

# NEBRASKA DEPARTMENT OF ROADS

## Report of research results

NUMBER: RHE-01

TITLE: Fertilizer Effects on Attaining Vegetation Requirements

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### Executive Summary

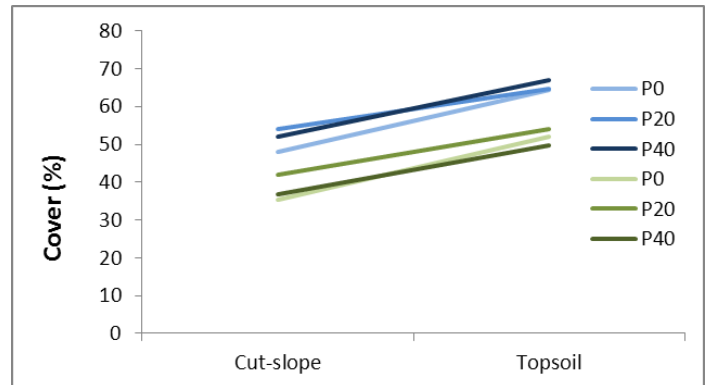
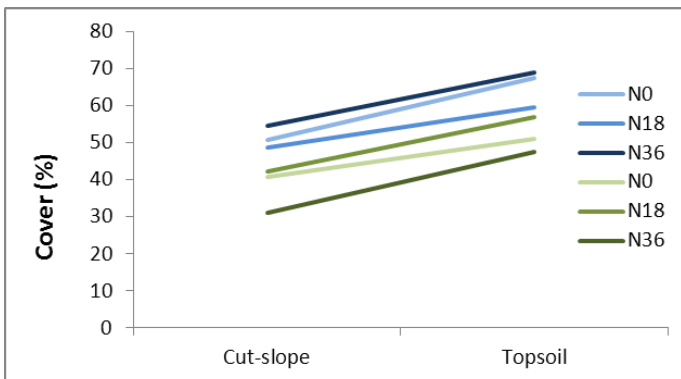
This project was developed to evaluate the effects of varying the substrate and fertilization regimes on the success of complex warm-season grass and forb seedings on recent roadside construction sites. Re-vegetating construction projects is required for complying with NDOR's environmental permits pertaining to wetlands, endangered or threatened species, and stormwater. The stormwater discharge permit specifies that 70% perennial cover relative to pre-construction foliar cover must develop prior to closing the permit. There are substantial costs associated with both ongoing monitoring of sites until they meet permit requirements and of reseeded if re-vegetation fails. The use of stockpiled or imported topsoil was expected to produce better stand establishment than existing cut-slope soils because construction methods often result in a surface layer of low-quality subsoil. Topsoil should provide better growing conditions, and is therefore expected to provide better stand establishment and foliar cover. Fertilizer treatments were tested because protocols were developed for low diversity cool-season grass seedings, and complex warm-season grass and forb mixes may respond differently to fertilization. Nitrogen fertilization (applied at 0, 18, and 36 lbs N/acre) was not expected to improve stand establishment in the complex warm-season mixes because faster growing annuals also take up nitrogen rapidly and compete with seeded species. In contrast, phosphorus fertilization (applied at 0, 20, and 40 lbs N/acre) was expected to improve stand establishment because it is thought to encourage rapid root development, which would favor the more substantial roots of most species in the complex warm-season mixes.

Two experimental sites were established near Ashland, Nebraska. Both sites were established on 3:1 backslope locations that had been successfully planted to warm-season mixes in 2005. Sites were disked repeatedly to kill existing vegetation in preparation for this study. Topsoil was purchased from a local business and applied to half of plot area. The area was seeded by NDOR contractors using NDOR protocols, after which fertilization treatments were applied by hand immediately after seeding. The first site (SAC) was planted in November 2009. The second site (Ashland) was planted in June 2010. Soil samples were collected from both existing cut-slope soils and imported topsoil to allow evaluation of soil fertility. After seeding, vegetation frequency data was collected twice per year: once in June and once in September through September 2012. Foliar cover data was collected once in August of 2012. Soil movement was tracked using erosion pins. In addition to the main project, a small side project at SAC tested the effect of adding mycorrhizae to the existing cut-slope soils with either no fertilization or standard rates of nitrogen and phosphorus. Only species frequency data was collected for this project.

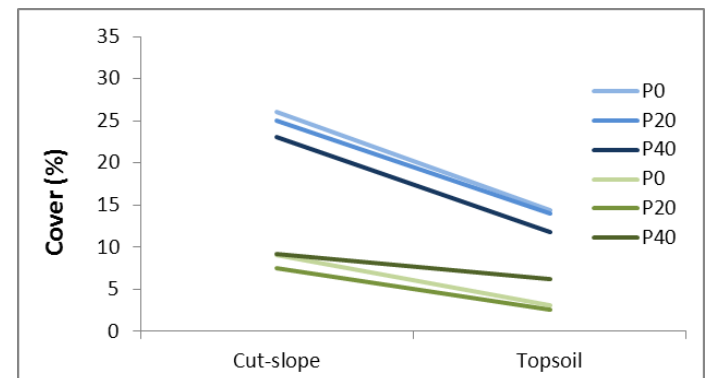
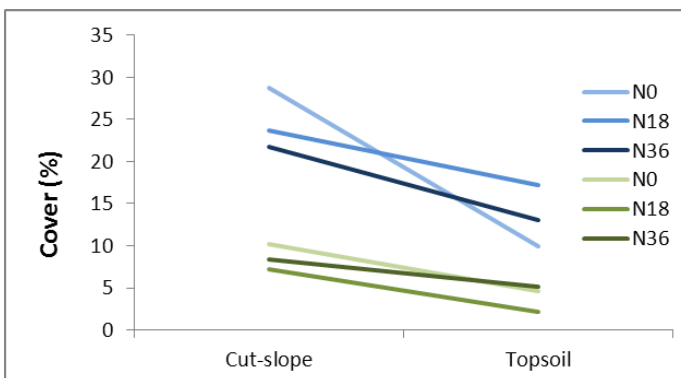
Overall, the results suggest no benefit in stand establishment with the use of nitrogen or phosphorus

fertilizer. Seeding into topsoil resulted in 15% higher cover of warm-season grasses (Figures 1A and 1B) and a 50% reduction in the amount of bare ground (Figures 2A and 2B) by the third growing season. However, there are two important issues to consider in these conclusions. First, the topsoil obtained for these sites was likely from a soybean field (based on presence of soybean residue). These imported soils, when compared with existing cut-slope soils, performed better in some but not all of the soil fertility tests performed. It is likely that stockpiled topsoil from an area previously dominated by grasses (as is the case in most local roadsides) would have been higher quality than the topsoil we used from cultivated fields because soils that have been cultivated repeatedly tend to have reduced soil fertility compared to untilled soils. Second, our conclusions are driven almost entirely by the cover data collected in the third year. Although frequency of occurrence of seeded species has been a good technique in previous studies, it was found to be insufficiently sensitive to detect differences in stand establishment that were clearly visible to the naked eye. The use of foliar cover as a measure of vegetation response is recommended for future studies. Determining foliar cover response to treatments on roadsides is particularly relevant to NDOR-funded projects because SWPPP requires seeded perennial vegetation to attain specified canopy cover levels, generally set at 70% perennial foliar cover relative to pre-project cover levels.

