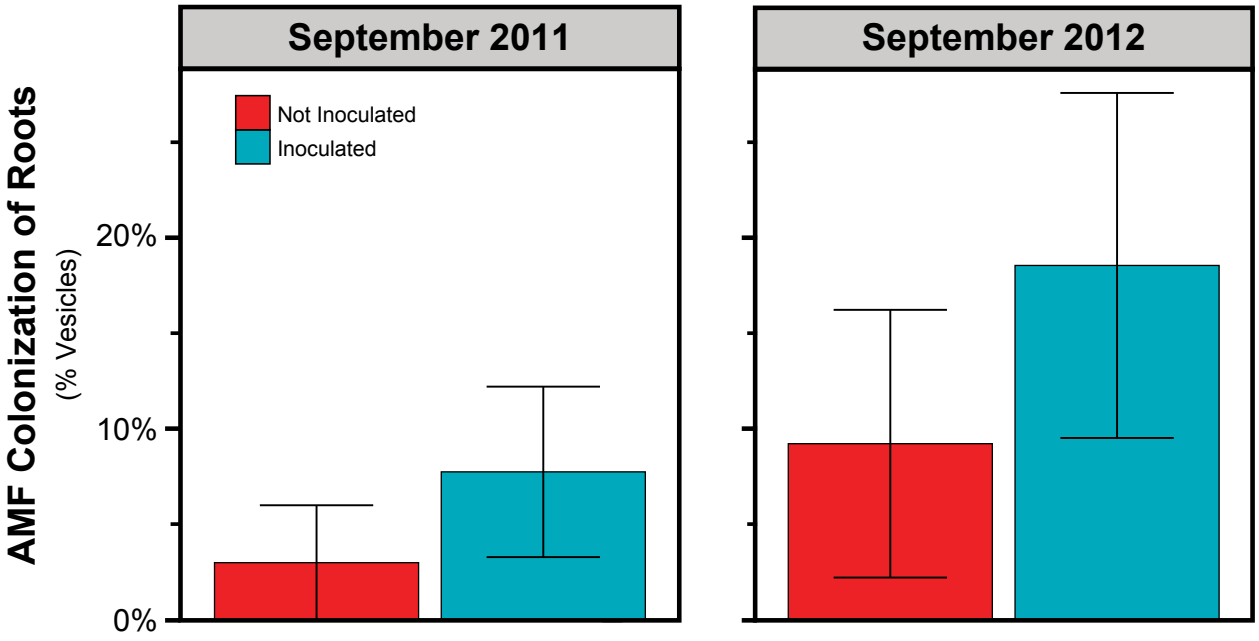


Treatment	Coeff. Est.	Coeff. Std Error	Pr(>|z|)	sig.
Main Effects				
(Intercept)	1.244	0.163	0.000	***
5BC	-0.004	0.202	0.985	n.s.
10BC	-0.082	0.205	0.689	n.s.
20CP	-0.080	0.207	0.700	n.s.
20CP + 5BC	-0.161	0.211	0.445	n.s.
20CP + 10BC	-0.419	0.223	0.061	(.)
Season	0.210	0.194	0.278	n.s.
AMF	1.492	0.092	0.000	***
Significant Interactions				
20CP x Season	0.670	0.260	0.010	**
20CP + 5BC x Season	0.748	0.262	0.004	**
20CP + 10BC x Season	0.845	0.275	0.002	**

Significance codes: *** < 0.001 | ** < 0.01 | * < 0.05 | . < 0.1 | n.s. > 0.1

Note: Significantly different intercepts with negative values in parentheses

Graph #4: AMF arbuscule percentage in the mixed community of roots collected from Exp #1. The left panel represents the first full growing season. Statistical significance based the most parsimonious model selected via model comparison (lowest AIC value) using a quasi-poisson distributed generalized linear mixed effects model. Coefficients estimates are relative to the model intercept (no amendment, no amf inoculation, first growing season). Error bars +/- 1 standard deviation. Each treatment level is replicated nine times (n=9). Abbreviations: none = no amendment, 5BC = 5 T/ha biochar, 10BC = 10 T/ha biochar, 20CP = 20 T/ha compost, 20CP + 5 BC = 20 T/ha compost + 5 T/ha biochar, 20CP + 10 BC = 20 T/ha compost + 10 T/ha biochar.



Treatment	Coeff. Est.	Coeff. Std Error	Pr(>|z|)	sig.
Main Effects				
(Intercept)	1.201	0.128	0.000	***
Season	0.816	0.111	0.000	***
AMF	0.899	0.119	0.000	***

Significance codes: *** < 0.001 | ** < 0.01 | * < 0.05 | . < 0.1 | n.s. > 0.1

Note: Significantly different intercepts with negative values in parentheses

Graph #5: AMF vesicle percentage in the mixed community of roots collected from Exp #1. The left panel represents the first full growing season. Statistical significance based the most parsimonious model selected via model comparison (lowest AIC value) using a quasi-poisson distributed generalized linear mixed effects model. Coefficients estimates are relative to the model intercept (no amf inoculation, first growing season). Error bars +/- 1 standard deviation. Each treatment level is replicated nine times (n=9).

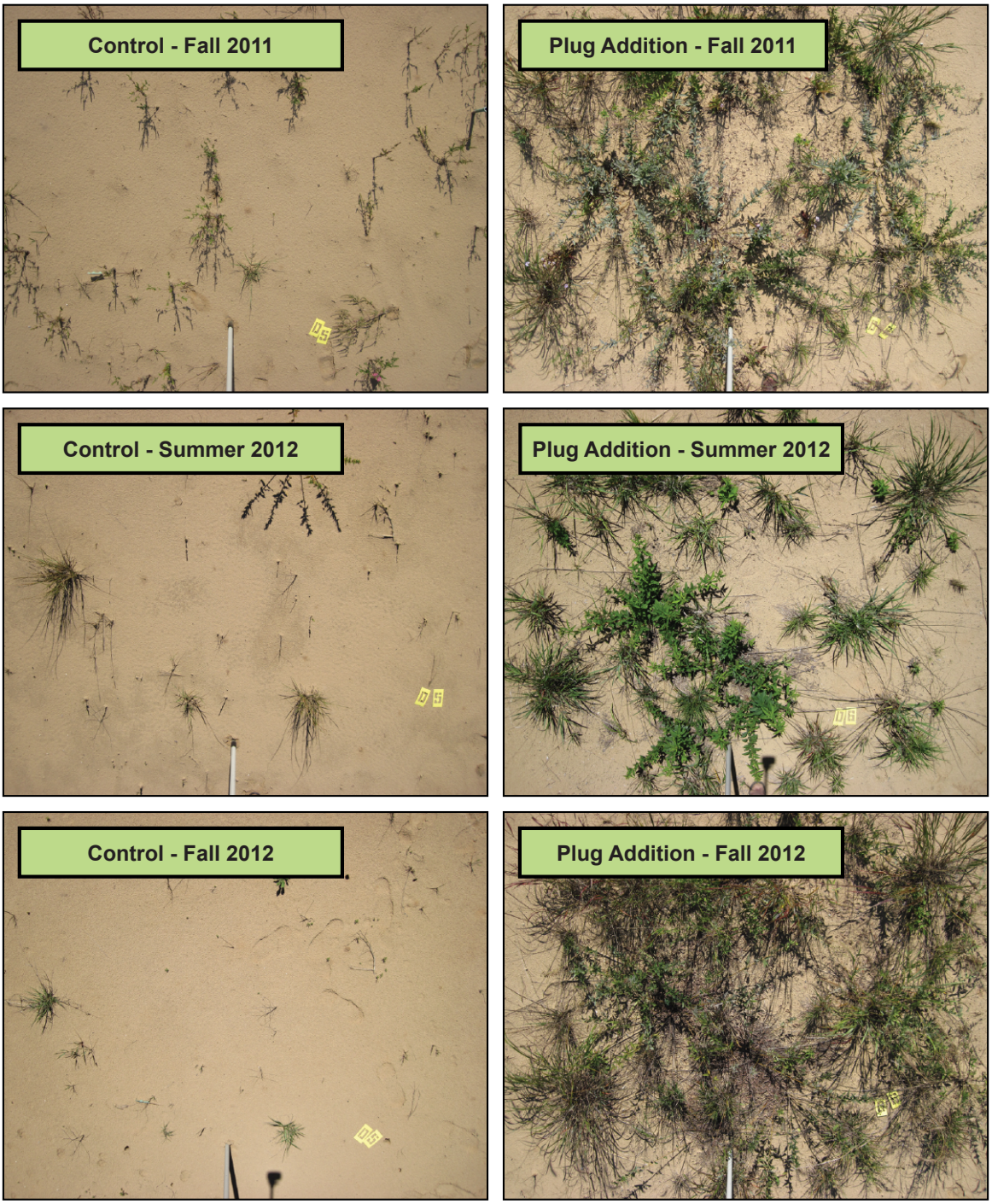
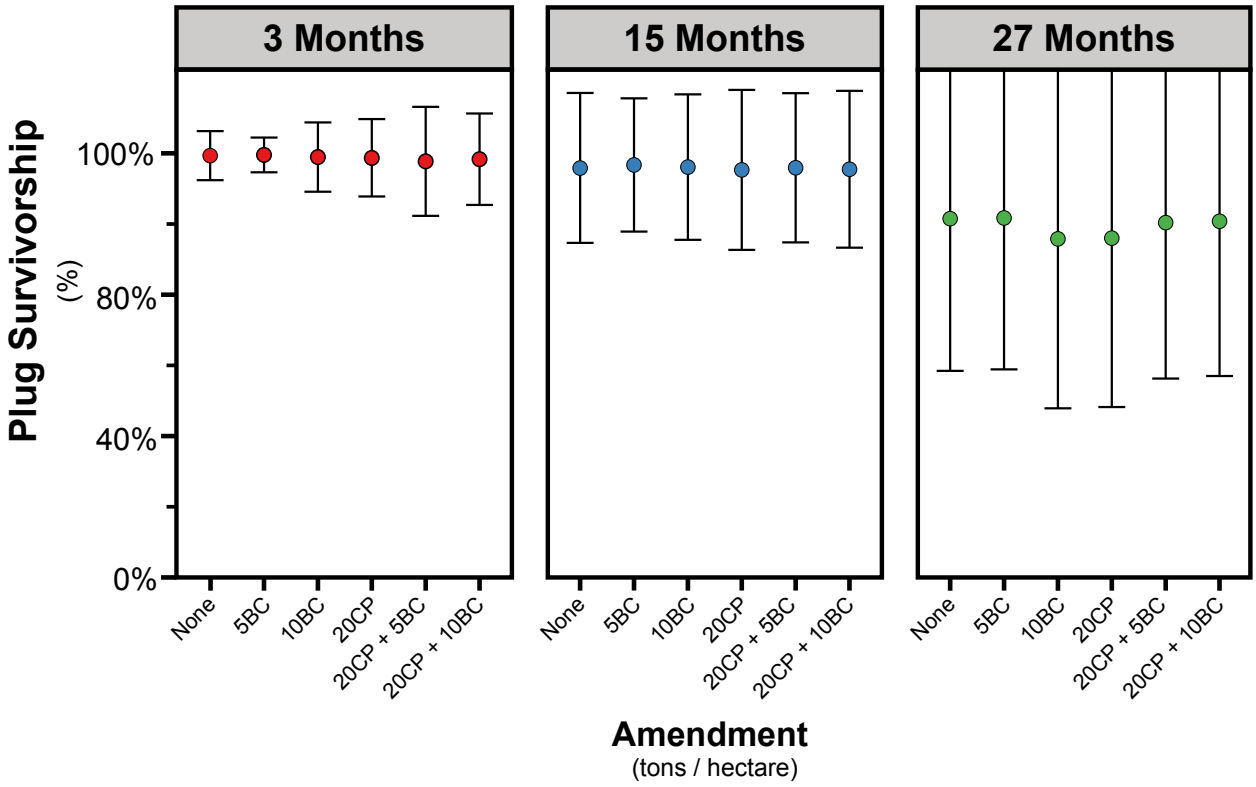
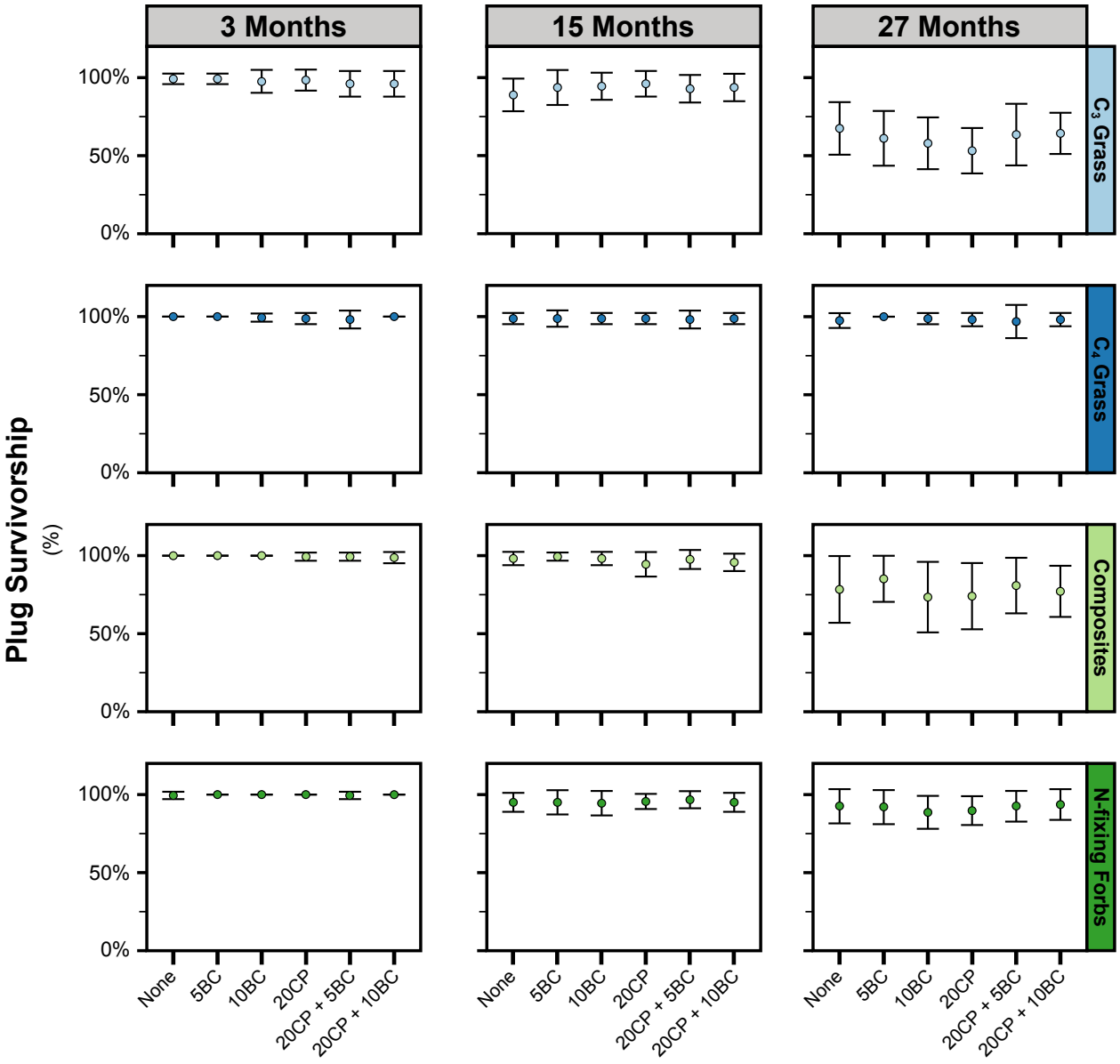


