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

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Photo: Tallgrass prairie restoration project (Photo taken September 2011)

EXECUTIVE SUMMARY

INTRODUCTION:

Depleted sand and gravel aggregate sites are good candidates for prairie restoration projects due to their 'open' nature, sandy substrata, and adaptability to management scenarios. The results of this study can be directly translated into the industrial-scale restoration of tallgrass prairies in post-extraction areas. This report describes the results and establishment protocol of a multi-year, large-scale grassland restoration project established on the Norfolk Sand Plain in southern Ontario.

Ontario's tallgrass prairies are treeless habitats dominated by native grasses and wildflowers. Interspersed among deciduous Carolinian forests, prairie vegetation is restricted to the dry conditions of well-drained, sandy soils. One of the largest bands of prairie vegetation in Ontario is found on the Norfolk Sand Plain [also the location of the research site]. Currently, these ecosystems are disappearing as a result of urban sprawl, agriculture, invasive species colonization, and fire suppression. Sand plain prairie habitat supports a high biodiversity of regionally unique plants, invertebrates and animals. Habitat loss has elevated the status of many grassland species to provincially endangered or rare. Furthermore, sand plain prairies are home to a large number of rare grassland birds and insects. Increasing habitat quality and quantity through prairie restoration in southern Ontario will help ensure the survival of the ecosystem in addition to Species-at-Risk and increase biodiversity.

RESEARCH OBJECTIVES:

A large-scale field study was established to test the best way to grow grassland plants in abandoned sand pits. This research tested the effect of soil supplements (municipal compost, biochar) and plant symbionts (arbuscular mycorrhizal fungi [AMF]) on prairie plant growth and soil rehabilitation. These treatments were hypothesized to cost-effectively increase plant growth. This research is significantly contributing to industry and scientific research by establishing cost-effective grassland restoration protocol. We have developed this protocol from research results conducted in the fields of ecological restoration, mycorrhizal ecology, and soil ecology.

This research answered 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?

Research objectives addressed in the project focused on:

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.

<u>TECHNICAL RECLAMATION (Components of the Research Project):</u>

Biochar is a relatively new soil amendment used for the management of agricultural soils and mine land rehabilitation. Biochar is created from heating organic matter at high temperatures ($500^{o}C$ – $700^{o}C$) with little oxygen. The remaining charcoal (a.k.a. biochar) is a carbon-rich, porous substance that can be added to soil. Benefits of biochar added to post-mine substrates include retaining essential plant nutrients, increasing water retention, and decreasing soil acidity. Biochar's use as a soil amendment in mine land restoration scenarios is limited. As a relatively new technology, costs are being reduced, thus increasing the feasibility of biochar addition at low rates in large-scale restoration projects. Initial biochar research has shown positive plant growth benefits in degraded, nutrient poor soils. It has been found that using biochar can significantly increase the biomass of agricultural crops, particularly in acidic, coarse textured soils.

Photo: Incorporating soil amendments into pit floor substrate. Each 10^2 meter plot will have 72 tallgrass prairie plant plugs. (Photo taken May 2010)

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 controlling the decomposition process of household and agricultural vegetative waste. As a soil amendment, compost increases water holding capacity and supplies plants with slow release nutrients. Compost is widely available due to community recycling programs. The addition of compost to a post mine sand pit ecosystem is a cost effective way to benefit long-term plant growth

Arbuscular mycorrhizal fungi (AMF) are fungal symbionts associating with the majority of terrestrial plant species. A symbiotic relationship occurs when a close interaction between two biological species benefits both partners. For instance, in exchange for photosynthetic sugars, arbuscular mycorrhizas transfer soil nutrients to plants, contribute to soil stability, and protect plants from pathogens. Plants and seeds can be inoculated with commercially-available arbuscular mycorrhizas allowing for their use in restoration projects.

Some post-mine areas are stressful environments to plants due to a lack of beneficial soil microbes such as arbuscular mycorrhizas. As most soil microbes associate with plant roots, the removal of topsoil in post-mine areas also removes beneficial microorganisms. There are many restoration scenarios indicating increased plant growth when adding AMF inoculum in post-mine areas. Commercial mycorrhizal inoculum is hypothesized to benefit seed and plant plug establishment and survival in severely degraded substrate.

Photo: Progress of amendment application. The dark hexagon has soil amendment mixed into the upper layer of pit floor substrate. In total, 150 plots were established for the plant plug experiment and 72 plots for the seed experiment. (Photo taken May 2010)

METHODS:

The Nature Conservancy of Canada (NCC) has granted us permission to conduct this research on their land holdings in St. Williams, Ontario near Port Rowan. The St. Williams 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.