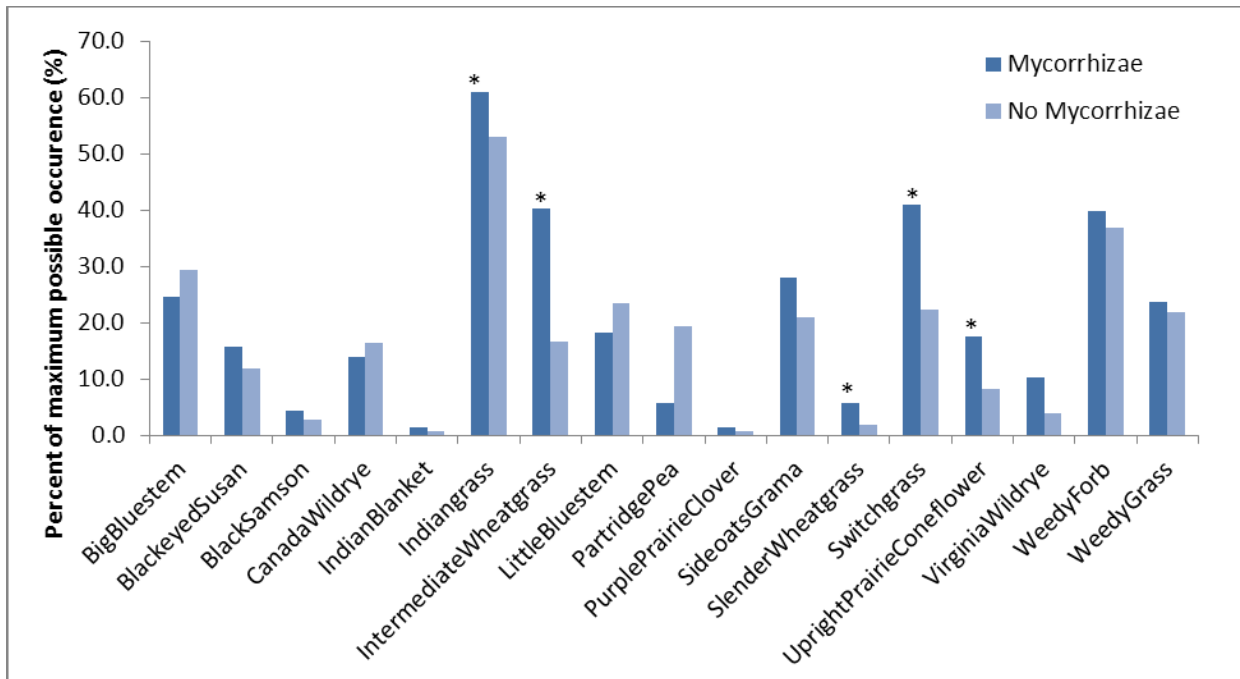
The mycorrhizae addition side project found that only a handful of species were significantly affected by mycorrhizae addition, and significant responses were found only in the first and third year (Figure 3). However, given the relative inability of frequency to identify species responses in the main project, and the fact that the third year data was collected under severe drought conditions, results suggest that mycorrhizae addition may benefit stand quality and is likely worthy of further exploration.



**Figures 1A and 1B:** Warm-season grass cover in the third growing season is higher in the presence of topsoil regardless of nitrogen or phosphorus level. Results are averaged across phosphorus levels on the right and averaged across nitrogen levels on the right. Ashland results are in blue, SAC results are in green



**Figures 2A and 2B:** Bare ground cover in the third growing season is lower in the presence of topsoil regardless of nitrogen or phosphorus level. Results are averaged across phosphorus levels on the right and averaged across nitrogen levels on the right. Ashland results are in blue, SAC results are in green



**Figure 3:** Species frequency in third growing season. \* indicates species with significantly higher frequency in mycorrhizae plots. No plots were significantly higher in the absence of mycorrhizae

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## **Introduction**

Establishing vegetation quickly on Nebraska Department of Roads (NDOR) roadsides is important for stabilizing slopes and reducing soil erosion. In the last few decades, the seed mixtures used on roadsides after construction have shifted from rapidly-establishing exotic cool-season grasses to complex mixtures of slower-establishing, native warm-season grasses and wildflowers. This shift is driven by increasing public and government agency appreciation of and interest in promoting native plant communities, and by the desirable characteristics of native warm-season grasses (e.g., drought resilience and deep root systems). Despite their desirable characteristics, the native grasses and wildflowers in these complex mixtures establish more slowly, have different fertilization requirements than the simple cool-season-only grass mixtures, and are more expensive than cool-season grass mixtures. It is important to examine ways in which both establishment success and growth rate can be increased in warm-season grass seedings in order to increase the likelihood of seeding success as measured by the ability to meet the requirements of the stormwater permit and other permits.

Stormwater Pollution Prevention Plans (SWPPPs) are required for most NDOR construction projects, as part of the stormwater permit. The SWPPP requires on-going inspections until perennial vegetation attains specified canopy cover levels, generally set at 70% perennial foliar cover relative to pre-project cover levels. Stormwater permits for projects that have not attained foliar cover goals cannot be closed, and must continue to be inspected until the cover threshold is attained. Timely closure of permits following construction reduces the NDOR staff time needed for conducting inspections and documents the accomplishment of environmental goals. The need to rapidly establish perennial plant cover has led to NDOR investigations of alternative seeding methodology and soil fertility practices. This project focuses on evaluating two main issues: benefits of fertilizer application and impacts of applying topsoil to roadside surfaces before seeding.