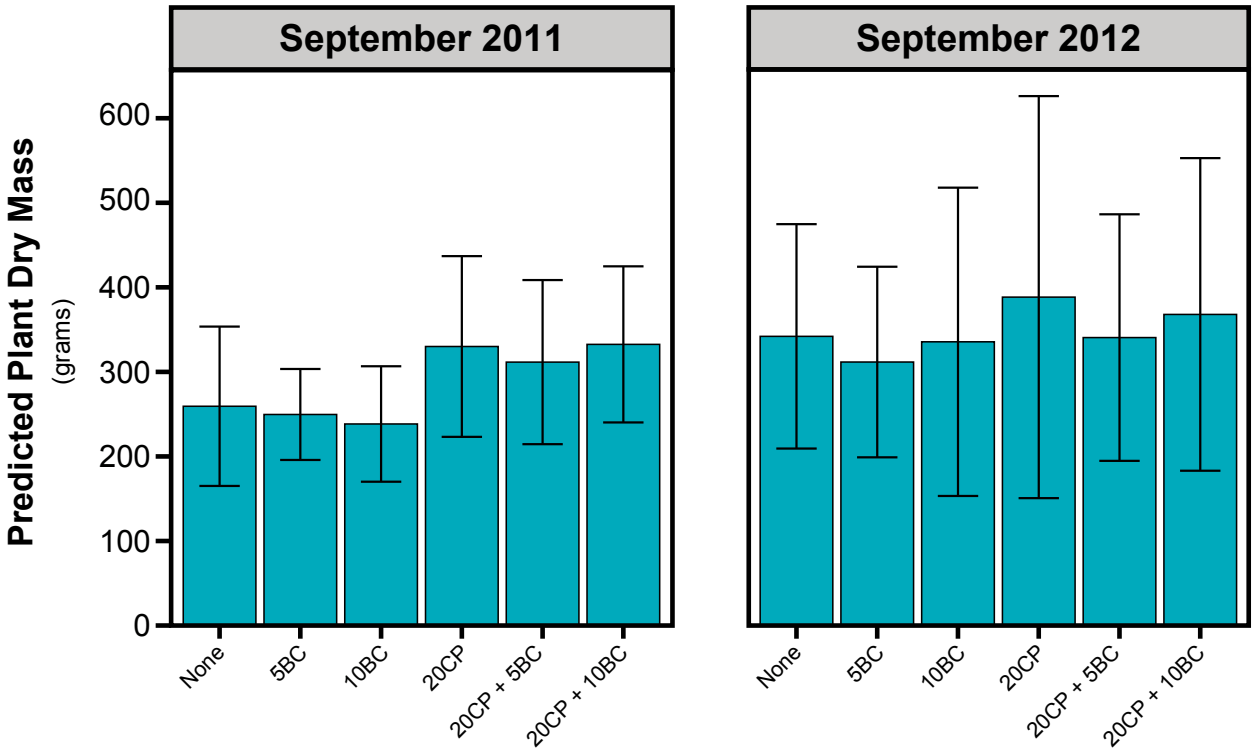
Figure #1: Photographic time series for two sets of plots in the plant plug experiment. The left set of pictures follows a control plot thru time. Therefore, no plant plugs, soil amendments, or mycorrhizas were added. The right hand plots follow a replicate with plant plugs addition only. Photos were taken after one year, one and a half years, and two years follwing plant plug installation in June 2010. Note the lack of plant growth in the control plots.



Graph #6: Total plant plug survivorship tracked for three growing seasons in *Exp #1*. Time of initial planting was June 2010. A mycorrhizal effect was not detected in plant survivorship. Only core plant plug survivorship was estimated (n = 33). Mycorrhizal and non-mycorrhizal replicates are pooled for this dataset. Error bars +/- 1 standard deviation. Abbreviations: none = no amendment, 5BC = 5 T/ha biochar, 10BC = 10 T/ha biochar, 20CP = 20 T/ha compost, 20CP + 5 BC = 20 T/ha compost + 5 T/ha biochar, 20CP + 10 BC = 20 T/ha compost + 10 T/ha biochar.



Graph #7: Plant plug survivorship by functional group tracked for three growing seasons in *Exp #1*. Time of initial planting was June 2010. C_4 Grasses (*Andropogon gerardii + Panicum virgatum*), C_3 Grasses (*Elymus canadensis + Bromus kalmii*), nitrogen-fixing legumes (*Lespedeza capitata +Desmodium canadense*), composite flowers (*Liatris cylindracea + Symphyotrichum laeve*). A mycorrhizal effect was not detected in plant survivorship. Only core plant plug survivorship was estimated (n = 33). Mycorrhizal and non-mycorrhizal replicates are pooled. Error bars +/- 1 standard deviation. Abbreviations: none = no amendment, 5BC = 5 T/ha biochar, 10BC = 10 T/ha biochar, 20CP = 20 T/ha compost, 20CP + 5 BC = 20 T/ha compost + 5 T/ha biochar, 20CP + 10 BC = 20 T/ha compost + 10 T/ha biochar.

