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# EFFECTS OF SEED MIXTURE COMPOSITION AND COVER CROP USAGE ON PRODUCTIVITY AND GROWTH OF NATIVE PRAIRIE FORBS AND GRASSES

Kimberly S. Larson

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#### EFFECTS OF SEED MIXTURE COMPOSITION AND COVER CROP USAGE ON PRODUCTIVITY AND GROWTH OF NATIVE PRAIRIE FORBS AND GRASSES

By

Kimberly S. Larson

#### THESIS

Submitted to Northern Michigan University In partial fulfillment of the requirements For the degree of

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#### ABSTRACT

EFFECTS OF SEED MIXTURE COMPOSITION AND COVER CROP USAGE ON PRODUCTIVITY AND GROWTH OF NATIVE PRAIRIE FORBS AND GRASSES

By

#### Kimberly S. Larson

Few studies have experimentally addressed the aboveground and belowground growth potential of various mixtures of forbs and grasses and the effectiveness of cover crops in the establishment of the target species. Thirty-six plots were seeded with varying native seed mixtures made up of native forbs and grasses. An annual cover crop, Elymus canadensis, was seeded in half of the seed mixture plots.

Overall, there was a significant difference among seed mixture treatments. In general, any seed mixture that contained at least one species of native forb had higher biomass production, with a 75% forb:25% grass mix having the highest aboveground yield. Significant differences were seen in root biomass for the seed mixture and cover crop interaction when seasons were pooled, with those containing both cover crop and some level of forbs having higher root biomass. This suggests forbs are better able to utilize the rooting zones of the dying cover crop over time.

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This thesis follows the format prescribed by Ecology.

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#### INTRODUCTION<sup>a</sup>

The use of native plants in restoration has become increasingly important as some plant communities (e.g. prairie, savanna) have diminished due to changes in land use. Native plants are better adapted to various conditions such as drought, wind, grazing, fire, and temperatures that occur in that plant's environment (Kline 1997). Native plantings are becoming a cost-effective and aesthetically pleasing way to revegetate other areas that have been heavily disturbed by humans (e.g. roadsides, landfills, corporate building grounds).

Recently, native plantings of prairie grasses and forbs have been thrust into the limelight because of their efficiency as a potential biofuel source. Tilman et al. (2006) recently published an article in Science outlining the benefits of grassland biomass as a potential biofuel. Thus, there is a ready need to understand the growth potential of various mixtures of forbs and grasses and the effectiveness of cover crops in the establishment of the target species.

The effectiveness of annual or short-lived perennial grasses as cover crops in native plant restorations is a controversial issue. Cover crops are defined as "crops

<sup>1</sup> **<sup>a</sup>** <sup>A</sup> glossary of relevant terms is located in Appendix A.

grown to cover the ground to protect the soil from erosion and from loss of plant nutrients through leaching and runoff" (Dabney et al. 2001). Often when a prairie mixture is planted, an annual cover crop is initially planted with it to prevent initial weed growth and help native plants take root. Grasses have long been used for erosion control (Kemper et al. 1992), but their effectiveness has been questioned due to population fluctuations of annual grasses (Talbot et al. 1939). Perennial grasses are thought to help with erosion control better over the long term than annual grasses.

The effects of various cover crops on the initial growth of native plants have not been studied in much depth. It is believed that some cover crops could be allelopathic (Morgan 1997), and could actually hinder the growth of native perennial seedlings. While most allelopathic effects are plant-plant interactions, allelochemicals in plants have been shown to cause nitrogen deficiency by stifling the growth of nitrification organisms (Chou 1999). Brown and Rice (2000) introduced the exotic grass, Vulpia myuros (zorro fescue), into areas seeded with native perennial grasses. Growth and performances of the native perennial grasses, along with the resident weeds, were evaluated to determine the

possibility of the exotic grass suppressing native plants. The native perennials were found to have decreased seedling survival and above-ground biomass compared to the exotic species (Brown and Rice 2000). This was also true for the weeds that naturally occurred in the plots.

Studies show conflicting evidence in the use of nitrogen (N) by not only cover crops but plants in general. Cover crops like grasses can be good at utilizing residual N in soil before it is leached out (Dabney et al. 2001); but due to the high C/N ratio of grasses, there is also the risk of short-term N immobilization (Odhiambo and Bomke 2001). Inouye et al. (1987) showed that during old-field succession, perennial plant cover had a positive correlation with average soil nitrogen, but annual plants had a negative correlation. Soil N can also mediate competition in plant communities. For example, Tilman (1987) found a decrease in species richness with an increase in total soil nitrogen.

It is also important to study the effectiveness of seed mixtures, especially different ratios of perennial grass and forbs, and how they interact with cover crops. Studies have found that diversity plays a key role in the productivity and stability of ecosystems. According to the diversity-stability hypothesis, ecosystems that are more

diverse will contain more species that thrive during some sort of environmental perturbation (Tilman and Downing 1994). Different species have different traits and therefore are able to compensate for perturbations. Tilman and Downing (1994) found that areas higher in species richness had greater resistance to drought because those areas contained more drought resistant species. The diversity-sustainability hypothesis also states that biodiversity has a direct effect on soil nutrient cycles and soil fertility (Tilman et al. 1996). Ecosystems with higher species diversity are better able to utilize nutrients, which reduces leaching (especially of N).

Chapin et al. (1997) stated that an increase in diversity could increase an ecosystem's stability in three ways. First, higher diversity could increase the number of trophic interactions and provide a more stable energy flow among trophic levels. Species diversity could also allow an ecosystem to be less susceptible to invasion by exotic species. Finally, if diversity increases the distances between conspecifics, it could reduce the risk of plant pathogens.

However, some scientists refute the studies of Tilman and others on the role of diversity per se on ecosystem processes. Aarssen (1997) suggested higher productivity is

simply due to the fact that certain species are able to produce more biomass. Although diversity per se is important in many ecosystem processes, others emphasize the need to understand 'functional groups' of species and how they contribute to productivity and stability of whole ecosystems. Wardle et al. (2000) suggested functional group diversity drives ecosystem properties rather than species diversity.

In grasslands, the representation of different functional groups influences many ecosystem properties (Pokorny et al. 2004). For example, grasses can be divided into two  $C_3$  and  $C_4$  based on their photosynthetic pathways.  $C_3$  grasses, which includes species such as Poa pratensis (Kentucky bluegrass)and Elymus canadensis (Canada wildrye), actively grow during the cool season, spring-thaw through early June and again later in September until snow cover. They have numerous shallow roots which tend not to overlap the deeper rooting zone of  $C_4$  grasses. Selective removal of  $C_3$  grasses reduces total root biomass and alters shoot-to-root biomass ratios (Wardle et al. 1999).  $C_4$ graminoids include well known perennial grasses such as Andropogon gerardii (big bluestem) and Schizachyrium scoparium (little bluestem). These grasses actively grow during the warmer months, June through August. Their root

system is much more deeply rooted than  $C_3$  grasses but less abundant in terms of biomass. However, aboveground biomass in  $C_4$  grasses is noticeably higher than in  $C_3$  grasses.  $C_4$ grasses are also more efficient in terms of transpiration. They can close their stomata to prevent water loss and thus slow the rate of transpiration.

Forbs can also be divided into functional groups. Legumes are forbs which are able to fix atmospheric nitrogen due to a symbiotic relationship with rhizobia found in their root nodules. Although there is no formal classification for root systems of forbs, there are various types present. Some plants such as the Aster species have short, fibrous roots systems while others like the Monarda species have deep, strongly branched roots.

There is a tendency to think in terms of the aboveground biomass as the important part of plants because it is used as a food source and as cover for animals. However, the root system is just as important, because many arthropods thrive belowground and utilize roots as a food source. Wardle et al. (1999) found that removing  $C_4$  grasses greatly enhanced arthropod diversity. By selectively removing  $C_4$  grasses, the  $C_3$  grasses were able to utilize the gaps created and increased their root biomass, which some arthropods preferred. Trying to include species from

different functional groups is therefore critical to the establishment of plantings. Each functional group can have an impact on various other plants, animals, insects, and microbes.

As just stated, what goes on belowground can be just as important as what is occurring aboveground. Mycorrhizal fungi are known to attach and penetrate the roots of plants in the soil, thereby increasing the length and surface area of the root and aiding the plant in facilitating the uptake of important nutrients. Therefore, the symbiotic associations between plants and arbuscular mycorrhizal (AM) fungi could have an effect on the overall ecosystem productivity and species composition. AM fungi are very common and are estimated to have associations with over 80% of terrestrial plant species (Smith and Read 1997) with more than 84% of all grass species estimated to have AM relationships (Newman and Reddel 1987). If certain grasses and forbs are used in seed mixtures, there may be a higher rate of AM fungi infectivity and therefore increased nutrient uptake by host plants. It has long been thought that AM fungi were not host-specific (Law 1988), but recent studies have shown a higher degree of host specificity than originally thought (Eom et al. 2000).

Plant species vary in their responses to AM fungi. Mycorrhizae can alter soil pH (Porter et al. 1987), soil moisture (Anderson et al. 1984), and total nitrogen (Johnson et al. 1991). Studies have indicated AM fungi have a significant effect on tallgrass prairie plant communities by mediating competitive interactions and ultimately influencing diversity (Wilson and Hartnett 1997; Wilson and Hartnett 1998). Due to the higher number of AM associations with grasses, some cover crops could potentially have long-term effects on community richness and productivity.