Current NDOR fertilization protocols call for the application of relatively high nitrogen fertilization rates at seeding (36 lbs N/acre). Nitrogen fertilizer application at this rate is appropriate for seeding exotic cool-season grasses (Rehm, 1990; Schuman et al., 1991) but may be detrimental to native warm-season grass seedings because it favors fast growing weeds that compete with seeded species (Anderson, 2007; Gillen and Berg, 1998). The competition slows stand establishment and may cause stand failure if planted perennial species are suppressed to a level in which they are unable to meet permit requirements. In contrast, phosphorus fertilization is considered to be of value for all perennial grass seedings because it encourages rapid root development. Specific research to evaluate the impacts of nitrogen and phosphorus fertilization on the establishment of complex seeding mixtures of grasses and wildflowers has not been conducted, and restoration guides for warm-season grass and wildflower dominated prairies do not provide fertilization recommendations for establishment (e.g., Packard and Mutel, 1997). As a result of this uncertainty, and in the face of recent significant increases in fertilizer prices, this project will evaluate the effectiveness of using nitrogen and phosphorus fertilizer at the time of seeding to achieve re-vegetation goals for permit closure.

Roadside seeding may take place under harsh conditions. Soils are severely disturbed and often compacted during construction, topsoil is not routinely replaced, and soil amendments (i.e., composted manure or mycorrhizal inoculum) are rarely used. As a result, the standard post-construction surface soils available often are a surface layer of low-quality subsoil that provide challenging growing conditions to post-construction seedings (existing cut-slope soils). During post-construction vegetation inspections in 2008, seven completed projects had inadequate vegetation cover meet requirements for permit closure. These unsuccessful seedings required expensive second seeding attempts and recurring site inspections. Improving soil fertility is one way to potentially increase seeding establishment. Relative to the existing cut-slope soils, topsoil can be expected to be less dense and higher in nitrogen, phosphorus, organic matter, cation exchange capacity, water holding capacity, and generally more favorable to plant growth in virtually any metric applied. However, quality topsoil

can be expensive and difficult to acquire, and stockpiling topsoil can substantially increase construction expenses. Currently, little research exists that evaluates the benefits of applying a layer of topsoil to the surface of roadside construction sites prior to seeding. Thus, we evaluated the benefits of topsoil addition and fertilizer treatments in this project.

The objective of this project was to evaluate the interacting effects of nitrogen and phosphorus fertilization on the establishment of NDOR's complex seeding mixture(s) (Type A) planted into existing cut-slope soils or planted into cut-slope soils amended with topsoil. In addition to evaluating stand establishment, we examined the effects of these treatments on soil movement within the treated plots. Specifically, we examined the effects of using existing cut-slope soils with or without a topsoil amendment, three levels of nitrogen addition, and three levels of phosphorus addition. The three levels of nitrogen addition include the standard NDOR application rate (36 lbs N/acre), no nitrogen addition (0 lbs N/acre), and an intermediate rate of nitrogen fertilization (18 lbs N/acre). The intermediate rate was included because establishment of warm-season grasses may respond favorably to low levels of nitrogen fertilizer (Anderson, 2007). The three levels of phosphorus addition included the standard NDOR application rate (40 lbs P/acre), no phosphorus addition (0 lbs P/acre), and an intermediate rate of phosphorus addition (20 lbs P/acre). As with the nitrogen, lower levels of phosphorus were included because grasses may respond to this lower rate of phosphorus fertilization.

Overall, we tested the following specific hypotheses on complex native warm-season grass and wildflower seedings into recent roadside construction sites:

- 1) The addition of nitrogen fertilizer will not result in better stand establishment.
- 2) The addition of phosphorus fertilizer will result in better stand establishment.
- 3) The presence of topsoil will result in better stand establishment.
- 4) Soil movement will be affected by the same factors that affect stand establishment.

In addition to the main project, we also evaluated the potential benefits of inoculating seeds with mycorrhizal fungi in a small side project. Brejda et al. (1998) reported a several-fold increase in shoot and root production of warm-season tallgrasses in the Great Plains in response to seed inoculation with mycorrhizal fungi. The objective of this side project was to evaluate the effects of mycorrhizal fungi inoculation alone and in combination with standard fertilization levels in existing cut-slope soils. It was hypothesized that mycorrhizae inoculated plots will result in better stand establishment in the non-fertilized plots but that the fertilizer addition would negate the effects.

## **Methods**

### ***Plot layout and establishment***

This research was conducted on two roadsides in eastern Nebraska, beginning in 2009 and 2010. Both sites were initially planted in September 2005 as part of the NDOR "Ashland South" projects on Hwy 66. The SAC site was established in November 2009 and the Ashland site was established in June 2010. The two establishment times represent the two most common roadside seeding seasons (late spring/early summer and fall), but with only two sites we are not able to distinguish between site effects and seasonal effects. Plots were laid out on a 3:1 backslope, and existing vegetation was repeatedly disked to prepare each site for seeding. The experimental design at each site was a randomized complete block with three replications.

The treatment design was a split-split-plot design with existing cut-slope soil or topsoil as the whole plot factor, 3 levels of nitrogen application as the split plot factor, and 3 levels of phosphorus application as the split-split-plot factors. Each of the three replications was established as a 360-foot long strip, 25 feet wide, running along the contour of the backslope (Figure 1). Each replication was divided into two 180-foot long plots,

randomly assigned to receive either standard post-construction soils (existing cut-slope soils) shaped by the contractor or 4 to 6 inches of topsoil spread on existing cut-slope soils (topsoil). An additional 40-foot strip at the SAC site was reserved for a mycorrhizae sub-project. Because these sites were not new construction, obtaining stockpiled topsoil from these sites was not an option. Instead, topsoil was obtained as “pulverized topsoil” from a local construction company. Although the precise origin of the soil could not be identified, the presence of soybean residue suggested a cropfield origin. This is supported by the relatively low soil organic matter content (Figure 2).

After main plots had been established and topsoil applied where appropriate, the entire plot area was seeded with Type A complex seeding mixture (Appendix 1) using a Brillion-type seeder according to NDOR specifications. Following seeding, each 180-foot long plot was divided into thirds (60 feet) and one of three nitrogen application rates (0, 18, or 36 lbs N/acre) was assigned randomly to each of the thirds. Fertilizer treatments were applied by hand. Nitrogen was applied in the form of 0, 39, or 78 lbs/acre urea. Each of the nitrogen fertilization split-plots was divided into three equal-size sub-plots (20 feet) and one of three phosphorus application rates (0, 20, or 40 lbs actual P/acre) was assigned randomly to each of these three split-split-plots (“plots”). Phosphorus was applied in the form of 0, 46, or 92 lbs/acre  $P_2O_5$ . Following seeding and fertilization, all plots were covered with prairie hay and crimped as is the common practice on roadside seedings.