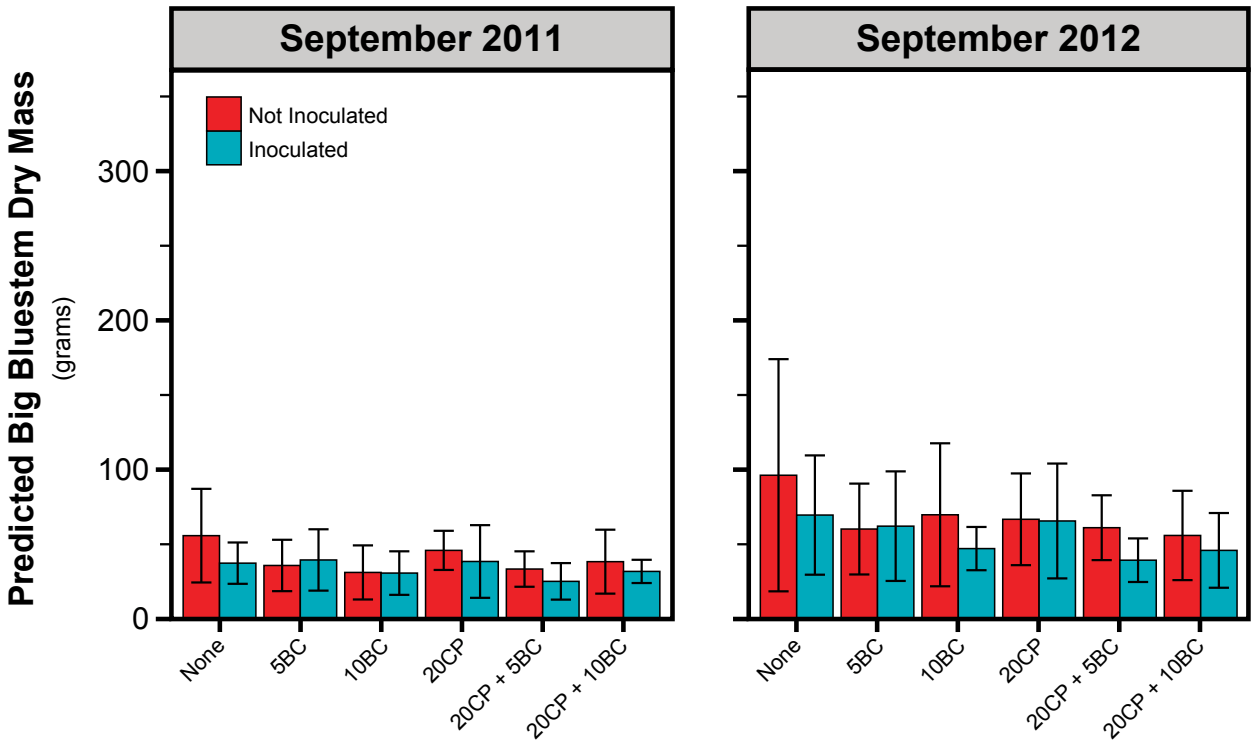


Treatment	Coeff. Est.	Coeff. Std Error	pMCMC	sig.
Main Effects				
(Intercept)	-0.063	0.002	0.000	***
5BC	-0.001	0.003	0.655	n.s.
10BC	-0.003	0.003	0.311	n.s.
20CP	0.004	0.003	0.073	.
20CP + 5BC	0.003	0.003	0.225	n.s.
20CP + 10BC	0.004	0.003	0.079	.
Season	0.004	0.001	0.005	**

Significance codes: *** < 0.001 | ** < 0.01 | * < 0.05 | . < 0.1 | n.s. > 0.1

Note: Significantly different intercepts with negative values in parentheses

Graph #8: Predicted pooled total plant dry mass (grams) from *Exp. #1*. The left panel represents results from the first full growing season. Statistical significance based the most parsimonious model selected via model comparison (lowest AIC value) using a Gaussian distributed linear mixed effects models. Coefficients estimates are relative to the model intercept (no amendment, first growing season). Error bars +/- 1 standard deviation. Nine replicates per treatment combination. Abbreviations: none = no amendment, 5BC = 5 T/ha biochar, 10BC = 10 T/ha biochar, 20CP = 20 T/ha compost, 20CP + 5 BC = 20 T/ha compost + 5 T/ha biochar, 20CP + 10 BC = 20 T/ha compost + 10 T/ha biochar.

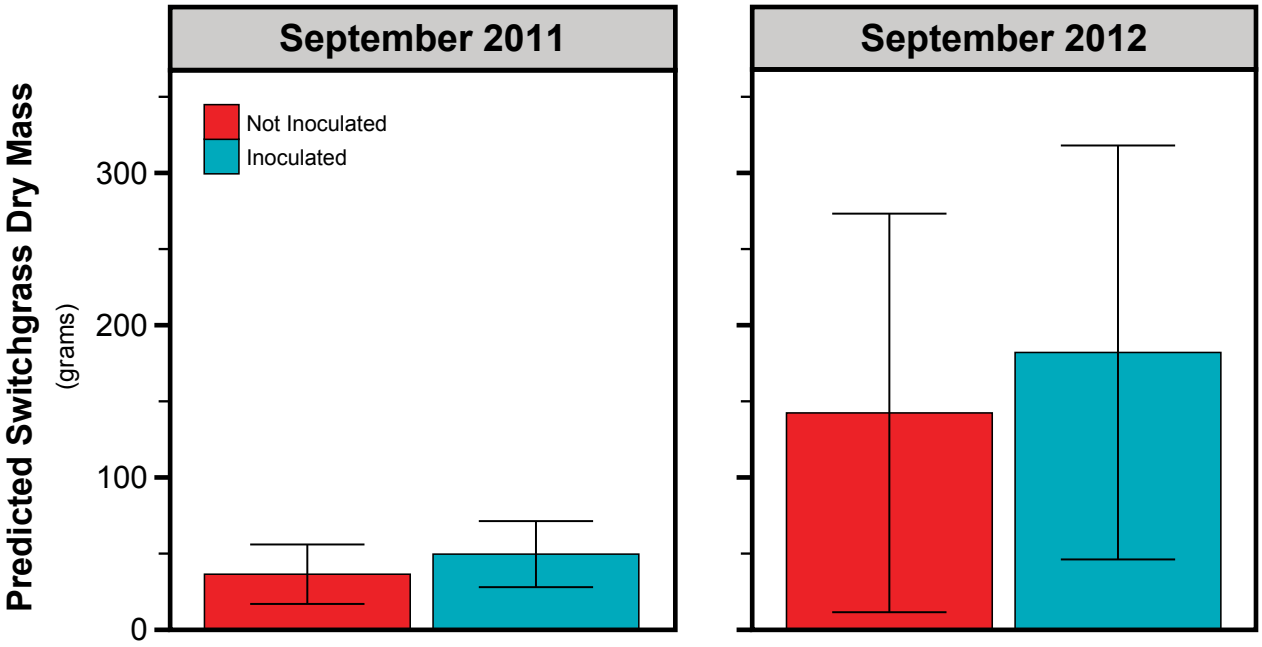


Treatment	Coeff. Est.	Coeff. Std Error	pMCMC	sig.
Main Effects				
(Intercept)	1.652	0.050	0.000	***
5BC	-0.091	0.064	0.066	(.)
10BC	-0.141	0.064	0.005	(**)
20CP	-0.041	0.064	0.398	n.s.
20CP + 5BC	-0.174	0.064	0.001	(**)
20CP + 10BC	-0.142	0.064	0.004	(**)
Season	0.209	0.017	0.000	***
AMF	-0.074	0.037	0.013	(*)

Significance codes: *** < 0.001 | ** < 0.01 | * < 0.05 | . < 0.1 | n.s. > 0.1

Note: Significantly different intercepts with negative values in parentheses

Graph #9: Predicted pooled big bluestem dry mass (C_4 grass) from *Exp. #1*. The left panel represents results from the first full growing season. Statistical significance based the most parsimonious model selected via model comparison (lowest AIC value) using a Gaussian distributed linear mixed effects models. Coefficients estimates are relative to the model intercept (no amendment, no amf inoculation, first growing season). Error bars +/- 1 standard deviation. Nine replicates per treatment combination. Abbreviations: none = no amendment, 5BC = 5 T/ha biochar, 10BC = 10 T/ha biochar, 20CP = 20 T/ha compost, 20CP + 5 BC = 20 T/ha compost + 5 T/ha biochar, 20CP + 10 BC = 20 T/ha compost + 10 T/ha biochar. Note: Graphs representing species biomass have identical y-axis scales.

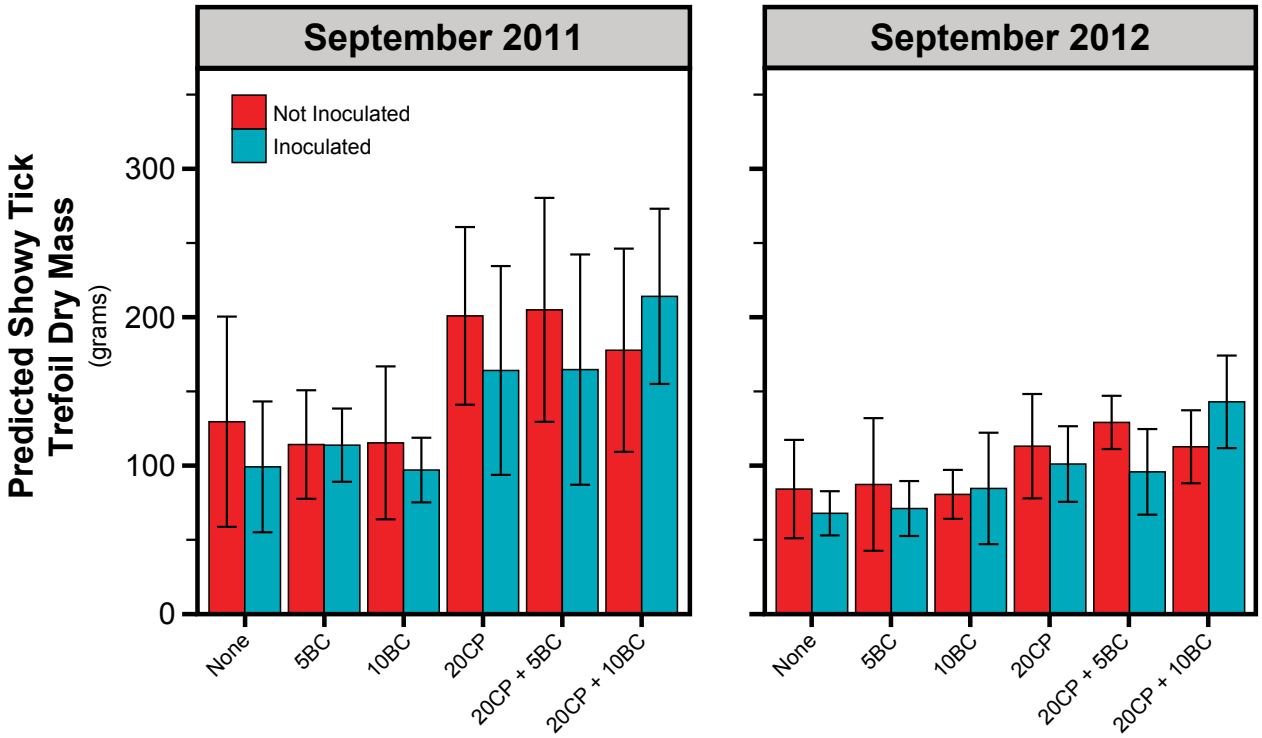


Treatment	Coeff. Est.	Coeff. Std Error	pMCMC	sig.
Main Effects				
(Intercept)	1.505	0.036	0.000	***
Season	0.492	0.021	0.000	***
AMF	0.163	0.048	0.000	***

Significance codes: *** < 0.001 | ** < 0.01 | * < 0.05 | . < 0.1 | n.s. > 0.1

Note: Significantly different intercepts with negative values in parentheses

Graph #10: Predicted pooled switchgrass dry mass (C_4 grass) from *Exp. #1*. The left panel represents results from the first full growing season. Statistical significance based the most parsimonious model selected via model comparison (lowest AIC value) using a Gaussian distributed linear mixed effects models. Coefficients estimates are relative to the model intercept (no amf inoculation, first growing season). Error bars +/- 1 standard deviation. Nine replicates per treatment combination. Abbreviations: none = no amendment, 5BC = 5 T/ha biochar, 10BC = 10 T/ha biochar, 20CP = 20 T/ha compost, 20CP + 5 BC = 20 T/ha compost + 5 T/ha biochar, 20CP + 10 BC = 20 T/ha compost + 10 T/ha biochar. Note: Graphs representing species biomass have identical y-axis scales.