Finally, recent studies have suggested using native perennial herbaceous grassland species as a source for biofuel. Tilman et al. (2006) studied the gross bio-energy yields of low-input, high-density (LIHD) plots. They found LIHD biomass was able to produce a 51% greater yield of usable energy per hectare compared to corn grain ethanol. These LIHD species do not need the fertile soils that corn and soybeans need and so are able to utilize degraded infertile soils. Higher energy yields are also produced because the plants are perennial and the entire aboveground biomass is utilized as opposed to only the fruit in corn and soybean biofuels.

#### **Objectives and Hypotheses**

The first objective of this study was to determine what effect, if any, seed mixture composition has on overall native plant growth. It has been shown that higher plant diversity leads to a higher productivity, which would be ideal, both ecologically and commercially. If an increase in biomass was shown in the most diverse seed mixtures, it would support this theory.

The second objective of the study was to determine whether the presence of a cover crop had a positive or negative impact on native perennial grass and forb biomass. The competitive effects of the cover crop could play an important role when associated with the native plants.

I hypothesized that there would be a difference in biomass productivity and total nitrogen among seed mixtures. Those mixtures containing a higher number of species would have an increase in biomass production and nitrogen levels. I also hypothesized that the use of Elymus canadensis as a grass cover crop would have a negative impact on the total native perennial plant biomass. Plots containing the cover crop would have less native forb and grass biomass due to suppression of the native plant seeds. Furthermore, I predicted that total nitrogen in the soil would be reduced by the cover crop.

This study aims to provide useful insight into the effects of seed mixtures and cover crops used in native plant restorations in Michigan. If a specific seed mix or the use of a cover crop does not significantly increase the overall productivity of the native plants, eliminating it from the procedures could save valuable time and money.

#### **METHODS**

#### **Study Area**

The study area was located in the southeast corner of Ionia County, Michigan (42°51'26"N, 84°51'06"W). The average monthly temperatures during the growing season ranged from 13°C in May to 20°C in August. Average annual precipitation was 87 cm per year. The experiment was conducted in an abandoned farm field composed of Fox sandy loam soil. The last crop planted was in 1981 when alfalfa was grown. At that point, the field was used as pasture for sheep until 1988. Since then, it has remained fallow, and is currently dominated by Euthamia graminifolia (flattop goldenrod), Daucus carota (Queen Anne's lace), Erigeron annuus (daisy fleabane), and Solidago spp. (goldenrods) (Appendix B).

#### **Site Preparation**

The area was first mowed using a tractor and mower and then sprayed with a non-selective herbicide (Buccaneer, Tenkoz Inc, Atlanta, GA, USA) to kill the remaining stubble left after mowing. The herbicide was applied twice with at least one week between sprayings. The site was then plowed and disked to break up the ground and dig up the roots. Plowing and disking in one direction, then in the opposite

direction and at a diagonal helped to ensure proper breakup of the ground. Subsequently, I raked the entire area to remove plant debris. Individual plots were also raked to help level them and provide a uniform planting surface.

#### **Preparation of Seed Mixtures and Planting**

Several grasses and forbs native to the study area were used, including four grass and five forb species. The native grasses used are all  $C_4$  species: Andropogon gerardii (big bluestem), Schizachyrium scoparium (little bluestem), Panicum virgatum (switch grass) and Sorghastrum nutans (Indian grass). Native forbs included Aster novae-angliae (New England aster), Coreopsis lanceolata (sand tickseed), Monarda fistulosa (bee balm), Ratibida pinnata (yellow coneflower) and Rudbeckia hirta (black-eyed susan). Elymus canadensis (Canada wild-rye), a  $C_3$  grass, was used as the cover crop. These species are commonly used in restoration projects in south-central Michigan (Esther Durnwald, Michigan Wildflower Farm, personal communication, May 4, 2002).

Seed mixtures were calculated using a recommended seed rate of about 538 seeds per square meter (Packard and Mutel 1997). For a 16  $m^2$  plot, the size used in this study, a total of 8608 seeds would be needed. However, the number of seeds per species depended on the seed ratios. An

estimate of the number of seeds per ounce of each species was obtained from the Prairie Moon Nursery catalog (Wade 2002). These estimates are from an industry-wide catalog used as a guidebook for most major native plant seed dealers. Seed amounts, in grams, were then calculated using this information. For example, in a 50:50 mix, 50% of the total seeds would be grass while the remaining 50% would be forbs. If there are five forbs used, each one would represent 10% of the total seed mixture. A total of 860 seeds per forb species would be needed. Because counting out all these seeds would be very tedious, I calculated the number of grams needed using the seeds per ounce estimates. If there are about 66,000 Aster novaeangliae seeds per ounce, I needed 0.368 g for each plot in this treatment. The Elymus canadensis seed was then added to these mixtures at a rate of 7.33 g per 16  $m^2$  (Durnwald 2002). See Appendix C for exact composition of each mixture used in the experiment.

Sawdust was used as a light medium that was added to the mixture to make it more visible on the ground. It also aided in the prevention of wind disbursement (Packard and Mutel 1997). The air was relatively still on the day of planting, so the medium's primary use was as a visual aid in planting.

Seeds were planted two weeks after the final round of herbicide application, sufficient time so the seeds were no longer affected by residual herbicide. After sowing, seeds were incorporated into the soil by lightly raking the plot. The seed mixtures were planted in the fall of 2002 to allow for natural stratification to occur during the winter.

#### **Study Design**

Thirty-six plots were established in the field using a randomized complete block design (Figure 1). Three blocks, each with 12 plots, were used to minimize extraneous soil variation across the study area. Each plot measured 4-m by 4-m with a half-meter buffer on all sides. This buffer was designed to help prevent invasion from surrounding plots and minimize other potential edge effects. A random numbers table was used to determine exact location of each treatment combination. All treatment combinations were present within each block, and there were two replicates of each treatment combination. The two treatments were randomly assigned within the three blocks. The randomized complete block design was designed to eliminate error due to heterogeneous site conditions such as slope or soil variations.

The seed mixtures were as follows: 75% grass:25% forb mix, 75% forb:25% grass mix, 50% forb:50% grass mix, all

grass mix, all forb mix and two types of controls (cover crop only and no cover crop or native seed planted).

#### **Sampling Procedures**

Above and belowground biomass and soil were sampled during the summers of 2003 and 2004. Sampling was originally scheduled once a month during June, July, and August. Due to weather conditions, sampling occurred only in August, 2003 and July and August, 2004. During the first sampling year, a late spring thaw and unusually cool temperatures delayed seed germination and hardly any biomass developed until August.

In the summer of 2004 an entirely different problem occurred during sampling. Average temperatures were much warmer but precipitation averages were also much higher. Over 30 cm of rainfall occurred in the month of May alone resulting in severe flooding and a "state of emergency" for the county. The soil was saturated until July and August, when the soil dried enough for sampling.

#### **Aboveground Biomass**

Aboveground biomass was harvested once during each sampling period. This was done by clipping a 0.1 by 3-m strip in a pre-determined location within the plot with a cordless electric vegetation clipper (Tilman et al. 1996). A different strip location was used each sampling period so

that no overlap occurred (Figure 2). Each strip was sorted by genus and most often to species (Appendix B). Special attention was given to separating target species, those in the seed mixtures, from non-target, predominately weed species. Each species was put into a paper bag or envelope, labeled, and dried at room temperature for at least one week. They were then dried an additional 24 hours in a drying oven at 150 ºC. After drying, each species was weighed on a scale to calculate aboveground biomass.

#### **Belowground Biomass**

Belowground biomass was found by taking root cores. Three 30-cm root cores were taken at evenly spaced intervals within the clip strip (Figure 2). A 5.1-cm (2 inch) diameter steel tube was pounded into the ground with a sledge hammer to a depth of 30 cm. A piece of rebar was inserted into holes that were drilled at the top of the steel tube. The rebar acted as a handle to pull the steel tube and root core sample out of the ground. The root core was removed from the tube and placed into a labeled plastic grocery bag. All three cores were combined into one bag for each plot. The majority of the soil was shaken off and then the samples were washed so only the roots remained. They were then dried and weighed in the same manner as the

aboveground biomass to obtain the total belowground biomass.

#### **Mycorrhizae**

A greenhouse study was performed to obtain estimates of mycorrhizal root colonization and infectivity. Since arbuscular mycorrhizae (AM) fungi can propagate and infect their hosts in many different ways, it is difficult to measure the density and species composition of the AM fungi (Jim Bever, Ph.D. Department of Biology, Indiana University, personal communication, February 20, 2003). However, a fairly accurate measure of abundance can be made by testing infectivity rates using inoculum from the field site soil and a standard plant bioassay such as corn (Nancy Johnson, Ph.D., Department of Biology, Northern Arizona University, personal communication, February 20, 2003). Soil was obtained from each plot in mid-October during the second sampling year and given a 48-day cold treatment in a 4ºC cooler (Johnson et al. 1991). A soil corer was used to collect soil in random locations throughout each plot which were then pooled as one sample per plot. The soil was then placed into labeled 15-cm pots. One week before the soil was ready to be potted, the corn seeds (Zea mays) were pregerminated by placing them in a moist paper towel and

sealing in Ziploc bags for one week. The bags were checked every day to add water if needed so the paper towel would not dry out.

