**TOARC Research Progress Report – Field Season #2 Completed** Date: December 10th, 2011

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*Project Goals:*

This medium-scale field trial is testing several land management strategies to establish tallgrass prairie habitat in an abandoned sand pit in southern Ontario. This project is testing the effect of soil amendments (biochar, compost) and plant symbionts (commercially-available arbuscular mycorrhizal fungi) on native prairie plant survival and growth. Results from this research will be directly applicable to the aggregate industry by devising best management practices for the restoration of tallgrass prairies in southern Ontario abandoned sand pits.

*Research Site Establishment:*

The research site, near St. Williams, Ontario, is constructed on land donated by the Nature Conservancy of Canada (NCC). The St. Williams, ON area is within the historic range of tallgrass prairie ecosystems in southern Ontario, making this location an ideal candidate for a tallgrass prairie restoration. The experimental site is setup on a recently active sand pit (0.5 hectares). In May 2010, an earthmover graded the sand pit in order to minimize soil topographical variability in the project. Topsoil was not replaced to mimic expended sand pit conditions. With the land surface homogenized, we began establishment of the experimental field plots in June 2010. To minimize ATV activity, a nine wire fence was installed around the perimeter of the experimental area (July 2010).

We are conducting two field trials on the NCC property: *Experiment #1 (Plant Plug Experiment)* and *Experiment #2 (Plant Seed Experiment)*. The following document is an update regarding the progress of each experiment.

Experiment #1 – Plant Plug Experiment:

*Experiment #1* was constructed during the spring 2010. One ton (T) of biochar, 1.5T of compost, and 8,640 plant plugs (8 grassland species) were utilized in *Exp. #1*. Plant plugs were grown by a commercial nursery (Pterophylla) using local prairie plant populations as a seed source.

This experiment used a fully-crossed factorial design. Factors were: (1) biochar (BC) and compost (CM) application rates per hectare (ha) [0, 5T $ha^{-1}$ BC, 10T $ha^{-1}$ BC, 20T $ha^{-1}$ CM, 5T $ha^{-1}$ BC + 20T $ha^{-1}$ CM, 10T $ha^{-1}$ BC + 20T $ha^{-1}$ CM], and (2) plant plug inoculation [± mycorrhizal fungi (*Glomus intraradices*)]. All treatments were replicated (n=10) and each replicate unit comprised a 10.2 $m^2$ plot. Thirty plots without plant plugs were established as controls. A total of 150 plots were set-up in a fully randomized order. Seventy-two plants were inserted into each plot (June 2010). The same spatial arrangement of plant plugs and plant identity was used in order to minimize within and among plot variability in the initial plant community.

Plant and soil data were collected after one full growing season (September 2011). Three types of samples / data were collected: (1) plant survivorship, (2) non-destructive aboveground biomass estimation, and (3) soil cores.

*Plant Survivorship*

Plant survivorship was estimated for the plant plug experiment. Thirty-six (36) plant plugs in the center of each plot were analyzed for new regrowth after winter 2010/2011.

*Non-destructive Biomass Estimation*

In order to measure plants in each plot for consecutive years, we needed to develop several minimally destructive techniques for accurately determining plant biomass.

- Technique #1 - Aboveground Photographs

  A photographic technique was implemented to estimate the percent cover of plant growth for each plot in experiment #1. To accomplish this, an apparatus was constructed to take overhead pictures in each plot. The photos will be analyzed for green pixel coverage to estimate the cover of photosynthetic (active) tissues. Data is currently being processed.

- Technique # 2 - Individual Biomass Estimation

  In order to estimate the biomass of individual plants in each plot, a suite of measurements (e.g. plant height, basal diameter, leaf number, stem height, etc) was collected from one complete set of the treatments. Approximately 36 plants from each species were measured with the various estimators. Once complete, the aboveground biomass of the measured plants were harvested, dried and weighed in the lab to determine true dry weights. Advanced statistical techniques (researched and developed in summer 2011) were then applied to the collected data to determine

the best measurements to estimate plant biomass. The appropriate measurements were then applied to the 32 individuals in the center of the plots. Approximately 3,900 individuals were measured during the field season. Data is currently being processed.

*Representative Soil Cores*

Soil cores were collected from each plot near representative plants. The individual soils were collected and pooled at the plot level. Approximately 1,500 soil cores were collected from the site. At the University of Guelph, roots were washed from the collected soils (October 2011) and stored for DNA analyses and AMF colonization. Soils and roots were shipped to UBC-O for analyses in our home lab. Microscopic and molecular analyses are currently in progress.