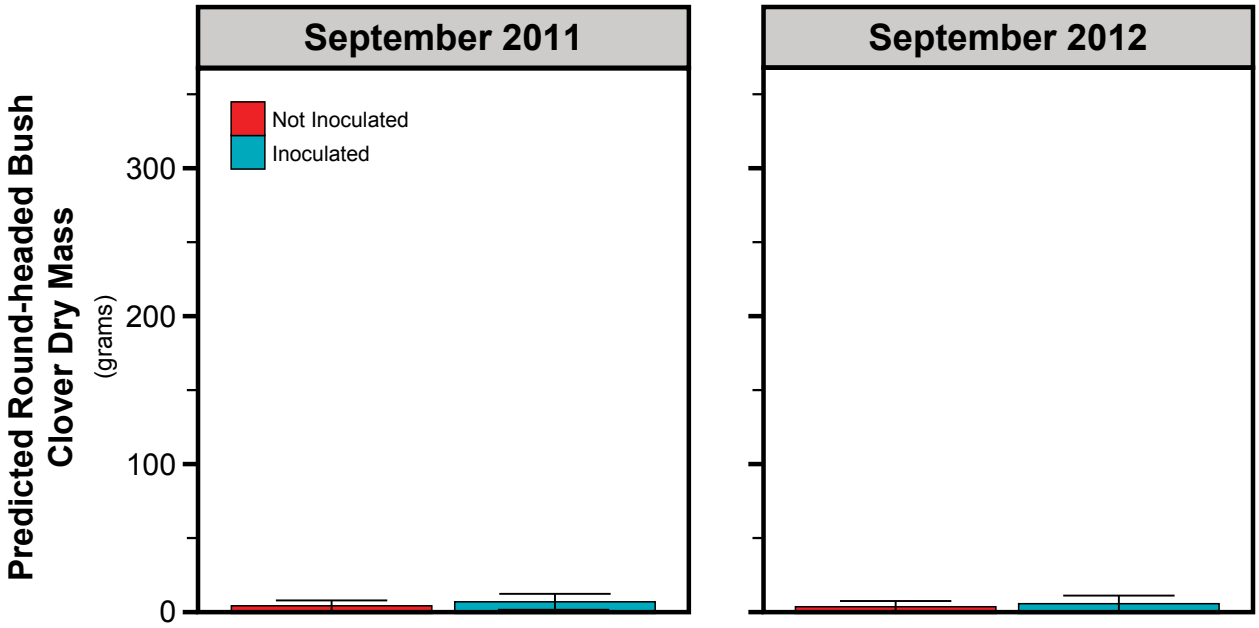


Treatment	Coeff. Est.	Coeff. Std Error	pMCMC	sig.
Main Effects				
(Intercept)	2.064	0.044	0.000	***
5BC	-0.013	0.062	0.789	n.s.
10BC	-0.028	0.062	0.600	n.s.
20CP	0.175	0.062	0.001	***
20CP + 5BC	0.216	0.062	0.000	***
20CP + 10BC	0.147	0.062	0.005	**
Season	-0.166	0.016	0.000	(***)
AMF	-0.093	0.062	0.077	(.)
Significant Interactions				
20CP + 10BC x AMF	0.196	0.087	0.009	**

Significance codes: *** < 0.001 | ** < 0.01 | * < 0.05 | . < 0.1 | n.s. > 0.1

Note: Significantly different intercepts with negative values in parentheses

Graph #11: Predicted pooled howy tick trefoil dry mass (N-fixing Forb) from *Exp. #1*. The left panel represents results from the first full growing season. Statistical significance based the most parsimonious model selected via model comparison (lowest AIC value) using a Gaussian distributed linear mixed effects models. Coefficients estimates are relative to the model intercept (no amendment, no amf inoculation, first growing season). Error bars +/- 1 standard deviation. Nine replicates per treatment combination. Abbreviations: none = no amendment, 5BC = 5 T/ha biochar, 10BC = 10 T/ha biochar, 20CP = 20 T/ha compost, 20CP + 5 BC = 20 T/ha compost + 5 T/ha biochar, 20CP + 10 BC = 20 T/ha compost + 10 T/ha biochar. Note: Graphs representing species biomass have identical y-axis scales.

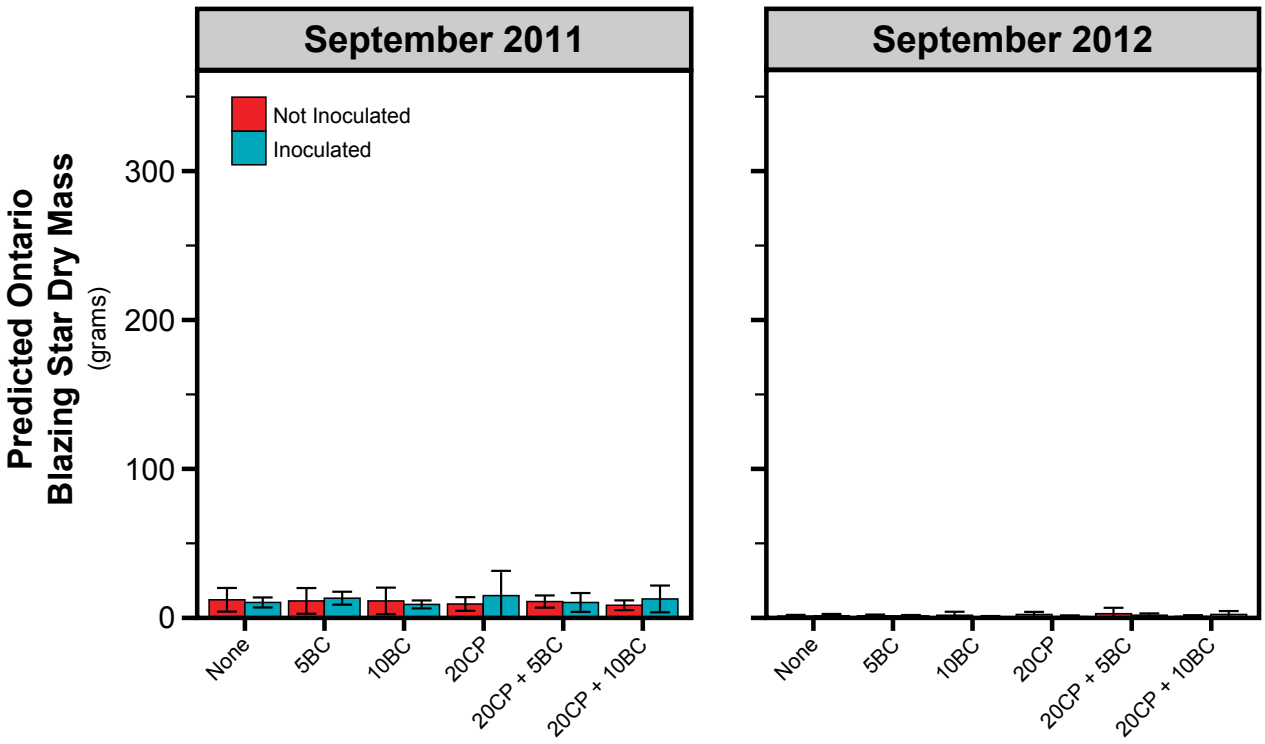


Treatment	Coeff. Est.	Coeff. Std Error	pMCMC	sig.
Main Effects				
(Intercept)	2.147	0.123	0.000	***
Season	-0.305	0.070	0.041	(***)
AMF	0.469	0.166	0.000	***

Significance codes: *** < 0.001 | ** < 0.01 | * < 0.05 | . < 0.1 | n.s. > 0.1

Note: Significantly different intercepts with negative values in parentheses

Graph #12: Predicted pooled round-headed bush clover dry mass (N-fixing Forb) from *Exp. #1*. The left panel represents results from the first full growing season. Statistical significance based the most parsimonious model selected via model comparison (lowest AIC value) using a Gaussian distributed linear mixed effects models. Coefficients estimates are relative to the model intercept (no amf inoculation, first growing season). Error bars +/- 1 standard deviation. Nine replicates per treatment combination. Abbreviations: none = no amendment, 5BC = 5 T/ha biochar, 10BC = 10 T/ha biochar, 20CP = 20 T/ha compost, 20CP + 5 BC = 20 T/ha compost + 5 T/ha biochar, 20CP + 10 BC = 20 T/ha compost + 10 T/ha biochar. Note: Graphs representing species biomass have identical y-axis scales.

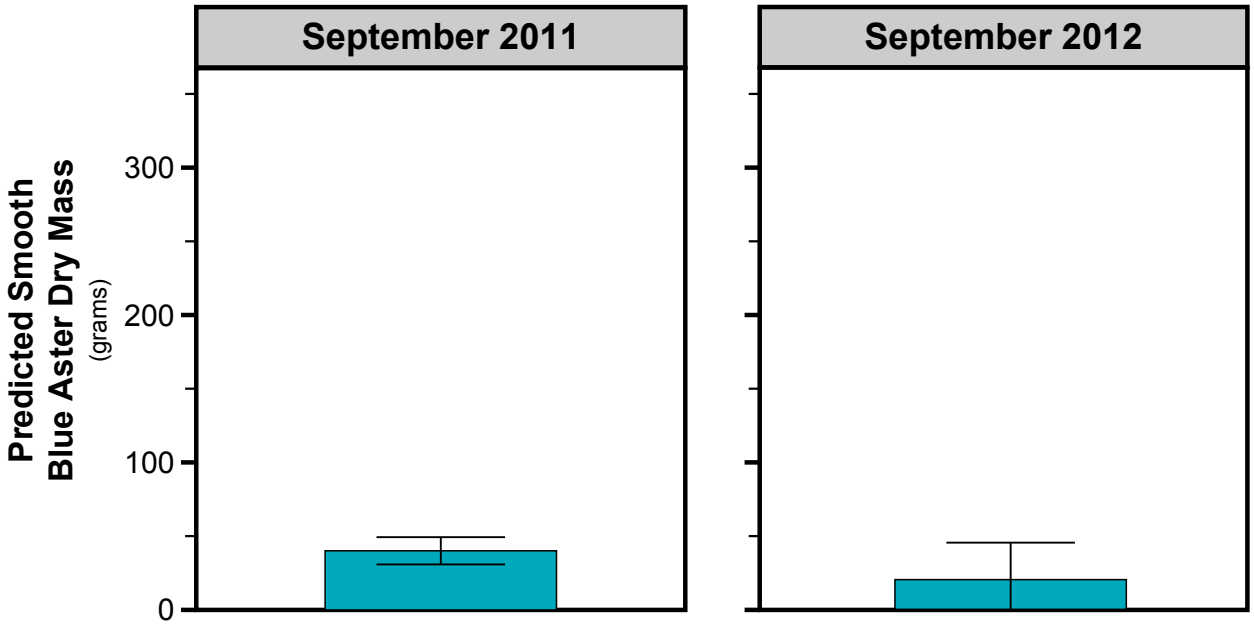


Treatment	Coeff. Est.	Coeff. Std Error	pMCMC	sig.
Main Effects				
(Intercept)	1.034	0.107	0.000	***
5BC	-0.049	0.151	0.738	n.s.
10BC	-0.052	0.151	0.718	n.s.
20CP	-0.122	0.151	0.414	n.s.
20CP + 5BC	-0.021	0.151	0.878	n.s.
20CP + 10BC	-0.133	0.151	0.367	n.s.
Season	-1.004	0.131	0.000	(***)
AMF	-0.033	0.151	0.813	n.s.
Significant Interactions				
20CP x Season	0.382	0.189	0.080	*
20CP x AMF x Season	-0.625	0.264	0.040	(*)