Experiment #1 – Plant Plugs Trial:

The plant plug experiment was constructed in 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. 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.

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:

<table>
<tr><th>Amendment Application</th><th>AMF Inoculum</th></tr>
<tr><td>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</td><td>Rhizophagus irregularis
Addition / No Addition</td></tr>
</table>

Each 10.2 m^2 plot was replicated ten times. Thirty plots without plant plugs were established as non-vegetated controls. A total of 150 plots were set-up in a fully randomized order.

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

Photo: Greenhouse grown plant plugs. (Photo taken May 2010) one set of the amendment application rates via a liquid medium containing spores. Seeds were applied at double the standard rate recommended for tallgrass prairie restoration projects to maximize plant coverage.

Measurements Conducted (See Full Report for Details):

- AMF colonization of roots was quantified for greenhouse grown plant plugs (June 2010) and field plots (September 2011 / 2012) for *Exp. #1*. To determine percent colonization, roots were dyed with a fungal specific stain and counted systematically under a microscope.

- Plant survivorship was estimated for *Exp. #1*. Thirty-six (36) plant plug locations in the center of each plot were analyzed for new growth each growing season. Survival of a plant plug was estimated by the presence of new, photosynthetically active leaf tissue for that growing season.

- A multivariate statistical method was developed to accurately predict plant biomass. For each species, the best biomass predictor variables were used to non-destructively measure plant plugs in the field. Approximately 3,900 individuals were measured during each field season.

- Plant cover was estimated using an aboveground photographic technique. This simple, non-destructive technique was used repeatedly throughout the experiment to track plant growth patterns. Percent cover measurements are based on the classification of 100 pixels per standardized photograph.

RESULTS AND DISCUSSION

Note: Graphical and statistical analysis are treated fully in the full report. The following sections synthesize the results for that report. Please see full report for details.

Mycorrhizal Colonization:

All greenhouse grown plant plugs were receptive to the AMF inoculum, *Rhizophagus irregularis*. Plant plug roots in the AMF inoculated treatment displayed a large increase in fungal colonization compared to the non-inoculated controls at the time of planting. These results indicate that an AMF inoculum treatment was successful for the *Exp. #1* field trial.

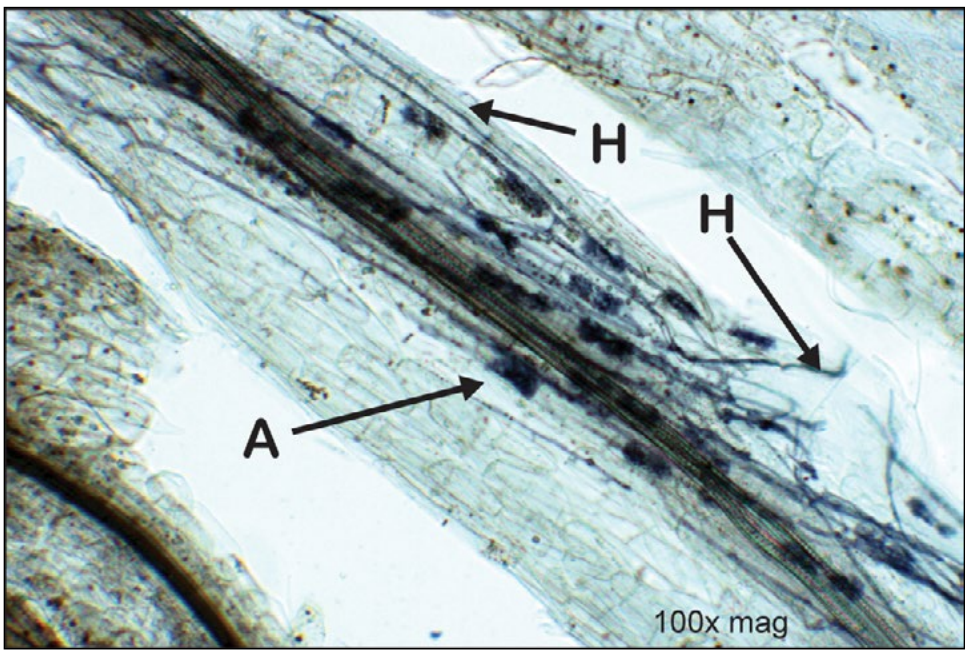

Photo: An example of roots colonized by arbuscular mycorrhizas visualized with a microscope (Magnification = 100x). Mycorrhizas are visualized with a fungal specific ink and vinegar stain. *Rhizophagus irregularis* (the experimental inoculum) is pictured here growing in the roots of *Plantago lanceolata*. 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)