Six pre-germinated corn seeds were sown into each pot and pushed into the soil so they were approximately 0.6-cm below the surface of the soil. Three pots per plot were prepared for a total of 108 pots. Watering occurred at least once a day, sometimes twice a day if needed, to ensure the soil did not dry out while the corn seedlings were being established. Once the corn seedlings were developing and growing well, each pot was thinned so only three healthy corn plants were in each one. They were allowed to grow for thirty days total from the time they were initially planted until they were harvested.

When the plants were ready to be harvested, they were pulled up from the soil and the roots cut off from the shoot. The roots were washed free of soil using distilled water, and then heated at 90ºC for one hour in a 10% KOH solution, which helps to remove the host cytoplasm and most of the nuclei in the roots. After heating, the roots were rinsed in distilled water and acidified with dilute HCl. They were then stained by simmering for five minutes in a 0.05% solution of trypan blue in lactophenol. After

staining, the roots were rinsed with clear lactophenol to remove any excess stain (Phillips and Hayman 1970).

Ten random, 1-cm long root pieces were mounted on labeled slides for each plot. The length of the infected cortex for each root piece was measured in mm. An average for all ten pieces on a slide was found and expressed as the percentage of infectivity for that plot (Giovannetti and Mosse 1980).

#### **Soil Properties**

#### **Soil Nitrogen**

Soil cores were also taken during the second year to test for changes in nitrogen (N) availability in the soil. Soil was tested for extractable  $NH_4^+$  and  $NO_3^-$ , using a 0.01-M KCl solution (Jones 2001; Tilman and Wedin 1991; Tilman et al. 1997). Four 20-cm cores were taken per plot during each sampling period using a 1.27-cm (0.5-inch) diameter soil corer. Once the four cores were taken, they were pooled together as one sample for each plot. A two tablespoon sub-sample was then taken from each of these and placed in a pre-weighed vial containing 50 ml of the KCl solution (Tilman and Wedin 1991). These were weighed again once the soil had been added. They were shaken for 30 minutes to ensure complete mixing and then refrigerated overnight to allow for extraction and settling to occur.

After a 24-hour period, the top liquid solution was pipetted out for analysis of the extracted  $NO_3^-$  and  $NH_4^+$ . The liquid solution was then analyzed using an Alpkem continuous flow spectrophotometer (O.I. Corporation, College Station, TX, USA). This analysis was performed by Steve Bauer of the University of Minnesota Cedar Creek Natural History Area Soil Chemistry Lab.

#### **pH Levels**

Levels of pH were found by using another sub-sample of soil taken from the soil coring. One tablespoon of soil was added to a vial containing 50 ml of  $0.1 \cdot M$  CaCl<sub>2</sub>. The vials were capped and shaken for 2 minutes by hand to allow proper mixing to occur. The suspension was then allowed to settle for 30 minutes. After settling occurred, the electrode of the pH meter was positioned into the supernatant and the pH value recorded.

#### **Soil Moisture**

Moisture levels for each plot were also measured during each sampling period using a HydroSense soil water content meter (Decagon Devices, Pullman, WA, USA). Four measurements per plot were taken from the same general area where the soil cores were obtained. These four measurements were averaged together to get one moisture reading per plot. The HydroSense measures soil moisture on
a 50% scale. Soil is expected to be composed of 50% solid soil particles and 50% pore space filled with air (Decagon Devices, 1999). Water fills the spaces that were occupied by air particles. A soil moisture reading is measured as percent volumetric water content (%VWC). This is a ratio of the volume of water in a specific soil sample to the total volume of that soil. When soil is completely saturated, all of its pore spaces are water-filled and so it is said to have a water content of 50%. Soil that is only half saturated would have a water content of 25%.

# **Statistical Analysis**

A randomized complete block design was used to assign the treatments to plots (Sokal and Rohlf 2000). Each treatment was a combination of two factors: (1) the presence/absence of a cover crop and (2) seed mixture composition (different ratios of grasses to forbs). The block effect was field location (3 levels).

The main effects and interactions were tested using a two-way factorial analysis of variance (ANOVA) with blocking. The seed mix had six levels (all forbs, 75% forbs:25% grasses, 50% forbs:50% grasses, 75% grasses:25% forbs, all grasses, and an unplanted control) while the second factor, the cover crop addition, had only two levels (present or absent). The cover crop

and seed mix were assigned as fixed factors, while the block effect was assigned as a random factor.

Although an annual cover crop was used, there was the possibility of observing it the following year. Some seeds may not have germinated the first season while others plants may have had enough of a growing season to actually produce viable seed which would germinate the following year. For this reason, a seasonal comparison was also conducted to determine the effects of cover crop on native plant biomass.

All analyses were conducted using the General Linear Models modules of SPSS ver. 13.0 (SPSS Inc., Chicago, IL, USA). Tukey HSD was used to perform post-hoc tests for determining specific differences.

#### **RESULTS**

Several statistical analyses were performed on the data to determine differences in the cover crop and seed mixture treatments. First, I tested the two factors (cover crop and seed mixture), and their interaction, for each sampling period (August 2003, July 2004, August 2004). Next, I included "year" in the model as a factor because annual cover crop effects may or may not extend into the second growing season. This was done by comparing the August samples (2003 and 2004). If any factor was determined to be significant, post-hoc tests were performed to identify which treatments differed. In the cover crop treatments, the control was identified as having no cover crop planted while the seed mixture treatments identified the control as having no native seeds planted.

## **Plant Productivity**

## **Aboveground Biomass**

Analyses were performed on three groups: the target native plant species (those actually planted), the nontarget ("weed") species, and overall biomass (target plus weed species combined). Only seed mix was significant for the native plant biomass. These differences persisted through all three sampling periods: August 2003

(F=17.434, df=5,22, P<0.001), July 2004 (F=101.242, df=5,22, P<0.001), and August 2004 (F=161.630, df=5,22, P<0.001) (Table 1, Figure 3).

Post-hoc tests were performed to determine which seed mixes differed in biomass production. Overall, there was a significant increase in native plant biomass for all seed mixtures when compared to the control (Tables 2, 3, and 4). The "all grass" treatment, however, was only significant from the control during the second season sampling periods (Tables 3 and 4). Likewise, all mixtures containing at least 50% forbs had significantly higher amounts of biomass than the 75% grass:25% forb mixture (Tables 3 and 4).

Cover crop, as well as the interaction between cover crop and seed mixture, was not significant during any sampling period. The block effect was not significant either, but was close in one sampling period (August 2004) and probably helped to control for some extraneous variation (e.g. soil differences) across plots.

Weed biomass was not significant for any treatment type during any sampling periods (Table 5). That is, none of the native seed mixtures differed with respect to their effects on weeds. There were no apparent trends among means for individual sampling periods.

Considering both target and non-target species together, overall biomass was not significant for any treatment type during any sampling period.

Finally, aboveground biomass data were combined for August 2003 and 2004 sampling periods and "year" was included in the model as a separate factor to assess temporal effects. Year was significant for weed biomass (F=7.095, df=1,46, P<0.05) (Table 6) and native plant biomass (F=456.555, df=1,46, P<0.001) (Table 7) with less weed biomass and more than two times the native plant biomass during the second year.

The seed mixture treatment was also significant for native plant biomass (Table 7). Tukey's post-hoc tests were performed on the seed mixture treatment for native plant biomass. The control had significantly less biomass than all mixes containing native plants while the "all grass" mixture had less biomass than any mixture containing forbs (Table 8). The interaction between year and seed mixture was also significant for native plant biomass (F=23.658, df=5,46, P<0.001), indicating that seed mixture affected native plant biomass differently for each year (Table 7). Most of this interaction was the trivial effect of biomass in the unplanted control not changing from year

1 to 2 while other plots with native mixtures increased dramatically between years. However, the "all forb", 75% forb:25% grass, and 50% forb:50% grass treatments increased more from year 1 to year 2 than other treatments (Figure 4).

## **Belowground Biomass**

Seed mixtures differed significantly with respect to belowground biomass during all sampling periods: August 2003 (F=3.078, df=5,22, P<0.05), July 2004 (F=3.001, df=5,22, P<0.05), and August 2004 (F=2.668, df=5,22, P<0.05) (Table 9). Specific differences among seed mixes could only be identified by Tukey's post-hoc analysis during the August 2003 sample. The "all grass" mixture had significantly greater root biomass than the 75% forb:25% grass mixture (Table 10).

Cover crop, as well as the cover crop and seed mixture interaction, were not significant for any individual sampling period (but see results for both years combined). The block effect was also not significant for any sampling periods.

"Year" was significant when the August samples were compared  $(F=64.112, df=1,46, P<0.001)$  with year 2 having higher mean weights for all seed mixes (Figure 5). Seed mix was also highly significant when years were pooled

(F=5.040, df=5,46, P=0.001) (Table 11). Tukey's post-hoc tests performed on the seed mixtures showed the "all grass" mixture has significantly greater root biomass than the "all forb"  $(P=0.044)$  and 75% forb:25% grass  $(P=0.005)$ mixtures (Table 12). The control and 50% forb:50% grass mixtures also had greater root biomass than the 75% forb:25% grass mixture. Finally, the interaction between cover crop and seed mixture was highly significant for root biomass (F=3.230, df=5,46, P<0.05) (Table 11). Marginal means show that the root biomass of the "all grass" mixture increased when a cover crop was not present while all other treatments decreased (Figure 6).