Significance codes: *** < 0.001 | ** < 0.01 | * < 0.05 | . < 0.1 | n.s. > 0.1

Note: Significantly different intercepts with negative values in parentheses

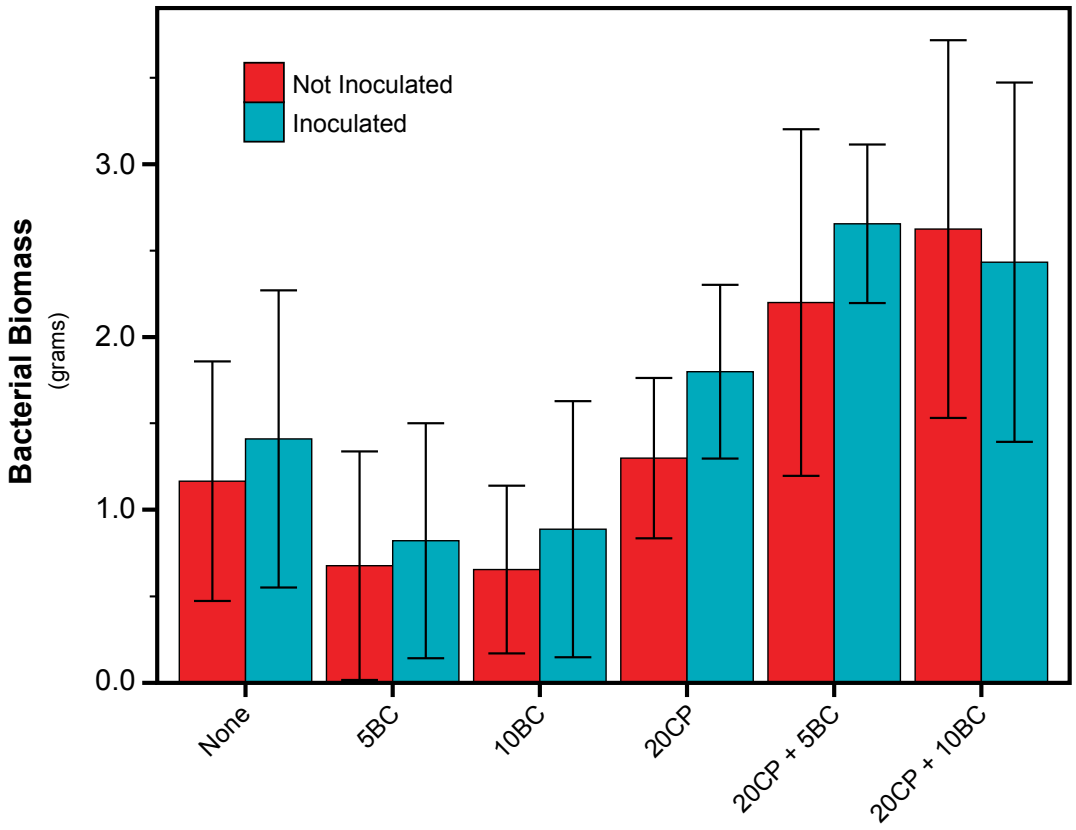
Graph #13: Predicted pooled Ontario blazing star dry mass (Composite Forb) from *Exp. #1*. The left panel represents results from the first full growing season. Statistical significance based the most parsimonious model selected via model comparison (lowest AIC value) using a Gaussian distributed linear mixed effects models. Coefficients estimates are relative to the model intercept (no amendment, no amf inoculation, first growing season). Error bars +/- 1 standard deviation. Nine replicates per treatment combination. Abbreviations: none = no amendment, 5BC = 5 T/ha biochar, 10BC = 10 T/ha biochar, 20CP = 20 T/ha compost, 20CP + 5 BC = 20 T/ha compost + 5 T/ha biochar, 20CP + 10 BC = 20 T/ha compost + 10 T/ha biochar. Note: Graphs representing species biomass have identical y-axis scales.



Treatment	Coeff. Est.	Coeff. Std Error	pMCMC	sig.
Main Effects				
(Intercept)	6.292	0.157	0.000	***
Season	-2.372	0.180	0.000	(***)

Significance codes: *** < 0.001 | ** < 0.01 | * < 0.05 | . < 0.1 | n.s. > 0.1
Note: Significantly different intercepts with negative values in parentheses

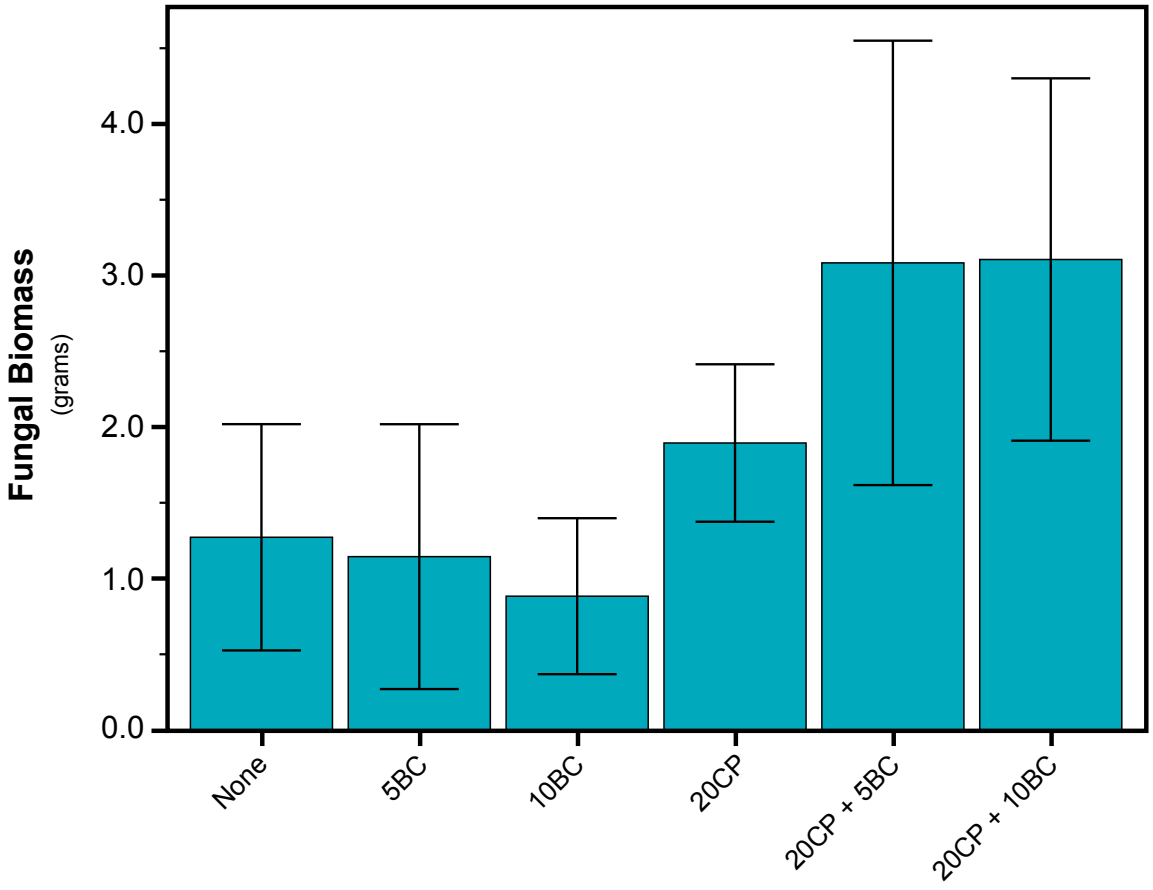
Graph #14: Predicted pooled smooth blue aster dry mass (Composite Forb) from *Exp. #1*. The left panel represents results from the first full growing season. Statistical significance based the most parsimonious model selected via model comparison (lowest AIC value) using a Gaussian distributed linear mixed effects models. Coefficients estimates are relative to the model intercept (first growing season). Error bars +/- 1 standard deviation. Nine replicates per treatment combination. Abbreviations: none = no amendment, 5BC = 5 T/ha biochar, 10BC = 10 T/ha biochar, 20CP = 20 T/ha compost, 20CP + 5 BC = 20 T/ha compost + 5 T/ha biochar, 20CP + 10 BC = 20 T/ha compost + 10 T/ha biochar. Note: Graphs representing species biomass have identical y-axis scales.



Treatment	Coeff. Est.	Coeff. Std Error	Pr(>|t|)	sig.
Main Effects				
(Intercept)	0.312	0.033	0.000	***
5BC	-0.116	0.044	0.009	(**)
10BC	-0.109	0.044	0.014	(*)
20CP	0.064	0.044	0.145	n.s.
20CP + 5BC	0.190	0.044	0.000	***
20CP + 10BC	0.194	0.044	0.000	***
AMF	0.044	0.025	0.085	.