*General Notes*

The native plant plugs had a high survivorship rate in the plots (~99%). The general health and vigor of the plant plugs after one growing season is positive. Also, there was an extremely low incidence of "misplants". Only 3 misplants were identified out of the approximately 3,900 plants measured.

*Experiment #2 – Plant Seed experiment:*

*Experiment #2*, adjacent to *Experiment #1*, used a fully-crossed experimental design. *Exp. 2* is testing the effect of amendment application rate and arbuscular mycorrhizae on native seed establishment and growth. One ton of biochar, 1.0 T of compost, and seeds of 8 grassland species are utilized in *Exp. #1*. Each amendment combination was replicated (n=2) for a total of seventy-two 10.2m$^2$ plots. The biochar and compost application rates were as follows:

| 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 |

Native prairie plant seeds are adapted to frozen winters followed by moist spring conditions. When restoring prairies, native plant seeds must be cold, moist stratified in order for germination to

occur. Seeds remained refrigerated until spring application to mimic cold stratification. This is a common practice in prairie restoration ecology.

To minimize overwintering losses from the plots, seeds were applied in early May 2011. The same eight plant species from *Exp. #1* were used in the seed experiment. Seeding rates were applied as per recommendation of local restoration ecologists (Ms. Mary Gartshore / Mr. Peter Carson). Mycorrhizal fungal inoculum (*Glomus intraradices*) was added to one set of the replicated plots in *Exp. #2* immediately after seed addition.

*General Notes*

Plant growth after five months of seed application was minimal, as expected. During the first year of plant growth, native grassland plants invest a lot of biomass belowground to prepare for the upcoming winter. Plants at this stage were difficult to identify so diversity analyses were not conducted. Each plot was photographed as described for experiment #1 to estimate percent cover.

*Photographs:*

Photo 1: A northwest view of the field site. The Plant Plug Experiment (foreground of fenced area) is indicated by vigorous plant growth at the end of the second growing season. Plant Seed Experimental plots are in the background of the fenced area. The green patches represent plots. (Photo Taken: September 2011)

Photo 2: Brian Ohsowski takes an overhead photograph to analyze plant cover. The overhead photograph shown here represents a high plant growth plot (Photo Taken: September 2011).

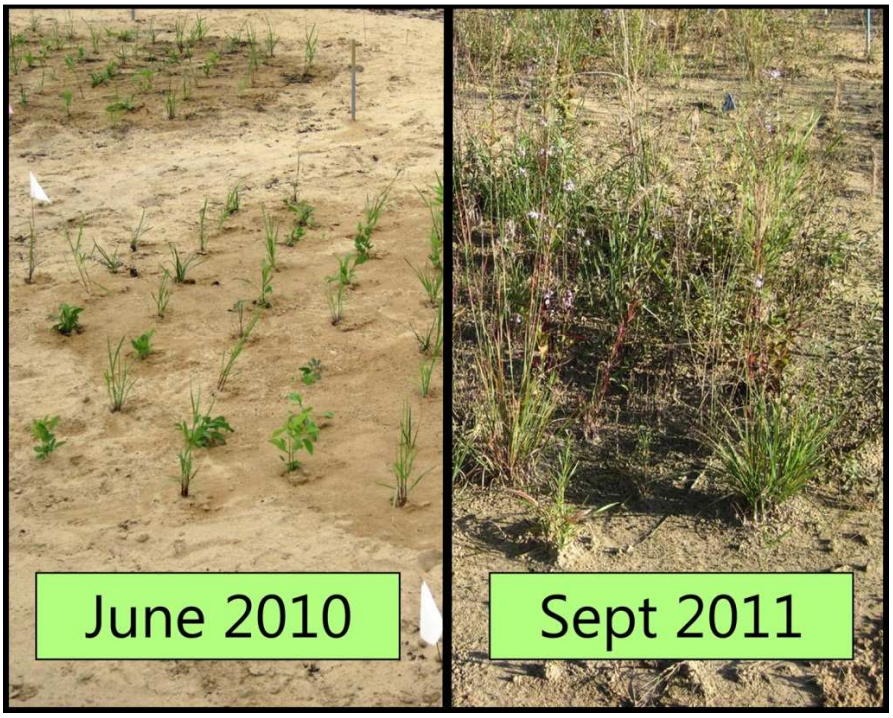


Photo 3: A before and after picture of a plot in the Plant Plug Experiment. The left half of the picture represents a newly planted plot. The right half of the picture represents plant growth after approximately one full growing season. Depending on treatment, plant growth results vary.

Photo 4: The seed experiment after five months of seed application. Notice the stunted growth at this point in time. Plant biomass is expected to dramatically increase next field season. (Photo Taken: September 2011)