Plots for the side project evaluating the effect of mycorrhizae were established at the SAC site only. Plots were established on existing cut-slope soils that received one of two treatments: mycorrhizae only or mycorrhizae plus standard levels of nitrogen and phosphorus (36 lbs N and 40 lbs P per acre). All site history and plot establishment protocols were the same as those used to establish the main project plots.

### ***Data collection***

Twelve vertical undisturbed soil cores (0.63 inch diameter x 6 inches long) were taken from throughout each whole plot (180 feet x 25 feet) before disking or the application of the treatments. The cores were divided into 2 depth increments: 0-3 and 3-6 inches. The subsample cores within each plot were composited, oven dried, and ground in a mortar to a fine powder. Samples were analyzed by AgSource Harris Lab for pH, organic matter content, nitrogen, phosphorus, potassium, and cation exchange capacity (CEC). Soil bulk density was also determined at each site. Three soil cores (2 inch diameter x 4 inch depth) were taken at random locations within each sub-sub-plot. Each core was placed in an airtight container, transported to the laboratory, and bulk density determined as a measure of soil compaction.

Vegetation data was collected in June and September for three growing seasons after seeding to monitor establishment after seeding. The methods were the same at both sites, except that the first vegetation data for the SAC site was collected in June 2010 and the first vegetation data for the Ashland site was collected September 2010 because of differences in seeding time. Frequency of occurrence of the seeded and volunteer (non-seeded) plant species were determined in 10 nested quadrats (20 x 20 inches) that were randomly placed in each plot. Planted and desirable volunteer species were recorded individually. Desirable volunteer species included perennial native species and exotic legumes. Annual forbs were lumped into a “weedy forb” group. Exotic cool-season grasses and annual grasses were lumped together in a “weedy grass” group. Nested frequency data was processed by determining the frequency of each species (or group of species) in each of the four frequency frame sizes (4 x 4, 10 x 10, 14 x 14, and 20 x 20 inches). The frequencies were then summed to generate a composite “sum of frequency” value (Smith et al., 1987). For ease of interpretation, the sum of frequency values were divided by 4 (the number of frame sizes used) to show average percent frequency across size classes. The average of the 10 frequency values per plot was used to evaluate treatment effects.

Mycorrhizae treated plots in the sub-study were compared with comparable plots in the main project.

Sampling protocols were the same for species frequency, but no other sampling was done on this sub-study.

In August of the third growing season (2012), we used an 8 x 20-inch Daubenmire frame (Daubenmire, 1959) to estimate percent foliar cover in 10 randomly selected sampling points per plot. Cover was estimated to the nearest 5% for major planted functional groups (cool-season grass, warm-season grass, and forbs), “weedy” functional groups (weedy forbs and weedy grasses). Areas not covered by standing vegetation were recorded as ground cover (bare ground or litter). Vegetation plus ground cover for each plot equaled 100%. Cover data was used to evaluate the treatment differences and determine if the stands had reached the foliar cover threshold (70% of pre-project cover) required as part of the Environmental Protection Agency specifications (stormwater permits).

Soil movement was estimated in all plots using erosion pins. Each “erosion pin” is a metal rod 18 inches in length that is pushed into the ground to a point where a mark on the rod (8 inches below the top) was flush with the soil surface. Shortly after seeding, ten pins were installed in each plot at regular intervals. Measurements from the top of the rod to the soil surface were taken at the same time as species frequency data were collected and were used to determine soil loss or accumulation.

### *Data analysis*

Data were analyzed using PROC GLIMMIX in SAS (SAS 9.3, Cary, NC, 2012) to assess the impact of site, soil, nitrogen, and phosphorus on the frequency of each species or group of species, the various types of canopy cover, and soil movement. There were frequent interactions between site and treatments, so we assessed the impact of the treatments separately for each site.

## **Results and Discussion**

### *Pre-treatment: cut-slope soil and topsoil characteristics*

Soil characteristics of existing cut-slope soil and topsoil were evaluated in order to ensure that the assumptions of differences between topsoil and existing cut-slope soils were supported. It was expected that topsoil would be higher than existing cut-slope soils in all aspects of soil fertility tested.

Topsoils at Ashland largely but not entirely met expectations, having higher nitrogen, phosphorus, potassium, and cation exchange capacity than the existing cut-slope soil. However, contrary to expectations, soil organic matter levels were similar between the Ashland existing cut-slope soil and topsoil (Table 1). Topsoils at SAC, like Ashland, did not entirely meet soil quality expectations. Although SAC topsoils had higher nitrogen, phosphorus, and soil organic matter than SAC existing cut-slope soils, the topsoil had lower potassium and cation exchange capacity than the existing cut-slope soil (Table 1).

Bulk density was tested only for the existing cut-slope soils. It was similar between Ashland and SAC sites, averaging 81.5 lbs/ft<sup>3</sup>, which is within a typical range for cultivated clay and silt loam soils (Brady and Weil, 1999), and below a threshold that would lead to restricted root growth (USDA Natural Resources Conservation Service, 2008).

Overall, the results of soil testing found that the topsoil applied in this project only partially met initial quality expectations. As a result, the ability of this project to detect benefits from topsoil addition may be limited. This situation highlights the difficulty in acquiring topsoil from alternative sources, and emphasizes the importance of using stockpiled topsoil if a surface layer of topsoil is desired.

### *Frequency of planted and volunteer plant species*

This project hypothesized that nitrogen fertilization would not benefit planted stand establishment, and that phosphorus fertilization or seeding into a topsoil substrate would result in better stand establishment. From the perspective of plant frequency, better stand establishment would be indicated by increased frequency of planted species and/or reduced numbers of weedy species.



Species frequency data collected included a large number of species, many of which occurred rarely (see tables in Appendix 2 for frequency of occurrence (%) of plant species in response to treatments). To simplify the analysis, we selected a subset of the frequency data for analysis, focusing on the most abundant species. We identified the three most frequent grasses and forbs and the most frequent legume at each site for each data collection period. Of these species, a few were common in only a single time period or site. The remainder of the frequent species was the focus of the statistical analysis. Overall, we selected the 10 most frequent species each at SAC and Ashland for use in the frequency analysis (Table 2).