Significance codes: *** < 0.001 | ** < 0.01 | * < 0.05 | . < 0.1 | n.s. > 0.1
Note: Significantly different intercepts with negative values in parentheses

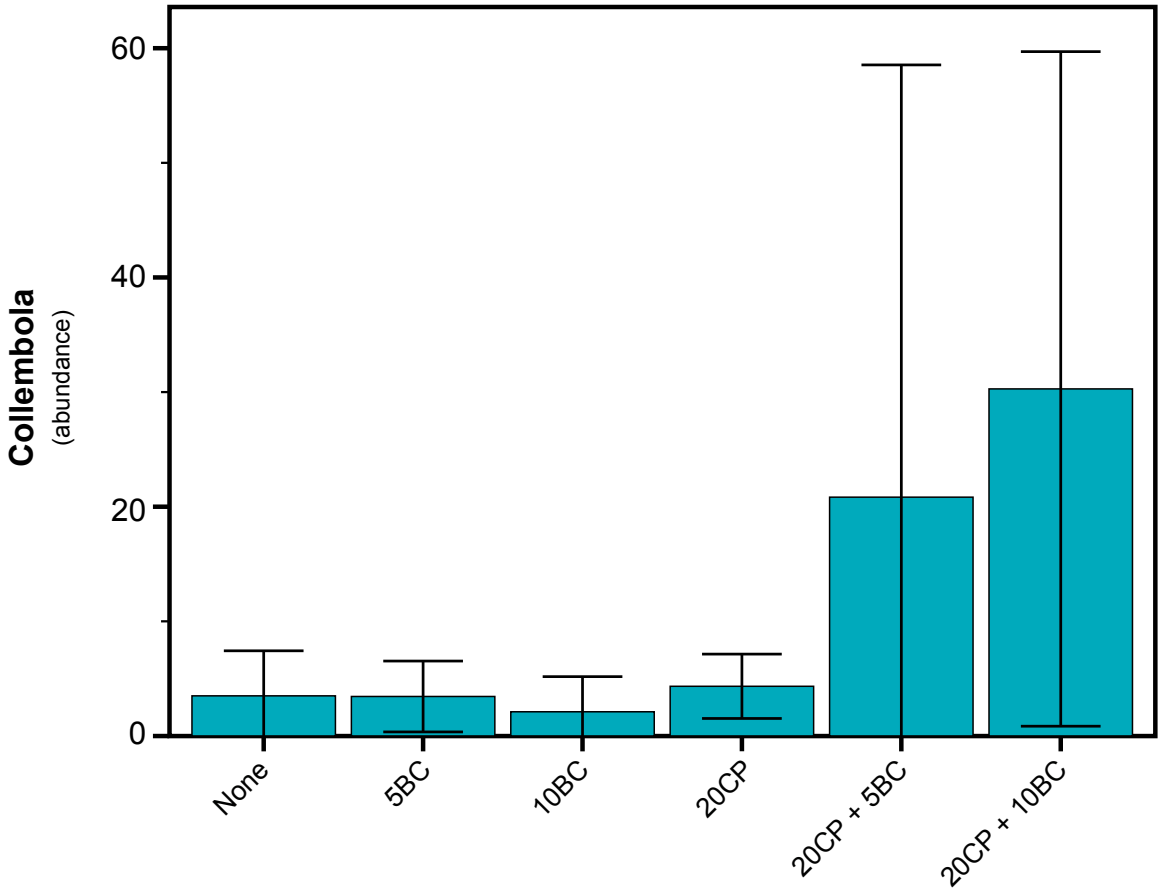
Graph #15: Plot of bacterial biomass data from *Exp. #1*. Soils were collected at the end of the second growing season (Sept. 2012). Statistical significance based the most parsimonious model selected via model comparison (lowest AIC value) using a Gaussian distributed linear model. Coefficients estimates are relative to the model intercept (no amendment, no inoculation). Error bars +/- 1 standard deviation. Nine replicates per treatment combination. Abbreviations: none = no amendment, 5BC = 5 T/ha biochar, 10BC = 10 T/ha biochar, 20CP = 20 T/ha compost, 20CP + 5 BC = 20 T/ha compost + 5 T/ha biochar, 20CP + 10 BC = 20 T/ha compost + 10 T/ha biochar.



Treatment	Coeff. Est.	Coeff. Std Error	Pr(>|t|)	sig.
Main Effects				
(Intercept)	0.333	0.033	< 2e-16	***
5BC	-0.036	0.046	0.441	n.s.
10BC	-0.073	0.046	0.119	n.s.
20CP	0.122	0.046	0.010	**
20CP + 5BC	0.252	0.046	0.000	***
20CP + 10BC	0.261	0.046	0.000	***

Significance codes: *** < 0.001 | ** < 0.01 | * < 0.05 | . < 0.1 | n.s. > 0.1
Note: Significantly different intercepts with negative values in parentheses

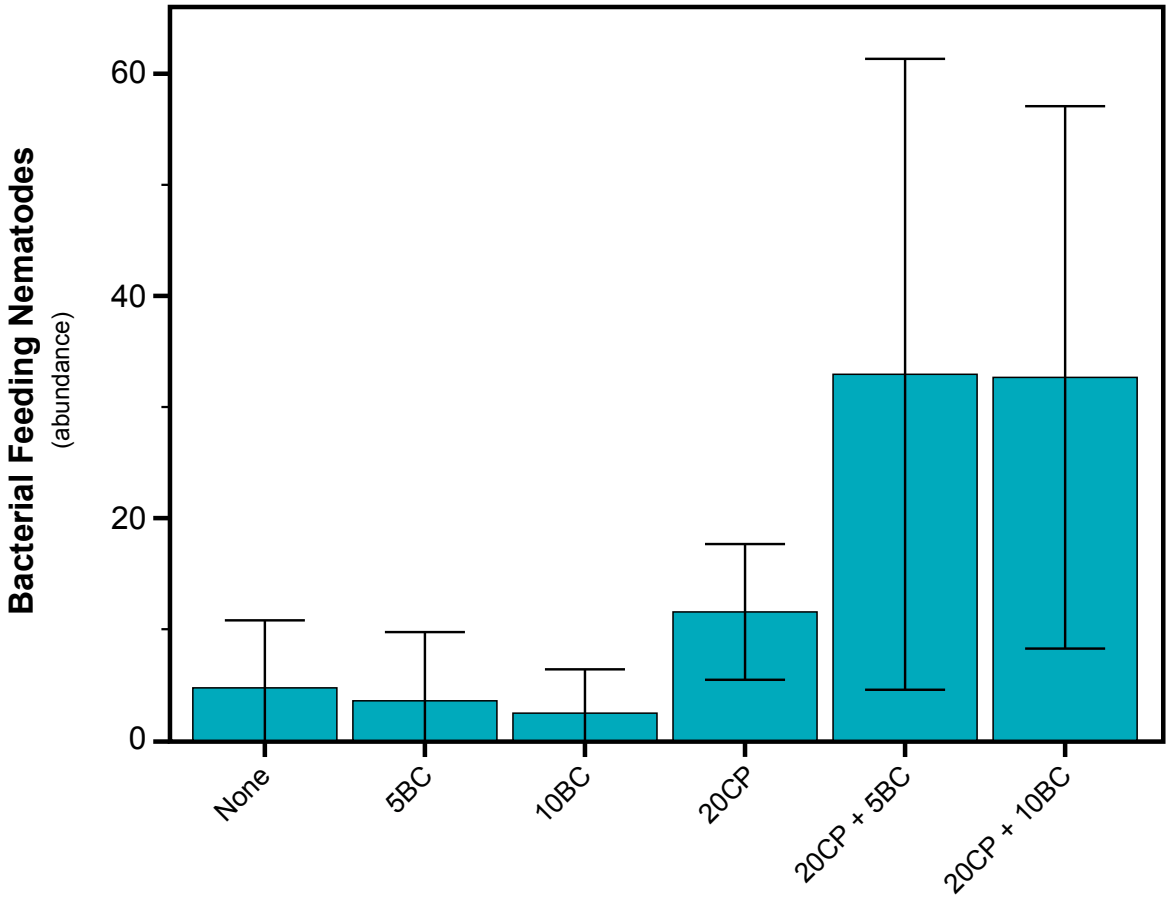
Graph #16: Plot of fungal biomass data from *Exp. #1*. Soils were collected at the end of the second growing season (Sept. 2012). Statistical significance based the most parsimonious model selected via model comparison (lowest AIC value) using a negative binomial distributed linear model. Coefficients estimates are relative to the model intercept (no amendment). Error bars +/- 1 standard deviation. Nine replicates per treatment combination. Abbreviations: none = no amendment, 5BC = 5 T/ha biochar, 10BC = 10 T/ha biochar, 20CP = 20 T/ha compost, 20CP + 5 BC = 20 T/ha compost + 5 T/ha biochar, 20CP + 10 BC = 20 T/ha compost + 10 T/ha biochar.



Treatment	Coeff. Est.	Coeff. Std Error	Pr(>|t|)	sig.
Main Effects				
(Intercept)	1.253	0.343	0.000	***
5BC	-0.016	0.485	0.974	n.s.
10BC	-0.506	0.496	0.308	n.s.
20CP	0.214	0.482	0.658	n.s.
20CP + 5BC	1.784	0.471	0.000	***
20CP + 10BC	2.158	0.470	0.000	***

Significance codes: *** < 0.001 | ** < 0.01 | * < 0.05 | . < 0.1 | n.s. > 0.1
Note: Significantly different intercepts with negative values in parentheses

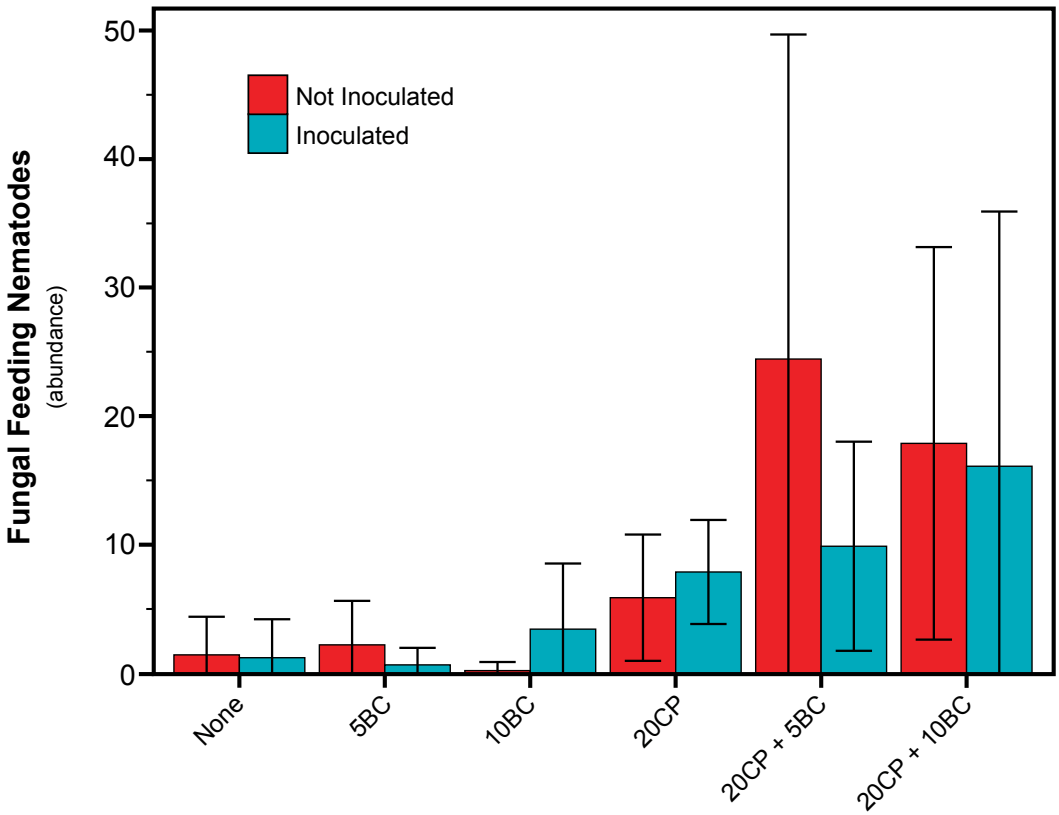
Graph #17: Plot of collembola biomass data from *Exp. #1*. Soils were collected at the end of the second growing season (Sept. 2012). Statistical significance based the most parsimonious model selected via model comparison (lowest AIC value) using a negative binomial distributed linear model. Coefficients estimates are relative to the model intercept (no amendment). Error bars +/- 1 standard deviation. Nine replicates per treatment combination. Abbreviations: none = no amendment, 5BC = 5 T/ha biochar, 10BC = 10 T/ha biochar, 20CP = 20 T/ha compost, 20CP + 5 BC = 20 T/ha compost + 5 T/ha biochar, 20CP + 10 BC = 20 T/ha compost + 10 T/ha biochar.