AMF in field roots were quantified and tracked for the first and second growing season. Low colonization rates were noted in the non-inoculated treatments. 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. *R. irregularis* inoculum was detected in the field after two growing seasons, nearly doubling in percent root colonization. In the second growing season, a significant increase in AMF colonization of roots was detected in treatments where 10T/ha biochar + 20T/ha compost was added. The concurrent addition of the biochar and compost in conjunction with AMF inoculum created more favorable conditions for the development of belowground mycorrhizal communities.

Plant Plug Survivorship:

Plants with analogous life history adaptations to abiotic and biotic influences are defined as functional groups. Remnant grasslands are composed of four main functional groups: (1)

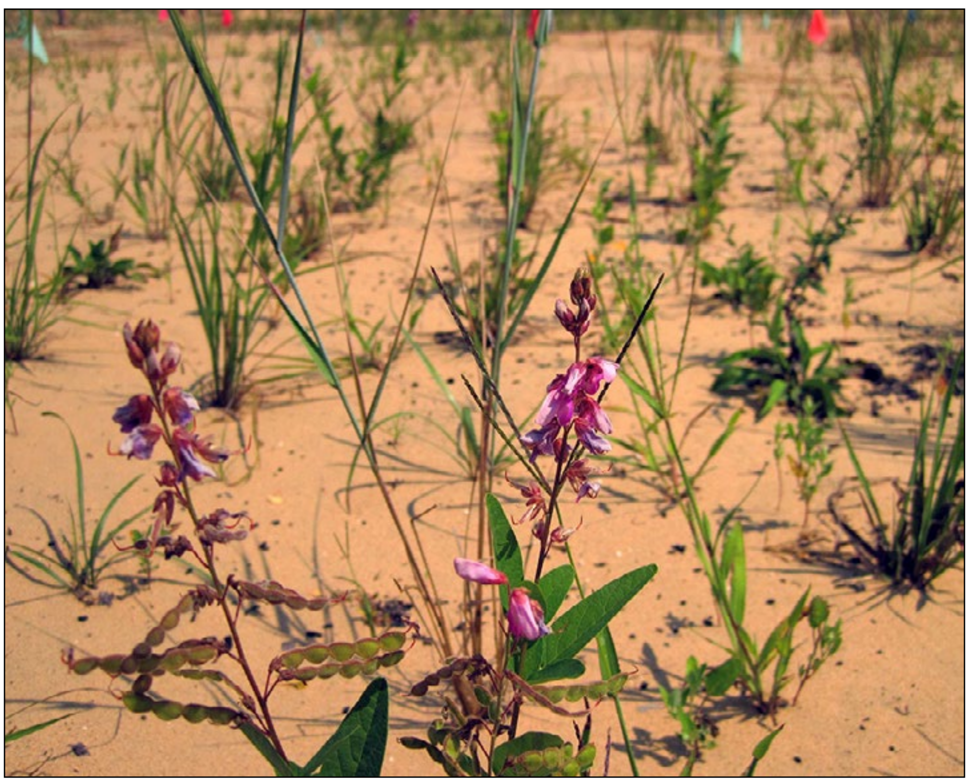

Photo: Plant plugs growing for approximately three months in pit floor substrate. (Photo taken September 2010) cool season [C_3] grasses, (2) warm season [C_4] grasses, (3) composite wildflowers, and (4) nitrogen [N]-fixing legumes. Cool season grasses provide spring/fall plant cover and herbivore fodder. Warm season grasses are drought resistant, provide herbivore fodder, and create habitat for grassland organisms. 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, contributing to the soil N pool.

At the time of initial sowing, all native plant plugs were alive in *Exp. #1*. No significant difference in mycorrhizal treatment was detected for plant plug survivorship. Plant plug survivorship was high during the first [mean ~ 95% survival] and second growing season [mean ~ 90% survival], regardless of soil amendment application. When analyzed as a functional group, C_4 grasses and nitrogen-fixing wildflowers had a consistently high survivorship across all growing seasons and treatment. A sharp decline in survivorship for composites [mean ~ 80% survival] and C_3 grasses [mean ~ 60% survival] was indicated during the 2012 growing season. 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 contributed to the decline in survivorship.