### **Mycorrhizae**

Mycorrhizae are organisms that can show a connection between the aboveground and belowground processes. Mycorrhizal infections were measured on a 5% incremental scale and ranged from 0% to 10% for this experiment. Mean percent infections for each seed mixture, with or without a cover crop, were all  $\leq 5\%$  with an overall percent infection of 4.028 for the entire experiment (Table 13). Statistical tests were not performed due to the low percentages of infectivity; however, 64% of plots in the experiment had some level of mycorrhizal infection.

## **Soil Properties**

#### **Soil Nitrogen**

Soil nitrogen levels were measured as the amount of  $\texttt{nitrates}$  (NO $_3$ ), ammonium (NH $_4^+$ ), and total nitrogen present  $(NO<sub>3</sub>^-$  plus  $NH<sub>4</sub>$ <sup>+</sup>). The August 2004 samples consistently had a higher mean level of total nitrogen present in all seed mixes when compared to the July sample of the same year (Figure 7). However, none were significantly different: July 2004 (F=0.486, df=5,22, P>0.05) and August 2004 (F=1.177, df=5,22, P>0.05) (Table 14).

The levels of soil nitrates in the soil were not significant for any treatment type during any sampling periods: July 2004 (F=0.532, df=5,22, P>0.05) and August 2004 (F=1.254, df=5,22, P>0.05) (Table 15). Ammonia levels also did not show any level of significance: July 2004 (F=0.774, df=5,22, P>0.05) and August 2004 (F=0.947, df=5,22, P>0.05) (Table 16). However, somewhat higher levels of ammonia were detected in August compared to July for all seed mixtures (Figure 8).

#### **pH Levels**

There was no significance in pH levels for any of the sampling periods (Table 17). Comparison among years also did not reveal any significant differences in pH

(Table 18). Mean pH levels varied little across treatments, ranging from 5.767 to 6.267 (Figure 9).

## **Soil Moisture**

Average percent moisture is measured as the mean percent volumetric water content (PVWC). Soil moisture levels for the cover crop treatment were significant both in August 2003 (F=12.670, df=1,22, P<0.05) and August 2004 (F=11.989, df=1,22, P<0.05) (Table 19). There were approximately 11-12% higher levels of soil moisture in the plots without a cover crop. Cover crop did not influence soil moisture in the July 2004 sample  $(F=1.768, df=1,22,$ P>0.05) (Table 19).

Soil moisture levels for the seed mixture treatment were also significant for both August sampling periods: August 2003 (F=6.077, df=5,22, P<0.001) and August 2004 (F=5.674, df=5,22, P<0.05) (Table 19, Figure 10). Post-hoc tests for both August sampling periods showed the moisture levels in the control and "all grass" mix were both significantly less than the moisture levels in the 75% grass:25% forb and 75% forb:25% grass mixtures but not to each other (Tables 20 and 21). This suggests some type of functional group dominance controlling soil moisture; however, it is not seen with the other mixes.

"Year" was significant for soil moisture levels when the August samples were compared (F=44.994, df=1,46, P<0.001) (Table 22) with higher moisture levels in year 2 compared to year 1 (Figure 11). Soil moisture levels for the cover crop  $(F=25.529, df=1,46, P<0.001)$  and seed mixture (F=12.000, df=5,46, P<0.001) treatments were also significant (Table 22). Tukey's post-hoc tests showed moisture levels in the control to be significantly lower than all mixes containing forbs (Table 23). The "all grass" mix moisture levels were also significantly less than all mixtures which contained any forbs (Table 23).

#### **DISCUSSION**

## **Importance of Including Functional Groups**

The notion of plant functional groups and their interactions is the key to understanding ecosystem productivity and stability. The ability of different functional groups to occupy diverse niches is directly related to overall plant productivity as well as weed invasibility. Plants can be classified into various functional groups based on morphological and physiological differences that can manipulate variations in life history, resource necessities, and seasonality of growth (Tilman et al. 1997). For example,  $C_3$  grasses have a different growing season than  $C_4$  grasses and therefore are employing different niches.  $C_3$  grasses have numerous shallow roots while  $C_4$ grasses have fewer, but deeper penetrating roots (Wardle et al. 1999). At the same time, forbs have various rooting depths and so are also making use of these different root niches.

Roots are not the only niche being occupied. Grasses are generally much taller than forbs and so a canopy level niche should also be considered. Forbs can be considered more of an understory species which would have reduced

light availability while grasses occupy the canopy level and are able to make the most of available light.

My research demonstrated that including forbs into seed mixtures increased the amount of native aboveground biomass produced in a season. While a mixture that contained only 25% forbs produced a significantly greater amount of biomass than one which contained only grasses, it was still not the ideal mixture. Increasing the percentage of forbs in the mixture to at least 50% almost doubled the amount of biomass produced (Figure 3). Hooper and Vitousek (1997) found that the composition of a particular functional group is just as important as the number of functional groups.

While belowground biomass was significantly different among seed mixes, no specific differences could be found between the individual mixes for a single sampling period. However, when seasons were pooled, an interaction effect between seed mixture and cover crop was seen (Figure 6). This suggests that any combination of both grasses and forbs appears to be more productive over time when a cover crop is added in terms of root biomass. However, this does not appear to be true for aboveground biomass. More studies would need to be done to see if this was indeed

true over the long-term or if it is just during plant establishment.

## **Native Plant Effects on Weeds and Invasibility**

Dukes (2001) noted that invasion success was reduced with high functional group diversity because resource availability was therefore reduced. Chapin et al. (1997) state that one key component to reducing the vulnerability of an ecosystem to invasion by unwanted species is to increase the species diversity which will, in turn, increase its stability. The diversity-stability hypothesis states that in order to promote resistance to disturbances, biodiversity needs to be increased (Tilman and Downing, 1994). High diversity also increases the likelihood of natives using vacated niches after disturbances (Tilman and Downing, 1994). This leads to a decrease in unoccupied openings for exotic species, which tend to be opportunistic, disturbance-adapted species.

However, my research suggests that species composition and functional group diversity have no effect, either positive or negative, on the invasibility of weed species during the establishment phase of restoration. There were no significant effects of seed mixture on weed biomass (Table 5). Less weed biomass was produced during the second season of sampling when compared to the weed biomass

produced the first season. However, weed biomass did not significantly differ between the unplanted control and plots with native plantings.

This brings up the importance of short-term versus long-term impacts on invasive species control. Symstad et al. (2003) stressed the significance of long-term data to understand how the stability of an ecosystem could be affected by biodiversity. Herbaceous ecosystems can have very slow responses to stability but are typically measured on a relatively short time frame when compared to the life of plants. This study only examined the first two seasons of native plant growth. Ecosystems are in a constant state of change and therefore impacts over a longer time scale should also be considered. The interaction between weeds and native seed mixtures could be a more important factor once the natives are fully developed and producing viable seed.

# **Cover Crop Effects on Native Plants**

Cover crops may have both positive and negative effects during restoration. The benefits include soil erosion and nutrient leaching control while the cons include suppression of desirable native plants. Morgan (1997) has suggested that some cover crops might be

allelopathic and are actually hampering the growth of native seedlings.

I originally hypothesized that the plots containing the cover crop would have less biomass due to these allelopathic effects. For aboveground biomass, however, this prediction was not supported. There were no significant differences in aboveground biomass (native, weed, and total) between plots with and without Elymus Canadensis (Tables 1 and 5). This suggests that Elymus canadensis does not have an allelopathic effect on native plants when used as a cover crop.

Conversely, belowground biomass may be affected by cover crop addition. While cover crop did not affect seed mixture during any specific sampling period, it did have an interaction effect when seasons were pooled. Over time, cover crop had a positive effect on native plant biomass when forbs were present in the mixture (Figure 6). However, when a mixture contains only grasses, there seems to be a negative effect. The increased growth of forb roots could be masking this effect on grasses in the other mixtures. This could also suggest that as the annual cover crop biomass dies out over time, the roots of the native forbs are better able to fill those spaces.

However, this is not to say cover crops have no other detrimental consequences in native plant restorations. In fact, my results suggest that cover crops exacerbate soil moisture levels and could limit the establishment of native seedlings by excessively using available water in the soil.

Cover crop will also be an important factor to consider when erosion control methods need to be implemented. Cover crops have long been used in farm practices to reduce soil erosion and runoff (Moomaw 1995). By showing there are no allelopathic effects, the use of Elymus canadensis as a cover crop would seem to benefit native plant restorations when used for soil erosion control if adequate soil moisture levels are maintained. However, more studies need to be done on the ability of Elymus canadensis to control soil erosion.

## **Impact of Plants on Soil Properties**

#### **Mycorrhizae**

Vesicular-arbuscular mycorrhizal infections were not as high as expected. VAM infections are the most common of all fungal associations and likely infect the majority of all plant species (Bever et al. 2001). Since it is well known that most grass species and a large percentage of forbs have mycorrhizal relationships, it was expected that

all seed mixes, especially the "all grass" mixture, would have a high rate of infectivity.