Treatment	Coeff. Est.	Coeff. Std Error	Pr(>|t|)	sig.
Main Effects				
(Intercept)	1.552	0.269	0.000	***
5BC	-0.284	0.385	0.461	n.s.
10BC	-0.659	0.394	0.095	(.)
20CP	0.895	0.371	0.016	*
20CP + 5BC	1.943	0.367	0.000	***
20CP + 10BC	1.934	0.367	0.000	***

Significance codes: *** < 0.001 | ** < 0.01 | * < 0.05 | . < 0.1 | n.s. > 0.1
Note: Significantly different intercepts with negative values in parentheses

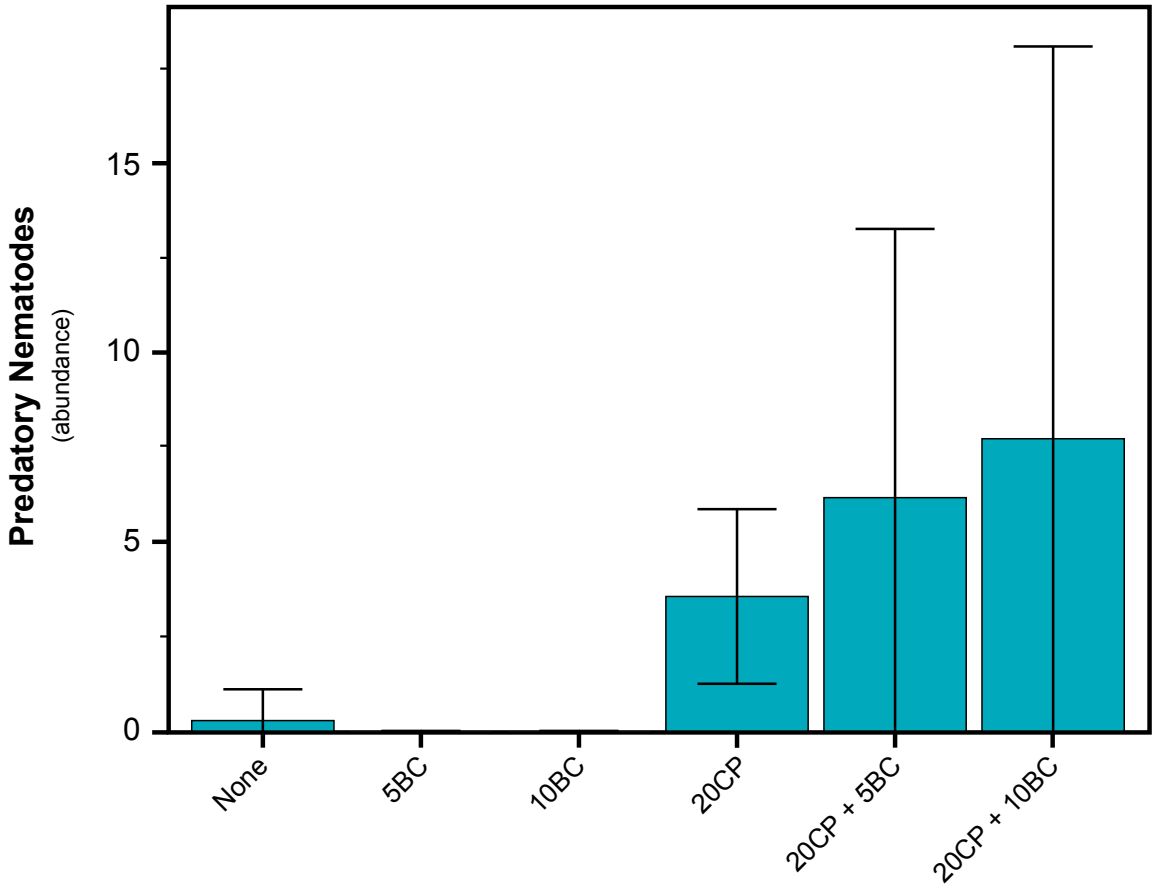
Graph #18: Plot of bacterial feeding nematode biomass data from *Exp. #1*. Soils were collected at the end of the second growing season (Sept. 2012). Statistical significance based the most parsimonious model selected via model comparison (lowest AIC value) using a negative binomial distributed linear model. Coefficients estimates are relative to the model intercept (no amendment). Error bars +/- 1 standard deviation. Nine replicates per treatment combination. Abbreviations: none = no amendment, 5BC = 5 T/ha biochar, 10BC = 10 T/ha biochar, 20CP = 20 T/ha compost, 20CP + 5 BC = 20 T/ha compost + 5 T/ha biochar, 20CP + 10 BC = 20 T/ha compost + 10 T/ha biochar.



Treatment	Coeff. Est.	Coeff. Std Error	Pr(>|t|)	sig.
Main Effects				
(Intercept)	0.368	0.479	0.443	**n.s.**
5BC	0.431	0.657	0.512	**n.s.**
10BC	-1.872	0.939	0.046	*
20CP	1.405	0.633	0.026	*
20CP + 5BC	2.829	0.622	0.000	***
20CP + 10BC	2.516	0.623	0.000	***
AMF	-0.16705	0.68761	0.808	**n.s.**
Significant Interactions				
10BC x amf	2.90789	1.14457	0.0111	*

Significance codes: *** < 0.001 | ** < 0.01 | * < 0.05 | . < 0.1 | n.s. > 0.1
Note: Significantly different intercepts with negative values in parentheses

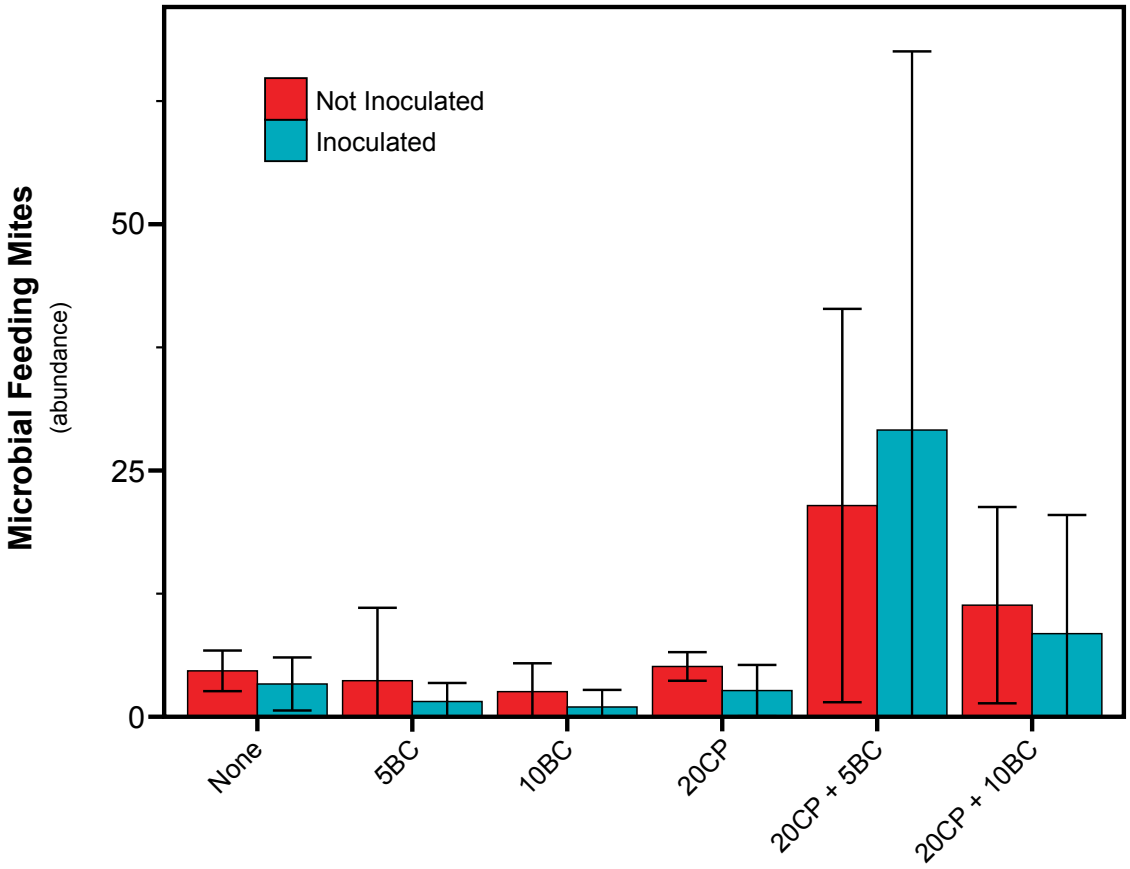
Graph #19: Plot of fungal feeding nematode biomass data from *Exp. #1*. Soils were collected at the end of the second growing season (Sept. 2012). Statistical significance based the most parsimonious model selected via model comparison (lowest AIC value) using a negative binomial distributed linear model. Coefficients estimates are relative to the model intercept (no amendment, no amf inoculation). Error bars +/- 1 standard deviation. Nine replicates per treatment combination. Abbreviations: none = no amendment, 5BC = 5 T/ha biochar, 10BC = 10 T/ha biochar, 20CP = 20 T/ha compost, 20CP + 5 BC = 20 T/ha compost + 5 T/ha biochar, 20CP + 10 BC = 20 T/ha compost + 10 T/ha biochar.