For many species, there were frequent interactions found among site, date, and treatments. Even when species responses were examined within a single site and date, there was either (1) no response to topsoil, nitrogen fertilization and/or phosphorus fertilization or (2) frequency responses to different combinations of topsoil, nitrogen fertilization, and phosphorus fertilization were not consistent (details in Appendices 2 and 3).

Despite the usefulness of measuring frequency of occurrence in other studies, and clear visual differences between topsoil and existing cut-slope soil plots, the frequency data collected in this project was generally not sensitive enough to identify clear patterns of response to the treatments (Table 3). Field observations suggested that there was better stand establishment in topsoil plots than in existing cut-slope soil plots even though the frequency of occurrence measurements did not detect differences. It is possible that plants had similar frequencies in topsoil and existing cut-slope soil plots but that plants in existing cut-slope soils were relatively small and had lower foliar cover. Therefore, we considered the frequency of occurrence data to be of limited value and that we should concentrate on the cover data for evaluating the impacts of the treatments.

### ***Cover***

This project hypothesized that nitrogen fertilization would not benefit planted species establishment, and that phosphorus fertilization and topsoil addition would result in better stand establishment. From the perspective of foliar cover, better stand establishment would be indicated by increased cover of desirable perennial plants (warm-season grasses, cool-season grasses, or forbs) and decreased cover of bare ground or litter.

As with frequency, interactions between site and treatment were common, so results were analyzed separately for the two sites, and conclusions are based on both the presence of and the pattern of significant responses to treatments.

Cover was strongly dominated by warm-season grasses, litter, and bare ground (Table 3). Total perennial foliar cover (the sum of warm-season grasses, forbs, and cool-season grasses) averaged 61% in Ashland plots and 45% in SAC plots. Depending on the pre-construction canopy cover, this may or may not have been enough to meet permit closure requirements. However, all cover data was collected in a year experiencing extreme to exceptional drought throughout much of the growing season, which likely reduced foliar cover relative to a more typical precipitation year. Warm-season grasses represented an average of 91% of the perennial foliar cover, making this the most critical functional group to evaluate in order to understand how treatments affect total perennial foliar cover. Statistical analysis of warm-season grass cover found that topsoil plots generally had significantly higher cover than existing cut-slope soil plots, but only at Ashland, and responses to fertilizer treatments were erratic. At SAC, statistical analysis found that plots with either no or intermediate levels of nitrogen fertilizer had more warm-season grass cover than plots with standard levels of nitrogen, but there were no significant responses to phosphorus or topsoil.

Because of the relatively low number of plots and the high frequency of interactions, it is critical to examine patterns and trends in the data that may not result in statistical differences (Table 3). We found that despite erratic statistical results, warm-season grass cover was an average of 14% higher in topsoil plots than in existing cut-slope soil plots at both Ashland and SAC (see the “Ave N” rows for the cut-slope soil and topsoil at

Ashland and SAC in Table 3). In addition, warm-season grass cover was higher in topsoil plots than existing cut-slope soil plots at every level of fertilization tested, suggesting that the lack of statistical differences may be more a result of low numbers of plots than lack of an effect of topsoil. These observations strongly support the initial hypothesis that topsoil addition results in better stand establishment.

Forb and cool-season grass cover was very low (averaging 3.25% overall). There were no significant responses or patterns of response by cool-season grasses or forbs to any treatment tested. Litter cover was different between sites, but was generally similar within a site regardless of treatment. At SAC, litter cover was not statistically affected by any treatments. At Ashland, the intermediate level of nitrogen (18 lbs/acre) resulted in statistically higher litter cover than no nitrogen or standard (36 lbs/acre) nitrogen treatments. However, an examination of the data finds that this is the result of a small number of plots with unusually high litter cover rather than a consistent pattern, and therefore may be driven more by random variation than treatment differences (Table 3).

Ashland had substantially greater bare ground than SAC (see the “Ave N” rows for the cut-slope soil and topsoil at Ashland in Table 3). None of the treatments at SAC resulted in statistical differences. At Ashland, statistical analysis found greater bare ground in existing cut-slope soil plots than in topsoil plots, higher bare ground in existing cut-slope soil plots receiving no nitrogen, and generally erratic results in response to phosphorus treatments. When the patterns of results were examined, it was clear that bare ground was consistently higher in existing cut-slope soil plots than in topsoil plots at both sites. In fact, the average amount of bare ground in existing cut-slope soil plots was nearly double that of topsoil plots in both sites despite substantial differences in the average amount of bare ground between sites (Table 3). This observation, taken in combination with the similar statistical results at Ashland, strongly supports the initial hypothesis that topsoil results in better stand establishment than existing cut-slope soils.

### ***Soil movement***

We expected factors that improve stand quality to reduce erosion. Therefore, we expected topsoil and phosphorus fertilization to reduce erosion, and we expected nitrogen fertilization to have no effect. For ease of understanding, we focused on three time periods: seeding to September 2010, Sept 2010 to Sept 2011, and Sept 2011 to Sept 2012.

There were no indications that phosphorus levels had any influence on soil movement, so analysis focused on the effects of topsoil addition and nitrogen fertilization. As was seen in other analysis, there were interactions between sites and treatments, so sites were analyzed separately.

At both Ashland and SAC, the effects of nitrogen fertilization on soil movement were erratic. In most of the data collection periods, there was no statistical effect of nitrogen on soil movement. There were also no clear patterns seen in examination of the data. Overall, nitrogen fertilization seemed to have little or no effect on soil movement.

Plots at Ashland tended to lose soil over time, as would be expected in a recent seeding in an erosion prone area. However, SAC plots unexpectedly tended to gain soil. Averaged across all plots, SAC plots gained a total of 0.76 in of soil between seeding and September 2012 while Ashland plots lost 0.98 in of soil (Table 4). Although we cannot empirically test it, this difference is likely the result of the substantial differences in the amount of bare ground and litter between sites. SAC averaged 6.5% bare and 31% litter while Ashland averaged 19% bare and 19% litter. The increased amount of litter and low amount of bare ground at SAC likely reduced potential for both wind and water erosion relative to Ashland. In addition, although not statistically significant, the data from Ashland consistently shows that soil loss was greater in topsoil (1.36 inch cumulative loss) plots than existing cut-slope plots (0.60 inch cumulative loss) despite lower levels of bare ground in topsoil. Topsoil tends to be less dense and less compacted than existing cut-slope soil, and therefore more

erodible. In contrast, topsoil plots at SAC gained soil at a slightly higher rate than existing cut-slope soil plots (Table 4), possibly benefiting from breakdown of existing litter or increased wind deposition due to the higher warm-season grass cover.