Low rates of infectivity could be due to many reasons. Daniels and Trappe (1980) reported that low endomycorrhizal colonization occurred when there were high soil moisture levels. Ebbers et al. (1987) suggested that when there are abundant levels of soil moisture and nutrients, VAM colonization is severely limited. VAM are obligate symbionts with their host plants but are not host specific. They have a mutualistic symbiosis where the host plant benefits from the relationship by an increased uptake of nutrients and water from the VAM in the roots. If these nutrients are not limiting, there is less need for mycorrhizae and VAM infections are much lower. During the second year of sampling, soil moisture levels were well above normal, which could explain the limited VAM colonization.

Another possible reason for low infectivity could be the sampling time period. The soil for this portion of the experiment was collected in early October. The native grasses used were all warm-season grasses. Bentivenga and Hetrick (1992) found that warm-season grasses had increased VAM infectivity during the summer months of July and August, while cool-season grasses showed a high occurrence

during early spring and early winter when soil temperatures were cooler. In other words, VAM infectivity was directly related to peaks in the productivity of host plants. The cover crop used in my experiment is a cool-season grass so fall soil samples should have displayed higher rates of infection. However, there were no significant differences in infection levels among plots with a cover crop compared to those without a cover crop.

Finally, the low infectivity rates could be due to the young age of my plants. Mycorrhizae may play a more important role once the plants are better established. Helm et al. (1996) stated ectomycorrhizal diversity increased from the early to later successional stages. The plants would then be expending extra energy and resources on flower and seed production and mycorrhizae would be more beneficial.

## **Soil Nitrogen and pH Levels**

One of the main functions of a cover crop species is its apparent ability to offer some protection against the loss of important plant nutrients due to leaching and runoff (Dabney et al. 2001). However, the effectiveness of this depends on a number of things including climate, precipitation, species used, growth stages, and management of the cover crop. Odhiambo and Bomke (2001) found that

cover crops used in areas of higher winter precipitation played an important role in inhibiting the leaching of  $\texttt{NO}_3^+$ from soils. If this were true, the plots with the cover crop treatment in this experiment should have had less  $NO_3^$ leached from the soil.

In this experiment, cover crop had no significant differences in soil nitrate, ammonia, total nitrogen, and pH levels for any sampling periods. Nitrogen levels and pH also did not differ among seed mix treatments (Tables 14, 15, 16, and 17). While the cover crop was expected to decrease soil nitrogen, the results were not entirely unexpected. The litter composition of these native plants is relatively similar in terms of decomposition outputs into the soil and is not known to acidify the soil. Also, none of the forbs used in the experiment where legumes, which fix N. The lequme Lupinus perennis (lupine) was available for this experiment; however, my plots were next to a horse pasture and lupine is extremely toxic to horses. Higher nitrogen levels would have been expected in the soil if a legume were present.

# **Soil Moisture**

The highly variable precipitation both years directly impacted the soil moisture part of the experiment. It is interesting to note that while the soil moisture levels

were much higher in year 2, they still exhibited the same distribution pattern among seed mixes and cover type.

The seed mixes with a cover crop had lower soil moistures than those without. This could be due to transpiration of the cover crop drying out the soils. This result was unexpected because I thought that shading by the cover crop might help retain soil moisture. However, transpiration rates are faster in  $C_3$  grasses like  $E1$ ymus. During the early establishment phase of native plants, soil moisture is critical for seed viability and seedling growth. Therefore, cover crops could potentially reduce germination and establishment of native seed mixes. This was not seen though when the cover crop treatment was examined for aboveground biomass. Lower water availability, however, has been associated with lower relative abundance of weeds (Kolb and Alpert 2003). Therefore, plots with a cover crop should have had lower weed biomass. However, there were no significant differences in the amount of weed biomass with and without a cover crop.

Soil moisture also varied among seed mix treatments, but in a curious way. The control and "all grass" mixes both had significantly less moisture than the 75% grass:25% forb and 75% forb:25% grass mixes but not from each other.

This was true in both 2003 and 2004 during the August samplings. It was expected that the control would be different from all seed mix combinations due to more ground being exposed to the elements. The sun would evaporate any moisture in the soil and yield a lower water content reading. However, the control was only different when all functional groups were present and one was dominate over another. Again, these results do not apply to the amount of aboveground biomass produced.

### **CONCLUSIONS**

The objectives of this study were to determine if the seed mixture composition or the presence of a cover crop had impacted the health of native perennial plants. The first was that differences in seed mixture composition made a difference in native plantings and that any mixture containing native forbs had a significantly greater biomass than treatments with only grasses. If overall biomass is the primary objective of restoration, a mixture containing at least 50% forbs would be ideal, as aesthetically pleasing.

The second objective was to determine if the presence of a cover crop had an impact on native plant productivity. Although I initially expected that the cover crop, Elymus canadensis, would have a significant negative impact on native plants, this was not the case. While the cover crop did decrease root biomass overall in the "all grass" mixture and decrease soil moisture available to the native plants by about 10%, it did not have an effect on native plant aboveground biomass produced. However, there was a subtle interaction between seed mix treatments and cover crop. Cover crop enhanced root biomass of forbs whereas it had a negative effect on root biomass of grasses.

Commercial operations such as the Michigan Department of Transportation could potentially eliminate the use of a cover crop in their native plantings and subsequently save significant amounts of money. However, a cover crop could still be effective in plantings where an erosion control method is needed if the seeding is not going to be done immediately. Finally, this study only focused on the first two years of plant growth and soil properties. Longer term studies are needed to fully evaluate the effects of treatments.

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Table 1. Native aboveground biomass

for each sampling period. ANOVA tables are from GLM analyses testing effects of block, cover, and seed.





(I)	(J) <b>SEED</b>	Mean Difference	Std.	Sig.	95% Confidence	
<b>SEED</b> $\star\,\star$					Interval	
	* *	$(I - J)$	Error		Lower	Upper
					Bound	Bound
	$1\,$	$-1.543$	0.630	0.182	$-3.505$	0.418
	$\overline{2}$	$-4.700$	0.630	$*0.000$	$-6.661$	$-2.739$
$\mathbf 0$	$\mathfrak{Z}$	$-2.670$	0.630	$*0.004$	$-4.631$	$-0.709$
	$\overline{4}$	$-4.468$	0.630	$*0.000$	$-6.430$	$-2.507$
	5	$-4.028$	0.630	$*0.000$	$-5.990$	$-2.067$
	$\boldsymbol{0}$	1.543	0.630	0.182	$-0.418$	3.505
	$\overline{c}$	$-3.157$	0.630	$*0.001$	$-5.118$	$-1.195$
$\mathbf 1$	$\overline{3}$	$-1.127$	0.630	0.492	$-3.088$	0.835
	$\overline{4}$	$-2.925$	0.630	$*0.002$	$-4.886$	$-0.964$
	5	$-2.485$	0.630	$*0.008$	$-4.446$	$-0.524$
	$\overline{0}$	4.700	0.630	$*0.000$	2.739	6.661
	$1\,$	3.157	0.630	$*0.001$	1.195	5.118
$\sqrt{2}$	$\mathfrak{Z}$	2.030	0.630	$*0.04$	0.069	3.991
	$\sqrt{4}$	0.232	0.630	0.999	$-1.730$	2.193
	5	0.672	0.630	0.889	$-1.290$	2.633
	$\overline{0}$	2.670	0.630	$*0.004$	0.709	4.631
	$1\,$	1.127	0.630	0.492	$-0.835$	3.088
3	$\overline{2}$	$-2.030$	0.630	$*0.04$	$-3.991$	$-0.069$
	4	$-1.798$	0.630	0.085	$-3.760$	0.163
	5	$-1.358$	0.630	0.296	$-3.320$	0.603
$\overline{4}$	$\mathbf 0$	4.468	0.630	$*0.000$	2.507	6.430
	$1\,$	2.925	0.630	$*0.002$	0.964	4.886
	$\overline{2}$	$-0.232$	0.630	0.999	$-2.193$	1.730
	$\mathfrak{Z}$	1.798	0.630	0.085	$-0.163$	3.760
	5	0.440	0.630	0.980	$-1.521$	2.401
5	$\mathbf 0$	4.028	0.630	$*0.000$	2.067	5.990
	$1\,$	2.485	0.630	$*0.008$	0.524	4.446
	$\overline{2}$	$-0.672$	0.630	0.889	$-2.633$	1.290
	$\mathfrak{Z}$	1.358	0.630	0.296	$-0.603$	3.320
	4	$-0.440$	0.630	0.980	$-2.401$	1.521

Table 2. Native aboveground biomass multiple comparisons of seed mix types for August 2003 using Tukey HSD.