Treatment	Coeff. Est.	Coeff. Std Error	Pr(>|t|)	sig.
Main Effects				
(Intercept)	-1.281	0.509	0.012	*
5BC	-19.511	4678.318	0.997	n.s.
10BC	-19.511	4678.318	0.997	n.s.
20CP	2.549	0.579	0.000	***
20CP + 5BC	3.100	0.573	0.000	***
20CP + 10BC	3.325	0.571	0.000	***

Significance codes: *** < 0.001 | ** < 0.01 | * < 0.05 | . < 0.1 | n.s. > 0.1
Note: Significantly different intercepts with negative values in parentheses

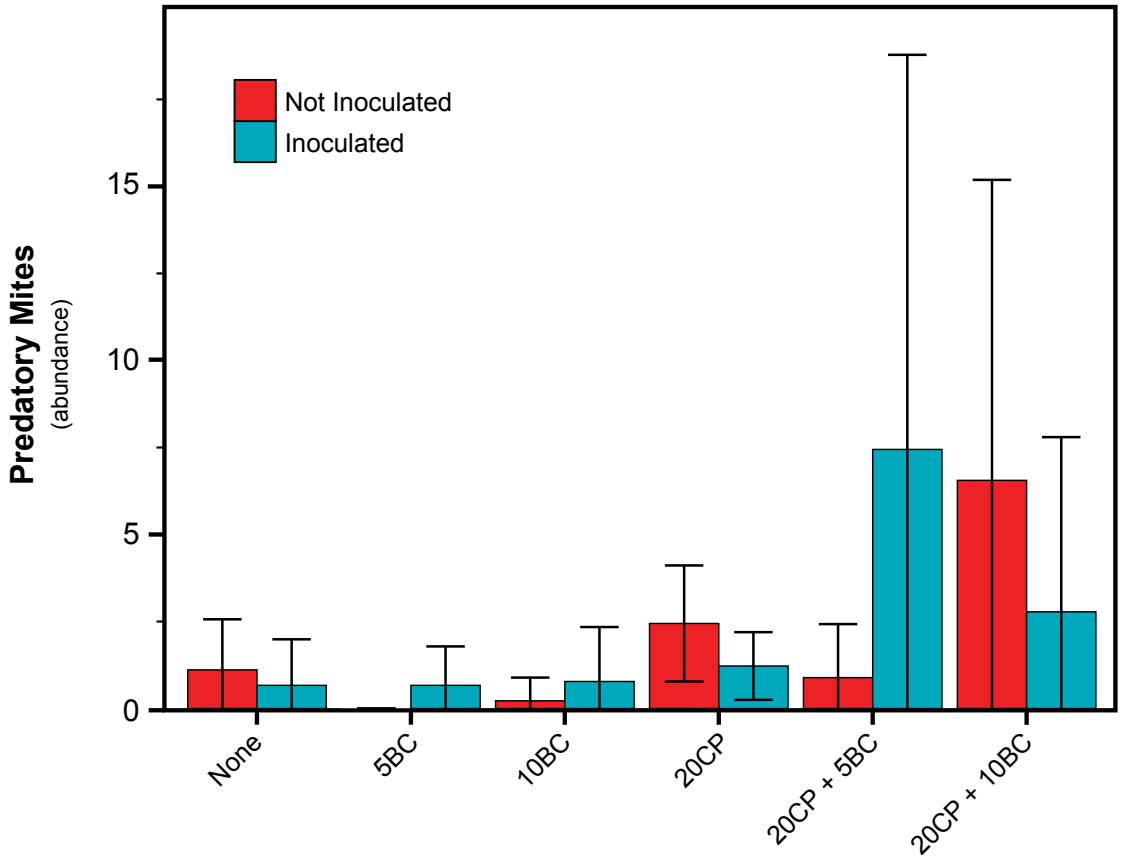
Graph #20: Plot of predatory nematode biomass data from *Exp. #1*. Soils were collected at the end of the second growing season (Sept. 2012). Statistical significance based the most parsimonious model selected via model comparison (lowest AIC value) using a negative binomial distributed linear model. Coefficients estimates are relative to the model intercept (no amendment). Error bars +/- 1 standard deviation. Nine replicates per treatment combination. Abbreviations: none = no amendment, 5BC = 5 T/ha biochar, 10BC = 10 T/ha biochar, 20CP = 20 T/ha compost, 20CP + 5 BC = 20 T/ha compost + 5 T/ha biochar, 20CP + 10 BC = 20 T/ha compost + 10 T/ha biochar.



Treatment	Coeff. Est.	Coeff. Std Error	Pr(>|t|)	sig.
Main Effects				
(Intercept)	1.579	0.305	0.000	***
5BC	-0.465	0.411	0.258	n.s.
10BC	-0.851	0.424	0.045	(*)
20CP	-0.054	0.402	0.893	n.s.
20CP + 5BC	1.908	0.386	0.000	***
20CP + 10BC	0.910	0.390	0.020	*
AMF	-0.414	0.232	0.074	(.)

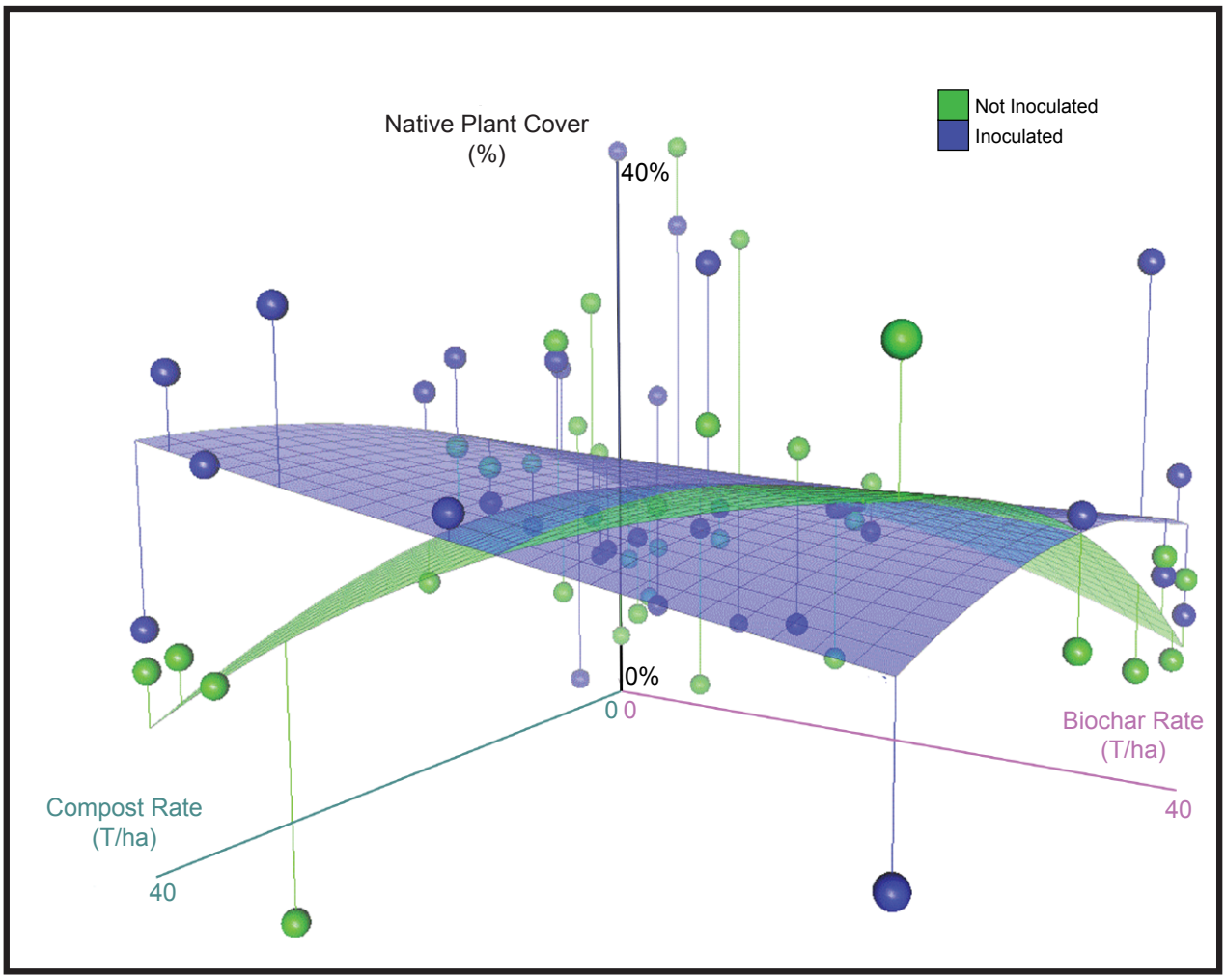
Significance codes: *** < 0.001 | ** < 0.01 | * < 0.05 | . < 0.1 | n.s. > 0.1
Note: Significantly different intercepts with negative values in parentheses

Graph #21: Plot of microbial feeding mites biomass data from *Exp. #1*. Soils were collected at the end of the second growing season (Sept. 2012). Statistical significance based the most parsimonious model selected via model comparison (lowest AIC value) using a negative binomial distributed linear model. Coefficients estimates are relative to the model intercept (no amendment, no amf inoculation). Error bars +/- 1 standard deviation. Nine replicates per treatment combination. Abbreviations: none = no amendment, 5BC = 5 T/ha biochar, 10BC = 10 T/ha biochar, 20CP = 20 T/ha compost, 20CP + 5 BC = 20 T/ha compost + 5 T/ha biochar, 20CP + 10 BC = 20 T/ha compost + 10 T/ha biochar.



Treatment	Coeff. Est.	Coeff. Std Error	Pr(>|t|)	sig.
Main Effects				
(Intercept)	0.105	0.514	0.838	**n.s.**
5BC	-19.408	3142.206	0.995	**n.s.**
10BC	-1.609	0.963	0.095	**(.)**
20CP	0.789	0.688	0.252	**n.s.**
20CP + 5BC	-0.223	0.744	0.764	**n.s.**
20CP + 10BC	1.775	0.667	0.008	******
AMF	-0.511	0.771	0.508	**n.s.**
Significant Interactions				
20CP + 5BC x AMF	2.636	1.031	0.011	*****

Graph #22: Plot of predatory mite biomass data from *Exp. #1*. Soils were collected at the end of the second growing season (Sept. 2012). Statistical significance based the most parsimonious model selected via model comparison (lowest AIC value) using a negative binomial distributed linear model. Coefficients estimates are relative to the model intercept (no amendment, no amf inoculation). Error bars +/- 1 standard deviation. Nine replicates per treatment combination. Abbreviations: none = no amendment, 5BC = 5 T/ha biochar, 10BC = 10 T/ha biochar, 20CP = 20 T/ha compost, 20CP + 5 BC = 20 T/ha compost + 5 T/ha biochar, 20CP + 10 BC = 20 T/ha compost + 10 T/ha biochar.



Graph #23: Three-dimensional graph of native plant % cover for the seed experiment. Data was collected at the end of the second growing season (September 2012). The y-axis ranges from 0% - 40% coverage of green, native plant tissue. Compost and biochar application rates are listed in Table 1. The smoothed plane represented a best-fit representation of the data. AMF inoculated plots are represented by the purple curve. Non-inoculated plots are represented by the green curve. Data points are shown as spheres.