Overall, fertilization had little effect on soil movement, but there is a suggestion that topsoil may be more prone to movement if stand establishment is poor or litter cover is sparse, resulting in bare ground.

### ***Mycorrhizae side project***

This project hypothesized that inoculating existing cut-slope soils would improve the seeding results, and that the effect of mycorrhizae addition would be less in the presence of fertilizer. To ensure mycorrhizae plot analysis was compatible with main project plots, species frequency data focused on the same group of most common species used in the main project (Table 2). In all but one species/time period examined, there was no statistical effect of fertilizer in any plot comparisons and no interactions between treatments. Instead, there were occasional effects of mycorrhizae in the first and third years, but no effect in the second year. All instances of significant differences between mycorrhizae treated and untreated plots supported improved stand quality. Species that were found more frequently in the presence of mycorrhizae included slender wheatgrass, intermediate wheatgrass, switchgrass, Indiangrass, and upright prairie coneflower (Figure 3). The only type of vegetation that was less frequent in the presence of mycorrhizae was the conglomerate of weedy grasses, and this effect was found only in the third year.

Overall, the results partially but not entirely support the initial hypotheses. There is some evidence of beneficial effects of mycorrhizae, but the effect is neither universal nor consistent through time. Contrary to the initial hypothesis, the effects of mycorrhizae addition did not appear to depend on the presence of fertilizer. Overall, the results suggest that mycorrhizae may be beneficial to stand establishment in roadside seedings. This is especially of interest because the third year of observations was both the year in which the benefits of mycorrhizae were most frequently identified and a year in which most of the area was in extreme or exceptional drought for a substantial portion of the growing season.

## **Conclusions**

- There is no benefit to applying nitrogen or phosphorus fertilizer.
- Availability of quality topsoil could limit both the ability to apply topsoil and the results obtained.
- Warm-season grass cover is greater in topsoil treated plots than in existing cut-slope plots.
- Bare ground is greater in existing cut-slope soil than in topsoil treated plots.
- Topsoil addition could make the difference between sites that do or do not meet vegetation requirements for permit closure.
- In areas with poor stand establishment, exposed topsoil may be more erosion prone than existing cut-slope soils.
- Mycorrhizal inoculation shows promise in increasing establishment of some but not all planted species, and may be particularly beneficial in drought conditions.
- Foliar cover was a better measurement than species frequency for evaluating treatment effects in this type of project.

Overall, the results suggest no benefit in stand establishment with the use of nitrogen or phosphorus fertilizer. Instead, standard NDOR nitrogen fertilization resulted in lower warm season grass cover than no or intermediate fertilizer rates at one of the sites. Seeding into topsoil resulted in 15% higher cover of warm-season

grasses and a 50% reduction in the amount of bare ground. However, there are two important issues to consider in these conclusions. First, the topsoil obtained for at least one of the sites was likely from a soybean field (based on presence of soybean residue). These imported soils, when compared with existing cut-slope soils, performed better in some but not all of the soil fertility tests performed. It is likely that stockpiled topsoil from an area previously dominated by grasses (as is the case in most local roadsides) would have been higher quality than the topsoil we used from cultivated fields because soils that have been cultivated repeatedly tend to have reduced soil fertility compared to untilled soils. Second, our conclusions are driven almost entirely by the cover data collected in the third year. Species frequency has been a good technique in previous studies, but it was found to be insufficiently sensitive to detect differences in stand establishment that were clearly visible to the naked eye. The use of foliar cover as a measure of vegetation response is recommended for future studies. Determining foliar cover response to treatments on roadsides is particularly relevant to NDOR-funded projects because SWPPP requires seeded perennial vegetation to attain specified canopy cover levels, generally set at 70% perennial foliar cover relative to pre-project cover levels.

The mycorrhizae addition side project found that only a handful of species were significantly affected by mycorrhizae addition, and significant responses were found only in the first and third year (Figure 3). However, given the relative inability of frequency measurement to identify species responses in the main project, and the fact that the third year data was collected under extreme to exceptional drought conditions, results suggest that mycorrhizae addition may benefit stand quality and is likely worthy of further exploration.

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## Tables and Figures

**Table 1: Pre-treatment cut-slope soil and topsoil characteristics.**

|                                    | Ashland cut-slope<br>May 2010 |            | Ashland<br>topsoil | SAC cut-slope<br>November 2009 |            | SAC<br>topsoil |
|------------------------------------|-------------------------------|------------|--------------------|--------------------------------|------------|----------------|
|                                    | 0-3 inches                    | 3-6 inches |                    | 0-3 inches                     | 3-6 inches |                |
| Bulk Density (lb/ft <sup>3</sup> ) | 80.5                          |            | --                 | 82.4                           |            | --             |
| Soil organic matter (%)            | 2.3                           | 1.9        | 2.3                | 1.3                            | 1.1        | 2.3            |
| Nitrate nitrogen (ppm)             | 2.7                           | 2.5        | 2.7                | 2.0                            | 1.6        | 11.9           |
| Phosphorus (ppm)*                  | 11                            | 6.7        | 11                 | 13                             | 13         | 30             |
| Potassium (ppm)                    | 191                           | 151        | 191                | 303                            | 271        | 160            |
| Cation exchange capacity           | 16.6                          | 15.4       | 16.6               | 20.5                           | 19.7       | 18.4           |

\*Phosphorus was assessed using the Bray-Kurtz P1 test, which is well correlated with plant responses to P fertilizer use

**Table 2: Species used in frequency analysis.**

| Plant type | Ashland                    | SAC                        |
|------------|----------------------------|----------------------------|
| Grasses    | Big bluestem               | Big bluestem               |
|            | Indiangrass                | Indiangrass                |
|            | Sideoats grama             | Intermediate wheatgrass    |
|            | Switchgrass                | Slender wheatgrass         |
|            | Weedy grass                | Switchgrass                |
|            |                            | Weedy grasses              |
| Forbs      | Black-eyed Susan           | Black-eyed Susan           |
|            | Indian blanket             | Upright prairie coneflower |
|            | Upright prairie coneflower | Weedy forbs                |
|            | Weedy forbs                |                            |
| Legumes    | Illinois bundleflower      | Partridge pea              |