(I)	(J) <b>SEED</b>	Mean Difference	Std.	Sig.	95% Confidence	
<b>SEED</b> $\star\,\star$					Interval	
	* *	$(I - J)$	Error		Lower	Upper
					Bound	Bound
$\overline{0}$	$1\,$	$-2.300$	0.415	$*0.000$	$-3.590$	$-1.000$
	$\overline{c}$	$-7.200$	0.415	$*0.000$	$-8.490$	$-5.910$
	3	$-4.530$	0.415	$*0.000$	$-5.820$	$-3.230$
	$\overline{4}$	$-7.370$	0.415	$*0.000$	$-8.660$	$-6.070$
	5	$-6.270$	0.415	$*0.000$	$-7.560$	$-4.980$
	$\mathbf 0$	2.300	0.415	$*0.000$	1.000	3.590
	$\overline{c}$	$-4.900$	0.415	$*0.000$	$-6.200$	$-3.610$
$\mathbf{1}$	3	$-2.230$	0.415	$*0.000$	$-3.530$	$-0.940$
	$\overline{4}$	$-5.070$	0.415	$*0.000$	$-6.370$	$-3.780$
	5	$-3.970$	0.415	$*0.000$	$-5.270$	$-2.680$
	$\overline{0}$	7.200	0.415	$*0.000$	5.910	8.490
	$\mathbf{1}$	4.900	0.415	$*0.000$	3.610	6.200
$\overline{2}$	3	2.670	0.415	$*0.000$	1.380	3.970
	4	$-0.170$	0.415	0.998	$-1.460$	1.130
	5	0.930	0.415	0.261	$-0.360$	2.220
	$\mathbf 0$	4.530	0.415	$*0.000$	3.230	5.820
	$\mathbf{1}$	2.230	0.415	$*0.000$	0.940	3.530
3	$\overline{c}$	$-2.670$	0.415	$*0.000$	$-3.970$	$-1.380$
	4	$-2.840$	0.415	$*0.000$	$-4.130$	$-1.550$
	5	$-1.740$	0.415	$*0.004$	$-3.040$	$-0.450$
$\overline{4}$	$\mathbf 0$	7.370	0.415	$*0.000$	6.070	8.660
	$\mathbf 1$	5.070	0.415	$*0.000$	3.780	6.370
	$\overline{c}$	0.170	0.415	0.998	$-1.130$	1.460
	3	2.840	0.415	$*0.000$	1.550	4.130
	5	1.100	0.415	0.128	$-0.200$	2.390
5	$\overline{0}$	6.270	0.415	$*0.000$	4.980	7.560
	$\mathbf 1$	3.970	0.415	$*0.000$	2.680	5.270
	$\overline{2}$	$-0.930$	0.415	0.261	$-2.220$	0.360
	3	1.740	0.415	$*0.004$	0.450	3.040
	$\overline{4}$	$-1.100$	0.415	0.128	$-2.390$	0.200

Table 3. Native aboveground biomass multiple comparisons of seed mix types for July 2004 using Tukey HSD.

	(J) <b>SEED</b>	Mean Difference	Std.	Sig.	95% Confidence	
$(\bot)$ <b>SEED</b> $\star\,\star$					Interval	
	$\star\star$	$(I - J)$	Error		Lower	Upper
					Bound	Bound
$\overline{0}$	$1\,$	$-5.118$	0.507	$*0.000$	$-6.697$	$-3.539$
	$\overline{2}$	$-11.468$	0.507	$*0.000$	$-13.047$	$-9.889$
	3	$-7.428$	0.507	$*0.000$	$-9.007$	$-5.849$
	$\overline{4}$	$-11.595$	0.507	$*0.000$	$-13.174$	$-10.016$
	5	$-10.602$	0.507	$*0.000$	$-12.181$	$-9.023$
	$\mathbf 0$	5.118	0.507	$*0.000$	3.539	6.697
	$\overline{c}$	$-6.350$	0.507	$*0.000$	$-7.929$	$-4.771$
$\mathbf{1}$	$\mathfrak{Z}$	$-2.310$	0.507	$*0.002$	$-3.889$	$-0.731$
	$\overline{4}$	$-6.477$	0.507	$*0.000$	$-8.056$	$-4.898$
	5	$-5.483$	0.507	$*0.000$	$-7.062$	$-3.904$
	$\mathbf 0$	11.468	0.507	$*0.000$	9.889	13.047
	$\mathbf 1$	6.350	0.507	$*0.000$	4.771	7.929
$\overline{2}$	3	4.040	0.507	$*0.000$	2.461	5.619
	$\sqrt{4}$	$-0.127$	0.507	1.000	$-1.706$	1.452
	5	0.867	0.507	0.540	$-0.712$	2.446
	$\mathbf 0$	7.428	0.507	$*0.000$	5.849	9.007
	$1\,$	2.310	0.507	$*0.002$	0.731	3.889
3	$\overline{2}$	$-4.040$	0.507	$*0.000$	$-5.619$	$-2.461$
	$\sqrt{4}$	$-4.167$	0.507	$*0.000$	$-5.746$	$-2.588$
	5	$-3.173$	0.507	$*0.000$	$-4.752$	$-1.594$
4	$\boldsymbol{0}$	11.595	0.507	$*0.000$	10.016	13.174
	$1\,$	6.477	0.507	$*0.000$	4.898	8.056
	$\overline{2}$	0.127	0.507	1.000	$-1.452$	1.706
	3	4.167	0.507	$*0.000$	2.588	5.746
	5	0.993	0.507	0.395	$-0.586$	2.572
5	$\overline{0}$	10.602	0.507	$*0.000$	9.023	12.181
	$1\,$	5.483	0.507	$*0.000$	3.904	7.062
	$\overline{2}$	$-0.867$	0.507	0.540	$-2.446$	0.712
	$\mathfrak{Z}$	3.173	0.507	$*0.000$	1.594	4.752
	$\overline{4}$	$-0.993$	0.507	0.395	$-2.572$	0.586

Table 4. Native aboveground biomass multiple comparisons of seed mix types for August 2004 using Tukey HSD.

Table 5. Weed aboveground biomass for each sampling period. ANOVA tables are from GLM analyses testing effects of block, cover, and seed.

August 2003							
Effect	đf	value F.	P value				
Block	2,22	6.926	0.005				
Cover	1,22	0.298	0.591				
Seed	5,22	0.674	0.647				
Cover*Seed	5,22	0.541	0.743				





Table 6. Weed aboveground biomass for August samples combined for yearly comparison. ANOVA tables are from GLM analyses testing effects of block, year, cover, and seed.







$(\bot)$	(J) <b>SEED</b>	Mean Difference	Std.	Sig.	95% Confidence	
<b>SEED</b> $\star\,\star$					Interval	
	* *	$(I - J)$	Error		Lower	Upper
					Bound	Bound
$\mathbf 0$	$1\,$	$-3.331$	0.395	$*0.000$	$-4.506$	$-2.155$
	$\overline{2}$	$-8.084$	0.395	$*0.000$	$-9.260$	$-6.909$
	$\mathfrak{Z}$	$-5.049$	0.395	$*0.000$	$-6.225$	$-3.874$
	$\overline{4}$	$-8.032$	0.395	$*0.000$	$-9.207$	$-6.856$
	5	$-7.315$	0.395	$*0.000$	$-8.490$	$-6.140$
	$\mathbf 0$	3.331	0.395	$*0.000$	2.155	4.506
	$\sqrt{2}$	$-4.753$	0.395	$*0.000$	$-5.929$	$-3.578$
$\mathbf{1}$	$\overline{3}$	$-1.718$	0.395	$*0.001$	$-2.894$	$-0.543$
	$\overline{4}$	$-4.701$	0.395	$*0.000$	$-5.876$	$-3.525$
	5	$-3.984$	0.395	$*0.000$	$-5.160$	$-2.809$
	$\mathbf 0$	8.084	0.395	$*0.000$	6.909	9.260
	$1\,$	4.753	0.395	$*0.000$	3.578	5.929
$\overline{2}$	$\mathfrak{Z}$	3.035	0.395	$*0.000$	1.860	4.210
	$\sqrt{4}$	0.053	0.395	1.000	$-1.123$	1.228
	5	0.769	0.395	0.389	$-0.406$	1.945
	$\overline{0}$	5.049	0.395	$*0.000$	3.874	6.225
	$1\,$	1.718	0.395	$*0.001$	0.543	2.894
3	$\overline{2}$	$-3.035$	0.395	$*0.000$	$-4.210$	$-1.860$
	$\sqrt{4}$	$-2.983$	0.395	$*0.000$	$-4.158$	$-1.807$
	5	$-2.266$	0.395	$*0.000$	$-3.441$	$-1.090$
$\overline{4}$	$\mathbf 0$	8.032	0.395	$*0.000$	6.856	9.207
	$\mathbf 1$	4.701	0.395	$*0.000$	3.525	5.876
	$\overline{2}$	$-0.053$	0.395	1.000	$-1.228$	1.123
	$\mathfrak{Z}$	2.983	0.395	$*0.000$	1.807	4.158
	5	0.717	0.395	0.468	$-0.459$	1.892
5	$\mathbf 0$	7.315	0.395	$*0.000$	6.140	8.490
	$\mathbf 1$	3.984	0.395	$*0.000$	2.809	5.160
	$\sqrt{2}$	$-0.769$	0.395	0.389	$-1.945$	0.406
	$\mathfrak{Z}$	2.266	0.395	$*0.000$	1.090	3.441
	$\sqrt{4}$	$-0.717$	0.395	0.468	$-1.892$	0.459

Table 8. Native aboveground biomass multiple comparisons of seed mix types for August samples combined for yearly comparison using Tukey HSD.