Plant Growth Dynamics in the Plant Plug Trial:

Results indicated that the plant plugs produced greater biomass when compost or compost + biochar were added. No significant AMF inoculum effect was detected on total plant dry weight. After one growing season, compost addition was the main driver of increased plant biomass. By the second growing season, no differences in total plant biomass were observed across soil amendment applications including controls. Despite this, plant species performance was variable in the presence of amendments and AMF inoculation. (See full report for details)

Overall, results indicated that the addition of 20 T/ha compost with or without 10T /ha biochar will translate into greater plant biomass, especially early in plant establishment. These amendment rates are under an ideal scenario for the establishment of native plants. The addition of 20T/ha compost achieves similar plant biomass responses compared to 20T/ha compost + 10T/ha biochar while being more cost effective. Although the individual species response may vary, increased plant community biomass is anticipated due to the slow release fertilizer effect, increased water retention, and microbial stimulation effect from compost addition.

Photo: Three of eight plant species growing at the experimental research cite. Common names from left to right: Smooth blue aster, switchgrass, and round-headed bushclover. (Photos taken September 2012)

Plant Growth Dynamics in the Seed Application Trial:

Trends indicated that native plant cover in *Exp. #2* increased as compost rates increased 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 aggregate sites.

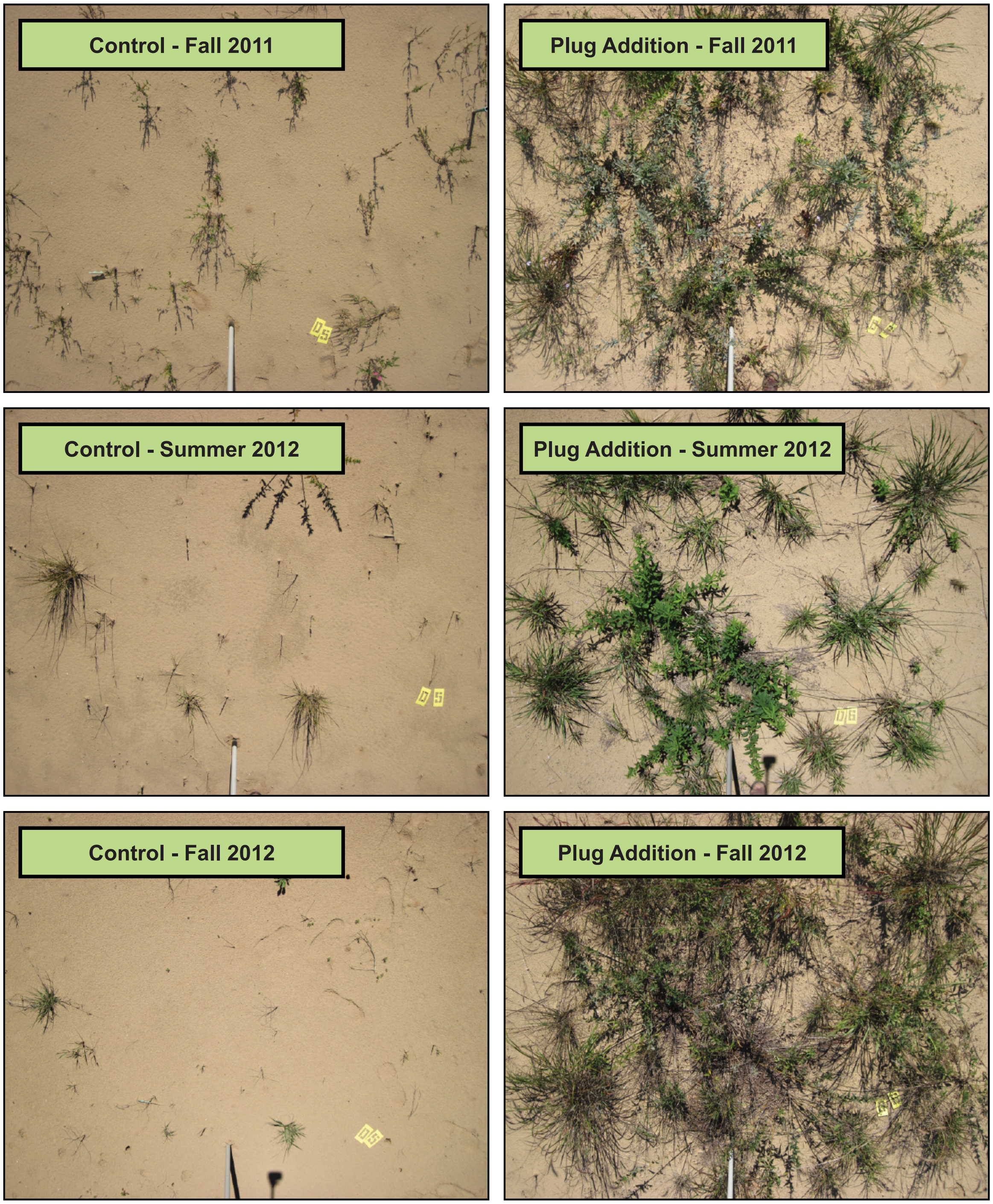