**Table 3: Cover data summary.**

|                |                 |                    | Bare            |                  |                  |                    | Cool-season grass |     |     |       | Forbs |     |     |       | Litter |     |     |       | Weedy forbs |     |     |       | Weedy grass |     |     |       | Warm-season grass |     |     |       |
|----------------|-----------------|--------------------|-----------------|------------------|------------------|--------------------|-------------------|-----|-----|-------|-------|-----|-----|-------|--------|-----|-----|-------|-------------|-----|-----|-------|-------------|-----|-----|-------|-------------------|-----|-----|-------|
|                |                 |                    | P0 <sup>5</sup> | P20 <sup>6</sup> | P40 <sup>7</sup> | Ave p <sup>8</sup> | P0                | P20 | P40 | Ave P | P0    | P20 | P40 | Ave P | P0     | P20 | P40 | Ave P | P0          | P20 | P40 | Ave P | P0          | P20 | P40 | Ave P | P0                | P20 | P40 | Ave P |
| Ashland        | Cut-slope soil  | N0 <sup>1</sup>    | 37              | 16               | 33               | 29                 | 1                 | 0   | 1   | 0     | 2     | 3   | 1   | 2     | 18     | 19  | 17  | 18    | 0           | 0   | 0   | 0     | 0           | 0   | 1   | 0     | 43                | 62  | 48  | 51    |
|                |                 | N18 <sup>2</sup>   | 23              | 32               | 16               | 24                 | 0                 | 0   | 0   | 0     | 1     | 3   | 4   | 3     | 25     | 17  | 31  | 24    | 0           | 1   | 0   | 1     | 0           | 0   | 0   | 0     | 50                | 47  | 49  | 49    |
|                |                 | N36 <sup>3</sup>   | 18              | 28               | 20               | 22                 | 1                 | 0   | 0   | 1     | 3     | 3   | 4   | 3     | 26     | 15  | 17  | 19    | 1           | 0   | 0   | 0     | 0           | 0   | 0   | 0     | 51                | 53  | 60  | 55    |
|                |                 | Ave N <sup>4</sup> | 26              | 25               | 23               | 25                 | 1                 | 0   | 0   | 0     | 2     | 3   | 3   | 3     | 23     | 17  | 21  | 20    | 0           | 1   | 0   | 0     | 0           | 0   | 0   | 0     | 48                | 54  | 52  | 51    |
|                | Topsoil         | Ave N              | 14              | 14               | 12               | 13                 | 0                 | 0   | 0   | 0     | 2     | 4   | 3   | 3     | 18     | 17  | 17  | 18    | 0           | 0   | 1   | 0     | 0           | 0   | 0   | 0     | 65                | 65  | 67  | 65    |
|                |                 | N0                 | 9               | 13               | 8                | 10                 | 0                 | 0   | 0   | 0     | 3     | 7   | 3   | 4     | 19     | 21  | 14  | 18    | 0           | 0   | 1   | 0     | 0           | 0   | 0   | 0     | 69                | 60  | 74  | 68    |
|                |                 | N18                | 21              | 16               | 15               | 17                 | 0                 | 0   | 0   | 0     | 2     | 2   | 3   | 2     | 25     | 18  | 19  | 21    | 0           | 0   | 0   | 0     | 0           | 0   | 0   | 0     | 53                | 63  | 63  | 60    |
|                |                 | N36                | 14              | 13               | 12               | 13                 | 0                 | 0   | 0   | 0     | 3     | 3   | 3   | 3     | 11     | 12  | 20  | 14    | 0           | 1   | 1   | 0     | 0           | 0   | 0   | 0     | 72                | 71  | 64  | 69    |
|                | Ashland overall |                    | 20              | 20               | 17               | 19                 | 0                 | 0   | 0   | 0     | 2     | 4   | 3   | 3     | 21     | 17  | 19  | 19    | 0           | 0   | 0   | 0     | 0           | 0   | 0   | 0     | 56                | 59  | 60  | 58    |
|                | SAC             | SAC overall        |                 | 6                | 5                | 8                  | 6                 | 6   | 5   | 5     | 6     | 4   | 4   | 3     | 4      | 30  | 29  | 32    | 31          | 9   | 7   | 7     | 7           | 1   | 2   | 2     | 2                 | 44  | 48  | 43    |
| Cut-slope soil |                 | N0                 | 13              | 10               | 8                | 10                 | 2                 | 2   | 2   | 2     | 4     | 2   | 4   | 3     | 28     | 30  | 35  | 31    | 8           | 15  | 11  | 12    | 1           | 1   | 1   | 1     | 43                | 39  | 40  | 41    |
|                |                 | N18                | 8               | 6                | 7                | 7                  | 6                 | 7   | 4   | 5     | 2     | 3   | 2   | 2     | 29     | 25  | 32  | 29    | 16          | 7   | 11  | 11    | 3           | 2   | 4   | 3     | 38                | 49  | 40  | 42    |
|                |                 | N36                | 6               | 6                | 13               | 8                  | 2                 | 3   | 3   | 3     | 7     | 4   | 6   | 6     | 36     | 31  | 38  | 35    | 22          | 16  | 9   | 16    | 1           | 3   | 1   | 2     | 25                | 38  | 31  | 31    |
|                |                 | Ave N              | 9               | 7                | 9                | 9                  | 3                 | 4   | 3   | 3     | 4     | 3   | 4   | 4     | 31     | 29  | 35  | 32    | 15          | 13  | 11  | 13    | 1           | 2   | 2   | 2     | 35                | 42  | 37  | 38    |
| Topsoil        |                 | Ave N              | 3               | 3                | 6                | 4                  | 9                 | 7   | 8   | 8     | 3     | 5   | 2   | 3     | 30     | 29  | 30  | 30    | 2           | 1   | 3   | 2     | 1           | 1   | 2   | 1     | 52                | 54  | 50  | 52    |
|                |                 | N0                 | 1               | 4                | 9                | 5                  | 8                 | 9   | 9   | 8     | 4     | 2   | 0   | 2     | 30     | 31  | 28  | 30    | 2           | 1   | 3   | 2     | 2           | 3   | 3   | 2     | 54                | 51  | 50  | 51    |
|                |                 | N18                | 1               | 1                | 5                | 2                  | 10                | 5   | 7   | 7     | 2     | 10  | 1   | 4     | 31     | 24  | 27  | 27    | 1           | 0   | 3   | 1     | 1           | 0   | 1   | 1     | 55                | 60  | 56  | 57    |
|                | N36             | 7                  | 4               | 5                | 5                | 8                  | 6                 | 9   | 8   | 4     | 4     | 4   | 4   | 28    | 32     | 35  | 32  | 3     | 1           | 3   | 3   | 1     | 1           | 2   | 1   | 48    | 52                | 43  | 48  |       |

Values show the average percent cover of a given functional group for each combination of treatments. Columns labeled Ave P show the average value across all phosphorus treatments. Rows labeled Ave N show average value across all nitrogen treatments.