(I)	(J)	Mean			95% Confidence		
<b>SEED</b>	<b>SEED</b>	Difference	Std.	Sig.	Interval		
* *	$\star\star$	$(I - J)$	Error		Lower	Upper	
					Bound	Bound	
$\mathbf 0$	$1\,$	$-0.347$	0.401	0.951	$-1.596$	0.903	
	$\overline{2}$	0.675	0.401	0.556	$-0.574$	1.924	
	$\overline{3}$	0.478	0.401	0.836	$-0.771$	1.728	
	$\sqrt{4}$	0.932	0.401	0.227	$-0.318$	2.181	
		$-0.103$	0.401	1.000	$-1.353$	1.146	
$\mathbf 1$	$\mathbf 0$	0.347	0.401	0.951	$-0.903$	1.596	
	$\sqrt{2}$	1.022	0.401	0.153	$-0.228$	2.271	
	$\mathfrak{Z}$	0.825	0.401	0.344	$-0.424$	2.074	
	$\sqrt{4}$	1.278	0.401	$*0.043$	0.029	2.528	
	5	0.243	0.401	0.989	$-1.006$	1.493	
$\overline{2}$	$\mathbf 0$	$-0.675$	0.401	0.556	$-1.924$	0.574	
	$\mathbf 1$	$-1.022$	0.401	0.153	$-2.271$	0.228	
	3	$-0.197$	0.401	0.996	$-1.446$	1.053	
	$\sqrt{4}$	0.257	0.401	0.987	$-0.993$	1.506	
	5	$-0.778$	0.401	0.405	$-2.028$	0.471	
3	$\mathbf 0$	$-0.478$	0.401	0.836	$-1.728$	0.771	
	$\mathbf 1$	$-0.825$	0.401	0.344	$-2.074$	0.424	
	$\overline{2}$	0.197	0.401	0.996	$-1.053$	1.446	
	$\overline{4}$	0.453	0.401	0.864	$-0.796$	1.703	
	5	$-0.582$	0.401	0.697	$-1.831$	0.668	
$\overline{4}$	$\mathbf 0$	$-0.932$	0.401	0.227	$-2.181$	0.318	
	$\mathbf 1$	$-1.278$	0.401	$*0.043$	$-2.528$	$-0.029$	
	$\overline{c}$	$-0.257$	0.401	0.987	$-1.506$	0.993	
	$\mathfrak{Z}$	$-0.453$	0.401	0.864	$-1.703$	0.796	
	5	$-1.035$	0.401	0.144	$-2.284$	0.214	
5	$\mathbf 0$	0.103	0.401	1.000	$-1.146$	1.353	
	$1\,$	$-0.243$	0.401	0.989	$-1.493$	1.006	
	$\sqrt{2}$	0.778	0.401	0.405	$-0.471$	2.028	
	$\mathfrak{Z}$	0.582	0.401	0.697	$-0.668$	1.831	
	$\overline{4}$	1.035	0.401	0.144	$-0.214$	2.284	

Table 10. Belowground biomass multiple comparisons of seed mix types during August 2003 using Tukey HSD.





(I)	(J) <b>SEED</b> $\star\star$	Mean Difference $(I - J)$	Std. Error	Sig.	95% Confidence Interval	
<b>SEED</b> $\star\star$					Lower	Upper
					Bound	Bound
	$1\,$	$-0.278$	0.462	0.990	$-1.653$	1.096
	$\overline{c}$	1.119	0.462	0.170	$-0.255$	2.494
$\mathbf 0$	3	0.968	0.462	0.308	$-0.406$	2.343
	$\overline{4}$	1.500	0.462	$*0.025$	0.126	2.874
	5	0.000	0.462	1.000	$-1.374$	1.374
	$\mathbf 0$	0.278	0.462	0.990	$-1.096$	1.653
	$\overline{c}$	1.398	0.462	$*0.044$	0.023	2.772
$1\,$	$\overline{3}$	1.247	0.462	0.095	$-0.128$	2.621
	$\overline{4}$	1.778	0.462	$*0.005$	0.404	3.153
	5	0.278	0.462	0.990	$-1.096$	1.653
	$\overline{0}$	$-1.119$	0.462	0.170	$-2.494$	0.255
	$\mathbf{1}$	$-1.398$	0.462	$*0.044$	$-2.772$	$-0.023$
$\overline{2}$	3	$-0.151$	0.462	0.999	$-1.525$	1.224
	$\overline{4}$	0.381	0.462	0.962	$-0.994$	1.755
	5	$-1.119$	0.462	0.170	$-2.494$	0.255
	$\overline{0}$	$-0.968$	0.462	0.308	$-2.343$	0.406
	$\mathbf 1$	$-1.247$	0.462	0.095	$-2.621$	0.128
3	$\overline{2}$	0.151	0.462	0.999	$-1.224$	1.525
	4	0.532	0.462	0.858	$-0.843$	1.906
	5	$-0.968$	0.462	0.308	$-2.343$	0.406
	$\mathbf 0$	$-1.500$	0.462	$*0.025$	$-2.874$	$-0.126$
	$\mathbf{1}$	$-1.778$	0.462	$*0.005$	$-3.153$	$-0.404$
$\overline{4}$	$\overline{c}$	$-0.381$	0.462	0.962	$-1.755$	0.994
	3	$-0.532$	0.462	0.858	$-1.906$	0.843
	5	$-1.500$	0.462	$*0.025$	$-2.874$	$-0.126$
5	$\overline{0}$	0.000	0.462	1.000	$-1.374$	1.374
	$\mathbf{1}$	$-0.278$	0.462	0.990	$-1.653$	1.096
	$\overline{2}$	1.119	0.462	0.170	$-0.255$	2.494
	$\mathfrak{Z}$	0.968	0.462	0.308	$-0.406$	2.343
	$\overline{4}$	1.500	0.462	$*0.025$	0.126	2.874

Table 12. Belowground biomass multiple comparisons of seed mix types for August samples combined for yearly comparison using Tukey HSD.



Table 13. Mean percent mycorrhizal infection for seed mixture treatments during October 2004.







































$(\bot)$	(J) <b>SEED</b> $\star\star$	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
<b>SEED</b> $\star\star$					Lower	Upper
		$(I - J)$			Bound	Bound
	$\mathbf 1$	0.500	1.176	0.998	$-3.163$	4.163
	$\overline{2}$	$-2.375$	1.176	0.363	$-6.038$	1.288
$\overline{0}$	3	$-3.958$	1.176	$*0.029$	$-7.622$	$-0.295$
	$\overline{4}$	$-4.583$	1.176	$*0.009$	$-8.247$	$-0.920$
	5	$-2.500$	1.176	0.311	$-6.163$	1.163
	$\boldsymbol{0}$	$-0.500$	1.176	0.998	$-4.163$	3.163
	$\sqrt{2}$	$-2.875$	1.176	0.184	$-6.538$	0.788
$\mathbf{1}$	3	$-4.458$	1.176	$*0.011$	$-8.122$	$-0.795$
	4	$-5.083$	1.176	$*0.003$	$-8.747$	$-1.420$
	5	$-3.000$	1.176	0.152	$-6.663$	0.663
	$\mathbf 0$	2.375	1.176	0.363	$-1.288$	6.038
	$1\,$	2.875	1.176	0.184	$-0.788$	6.538
$\overline{2}$	$\mathfrak{Z}$	$-1.583$	1.176	0.757	$-5.247$	2.080
	$\sqrt{4}$	$-2.208$	1.176	0.441	$-5.872$	1.455
	5	$-0.125$	1.176	1.000	$-3.788$	3.538
	$\mathbf 0$	3.958	1.176	$*0.029$	0.295	7.622
	$1\,$	4.458	1.176	$*0.011$	0.795	8.122
3	$\overline{2}$	1.583	1.176	0.757	$-2.080$	5.247
	$\sqrt{4}$	$-0.625$	1.176	0.994	$-4.288$	3.038
	5	1.458	1.176	0.813	$-2.205$	5.122
	$\mathbf 0$	4.583	1.176	$*0.009$	0.920	8.247
$\overline{4}$	$1\,$	5.083	1.176	$*0.003$	1.420	8.747
	$\overline{2}$	2.208	1.176	0.441	$-1.455$	5.872
	3	0.625	1.176	0.994	$-3.038$	4.288
	5	2.083	1.176	0.503	$-1.580$	5.747
5	$\mathbf 0$	2.500	1.176	0.311	$-1.163$	6.163
	$1\,$	3.000	1.176	0.152	$-0.663$	6.663
	$\overline{2}$	0.125	1.176	1.000	$-3.538$	3.788
	$\overline{3}$	$-1.458$	1.176	0.813	$-5.122$	2.205
	$\sqrt{4}$	$-2.083$	1.176	0.503	$-5.747$	1.580

Table 20. Percent volumetric water content multiple comparisons of seed mix types for August 2003 using Tukey HSD.