Figure #1: Photographic time series for two sets of plots in the plant plug experiment. The left set of pictures follows a control plot thru time. Therefore, no plant plugs, soil amendments, or mycorrhizas were added. The right hand plots follow a replicate with plant plugs addition only. Photos were 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.

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.

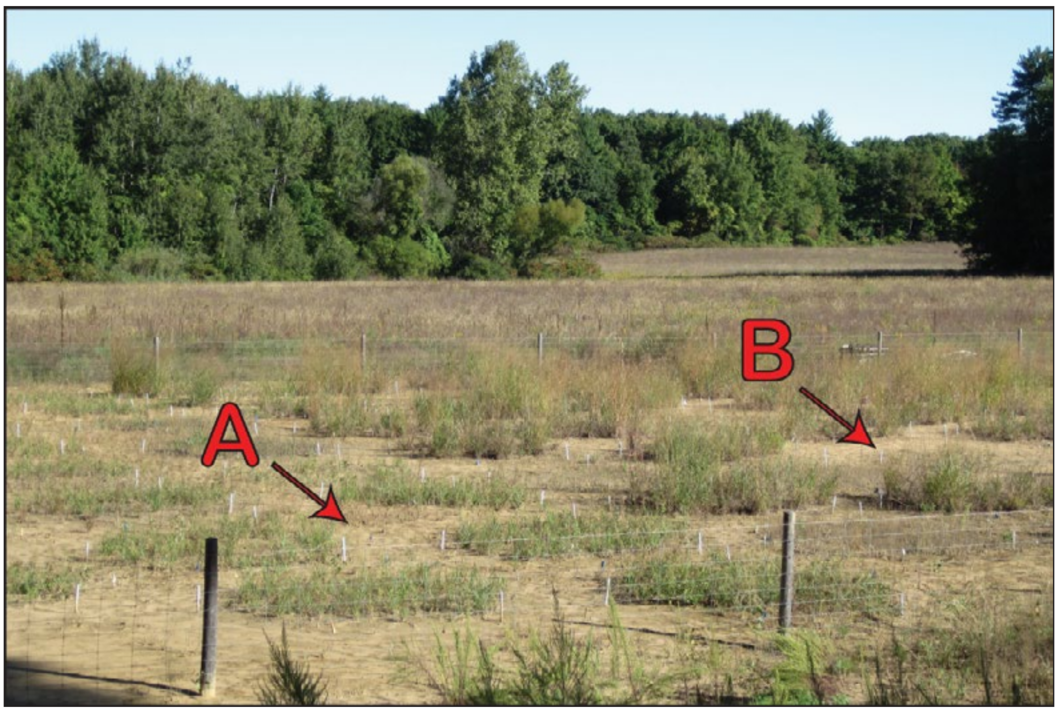

Photo: The seed experiment one year and five months after seed application (A). Plants are established and have sent deep roots into the sand substrate. Compare and contrast the plot biomass from each experiment. Plots with plugs are indidated by (B). During a restoration, plant plugs will yield faster, more dramatic results. (Photos taken September 2012)

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.

<u>Recommendations for Establishing Tallgrass Prairie:</u>

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. For further information on plant selection and growth habits, refer to the full report.

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, the Ontario Society for Ecological Restoration maintains a list of native plant suppliers (http://www.serontario.org/publications.htm). It is recommended that local native plant nurseries are contacted up to a year in advance as they will supply a quote, plant selection recommendations, and seed availability for the 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 compost cost is $40 - $50 per metric ton plus delivery. Compost is typically generated from municipal waste collection streams. Compost should be incorporated into the upper 10cm of substrate at a rate of approximately 20T/ha–30T/ha before sowing and 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 dry, nutrient poor conditions 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 sand pit 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 substrate. 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