$(\bot)$	(J)	Mean Difference $(I - J)$	Std. Error	Sig.	95% Confidence	
<b>SEED</b> $\star\star$	<b>SEED</b>				Interval	
	$\star\star$				Lower	Upper
					Bound	Bound
	$1\,$	$-0.833$	1.209	0.981	$-4.599$	2.933
	$\overline{2}$	$-3.125$	1.209	0.143	$-6.891$	0.641
$\overline{0}$	3	$-4.917$	1.209	$*0.006$	$-8.683$	$-1.151$
	$\overline{4}$	$-4.875$	1.209	$*0.006$	$-8.641$	$-1.109$
	5	$-3.167$	1.209	0.134	$-6.933$	0.599
	$\mathbf 0$	0.833	1.209	0.981	$-2.933$	4.599
	$\overline{c}$	$-2.292$	1.209	0.431	$-6.058$	1.474
$\mathbf{1}$	3	$-4.083$	1.209	$*0.028$	$-7.849$	$-0.317$
	4	$-4.042$	1.209	$*0.031$	$-7.808$	$-0.276$
	5	$-2.333$	1.209	0.411	$-6.099$	1.433
	$\mathbf 0$	3.125	1.209	0.143	$-0.641$	6.891
	$1\,$	2.292	1.209	0.431	$-1.474$	6.058
$\overline{2}$	$\mathfrak{Z}$	$-1.792$	1.209	0.679	$-5.558$	1.974
	$\sqrt{4}$	$-1.750$	1.209	0.699	$-5.516$	2.016
	5	$-0.042$	1.209	1.000	$-3.808$	3.724
	$\overline{0}$	4.917	1.209	$*0.006$	1.151	8.683
	$\mathbf 1$	4.083	1.209	$*0.028$	0.317	7.849
3	$\overline{2}$	1.792	1.209	0.679	$-1.974$	5.558
	$\overline{4}$	0.042	1.209	1.000	$-3.724$	3.808
	5	1.750	1.209	0.699	$-2.016$	5.516
	$\mathbf 0$	4.875	1.209	$*0.006$	1.109	8.641
$\overline{4}$	$\mathbf 1$	4.042	1.209	$*0.031$	0.276	7.808
	$\overline{2}$	1.750	1.209	0.699	$-2.016$	5.516
	$\mathfrak{Z}$	$-0.042$	1.209	1.000	$-3.808$	3.724
	5	1.708	1.209	0.719	$-2.058$	5.474
5	$\mathbf 0$	3.167	1.209	0.134	$-0.599$	6.933
	$\mathbf 1$	2.333	1.209	0.411	$-1.433$	6.099
	$\overline{2}$	0.042	1.209	1.000	$-3.724$	3.808
	$\mathfrak{Z}$	$-1.750$	1.209	0.699	$-5.516$	2.016
	4	$-1.708$	1.209	0.719	$-5.474$	2.058

Table 21. Percent volumetric water content multiple comparisons of seed mix types for August 2004 using Tukey HSD.

Table 22. Percent volumetric water content for August samples combined for yearly comparison. ANOVA tables are from GLM analyses testing effects of block, year, cover, and seed.

Effect	df	F value	P value
Block	2,46	23.924	0.000
Year	1,46	44.994	0.000
Cover	1,46	25.529	0.000
Seed	5,46	12.000	0.000
Cover*Seed	5,46	0.716	0.614
Year*Cover	1,46	0.000	1.000
Year*Seed	5,46	0.163	0.975
Year*Cover*Seed	5,46	0.234	0.946

(I)	(J)	Mean Difference $(I - J)$	Std. Error	Sig.	95% Confidence	
<b>SEED</b> $\star\star$	<b>SEED</b>				Interval	
	* *				Lower	Upper
					Bound	Bound
	$1\,$	$-0.167$	0.828	1.000	$-2.630$	2.296
	$\overline{c}$	$-2.750$	0.828	$*0.021$	$-5.213$	$-0.287$
$\overline{0}$	3	$-4.438$	0.828	$*0.000$	$-6.901$	$-1.974$
	$\overline{4}$	$-4.729$	0.828	$*0.000$	$-7.192$	$-2.266$
	5	$-2.833$	0.828	$*0.016$	$-5.296$	$-0.370$
	$\mathbf 0$	0.167	0.828	1.000	$-2.296$	2.630
	$\overline{c}$	$-2.583$	0.828	$*0.035$	$-5.046$	$-0.120$
$\mathbf 1$	3	$-4.271$	0.828	$*0.000$	$-6.734$	$-1.808$
	$\overline{4}$	$-4.563$	0.828	$*0.000$	$-7.026$	$-2.099$
	5	$-2.667$	0.828	$*0.027$	$-5.130$	$-0.204$
	$\overline{0}$	2.750	0.828	$*0.021$	0.287	5.213
	$\mathbf{1}$	2.583	0.828	$*0.035$	0.120	5.046
$\overline{2}$	$\overline{3}$	$-1.688$	0.828	0.338	$-4.151$	0.776
	$\overline{4}$	$-1.979$	0.828	0.181	$-4.442$	0.484
	5	$-0.083$	0.828	1.000	$-2.546$	2.380
	$\overline{0}$	4.438	0.828	$*0.000$	1.974	6.901
	$\mathbf 1$	4.271	0.828	$*0.000$	1.808	6.734
$\overline{3}$	$\overline{c}$	1.688	0.828	0.338	$-0.776$	4.151
	4	$-0.292$	0.828	0.999	$-2.755$	2.171
	5	1.604	0.828	0.394	$-0.859$	4.067
	$\mathbf 0$	$\overline{4}$ .729	0.828	$*0.000$	2.266	7.192
$\overline{4}$	$\mathbf 1$	4.563	0.828	$*0.000$	2.099	7.026
	$\overline{c}$	1.979	0.828	0.181	$-0.484$	4.442
	$\frac{3}{5}$	0.292	0.828	0.999	$-2.171$	2.755
		1.896	0.828	0.220	$-0.567$	4.359
5	$\mathbf 0$	2.833	0.828	$*0.016$	0.370	7.192
	$\mathbf{1}$	2.667	0.828	$*0.027$	0.204	7.026
	$\overline{c}$	0.083	0.828	1.000	$-2.380$	4.442
	3	$-1.604$	0.828	0.394	$-4.067$	2.755
	$\overline{4}$	$-1.896$	0.828	0.220	$-4.359$	4.359

Table 23. Percent volumetric water content multiple comparisons of seed mix types for August samples combined for yearly comparison using Tukey HSD.



Figure 1. Randomized block layout of plots.



Figure 2. Individual plot layout with clip strips and root cores mapped.



Figure 3. Mean weight of native aboveground biomass by seed mix type for all sampling periods.



Figure 4. Estimated marginal means of total native aboveground biomass for all seed mixtures for each year.



Figure 5. Mean weights of root biomass by year for all seed mix types.



Figure 6. Estimated marginal means of belowground biomass for all seed mixtures for each cover crop treatment.



Figure 7. Mean ppm of total soil nitrogen by seed mix type for all sampling periods.



Figure 8. Mean ppm of soil ammonia by seed mix type for all sampling periods.



Figure 9. Mean pH value by seed mix type for all sampling periods.



Figure 10. Mean percent volumetric water content by seed mix type for all sampling periods. Means with the same letter are not significantly different from each other based on Tukey's post-hoc analysis. Significance is for August samples only.



Figure 11. Mean percent volumetric water content by year for all seed mix types.

Appendix A. Glossary of relevant terms.

**Allelopathy** – An interaction in which one plant inhibits the growth of another plant by releasing biomolecules called allelochemicals.

**C3 Plants** – Cool-season plants use a C3 pathway to convert sunlight into carbohydrates using chlorophyll. They often grow best during the spring or fall when the weather is cool and moist. Most forbs and some grasses and sedges have a C3 metabolism. The chemical pathway of C3 metabolism is slightly different from that of C4 metabolism (see the description below).

**C4 Plants** – Warm-season plants use a C4 pathway to convert sunlight into carbohydrates using chlorophyll. These plants often grow best during the summer when the weather is warm and somewhat dry. Some grasses and most Cyperus spp. (Flat Sedges) have a C4 metabolism.

**Fibrous** – The root system consists of a loose collection of more or less thin branching roots that originate from the base of the plant.

**Forbs** – In general, a non-woody plant that is not a grass. These are plants that produce flowers with conspicuous petals and/or sepals; the flowers of such plants are often showy and insect-pollinated.

**Functional Group** – Collections of organisms based on morphological, physiological, behavioral, biochemical, or environmental responses.

**Perennial** – A plant that lives for several years, often producing flowers on an annual basis.

**Restoration** – Recuperating degraded, damaged, or destroyed ecosystems through active human intervention.

**Weed** - A plant considered undesirable, unattractive, or troublesome, especially one growing where it is not wanted, as in a garden.

Appendix B. Scientific and common names of species found in experiment. Target species which were planted are starred.

## **Scientific Name Common Name**

Agropyron repens Quackgrass Ambrosia coronopofolia Cuman Ragweed \*Andropogon gerardii Big Bluestem \*Aster novae-angliae New England Aster Chenopodium leptophyllum Narrowleaf Goosefoot Cirsium spp. Thistles \*Coreopsis lanceolata Sand Tickseed Cyperus spp. Sedges Daucus carota **Queen Anne's Lace** \*Elymus canadensis Canada Wild Rye Erigeron annuus Daisy Fleabane Euthamia graminifolia Flat-Top Goldenrod Medicago lupulina Black Medic Medicago sativa and Alfalfa \*Monarda fistulosa Beebalm Oxalis stricta Common Yellow Oxalis \*Panicum virgatum Switchgrass Phalaris arundinacea Reed Canarygrass Phleum pratense Timothy Grass Plantago major Common Plantain Poa pratensis Kentucky Bluegrass Polygonum convolvulus Black Bindweed Potentilla simplex Common Cinquefoil \*Ratibida pinnata Yellow Coneflower \*Rudbeckia hirta Black-Eyed Susan \*Schizachyrium scoparium Little Bluestem Solidago spp. Goldenrods \*Sorghastrum nutans Indiangrass Taraxicum officinale Common Dandelion Trifolium pratense Red Clover Trifolium repens White Clover Verbascum thapsus Common Mullein