<sup>1</sup> N0- Plots receiving 0 lbs nitrogen per acre.

<sup>2</sup> N18- Plots receiving 18 lbs nitrogen per acre.

<sup>3</sup> N36- Plots receiving 36 lbs nitrogen per acre.

<sup>4</sup> Ave N- averaged across all rates of nitrogen.

<sup>5</sup> P0- Plots receiving 0 lbs phosphorus per acre.

<sup>6</sup> P20- Plots receiving 20 lbs phosphorus per acre.

<sup>7</sup> P40- Plots receiving 40 lbs phosphorus per acre.

<sup>8</sup> Ave P- averaged across all rates of phosphorus.

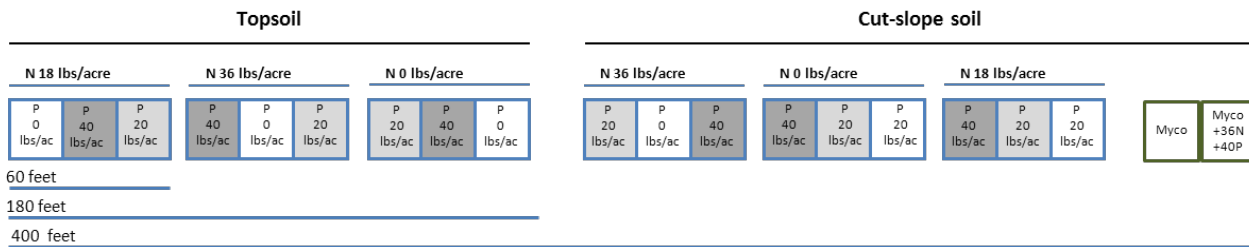
**Table 4: Soil movement.**

|                                  | Ashland        |         |         | SAC            |         |         |
|----------------------------------|----------------|---------|---------|----------------|---------|---------|
|                                  | Cut-slope soil | Topsoil | Average | Cut-slope soil | Topsoil | Average |
| Seeding to September 2010*       | 0.11           | -0.05   | 0.03    | 0.30           | 0.45    | 0.37    |
| September 2010 to September 2011 | -0.45          | -0.83   | -0.64   | 0.15           | 0.36    | 0.25    |
| September 2011 to September 2012 | -0.26          | -0.48   | -0.37   | 0.08           | 0.21    | 0.14    |
| Seeding to September 2012        | -0.60          | -1.36   | -0.98   | 0.53           | 1.03    | 0.76    |

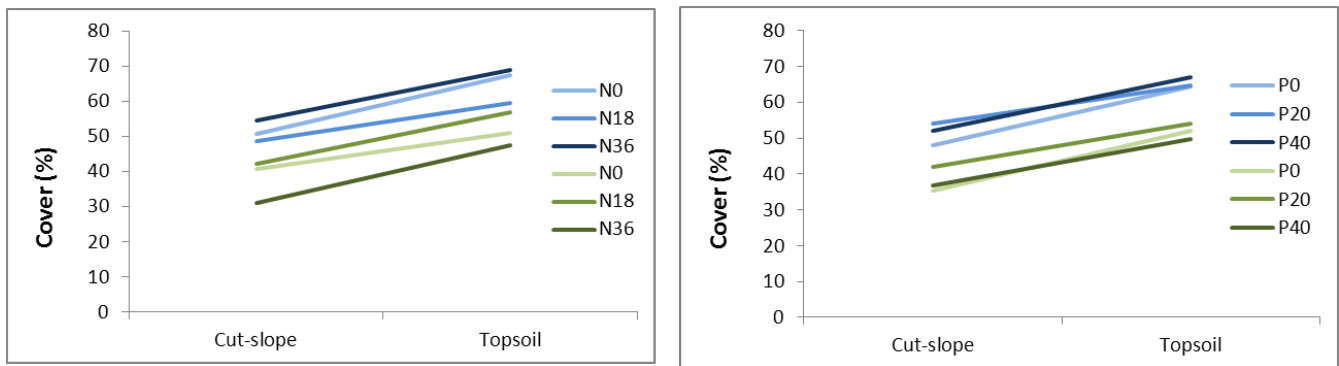
Cumulative soil movement (inches) within each period. Negative values indicate soil loss.

\*Seeding was November 2009 for SAC and June 2010 for Ashland.



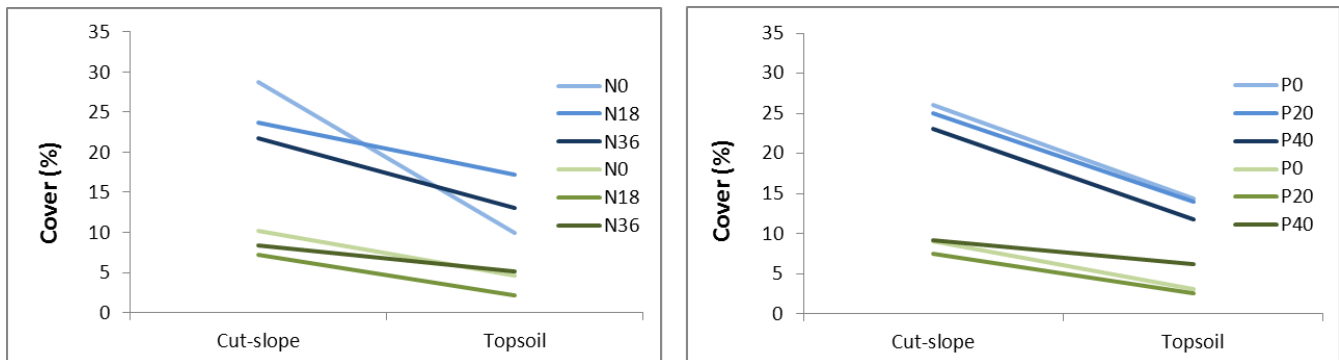


**Figure 1: Plot layout at SAC.**



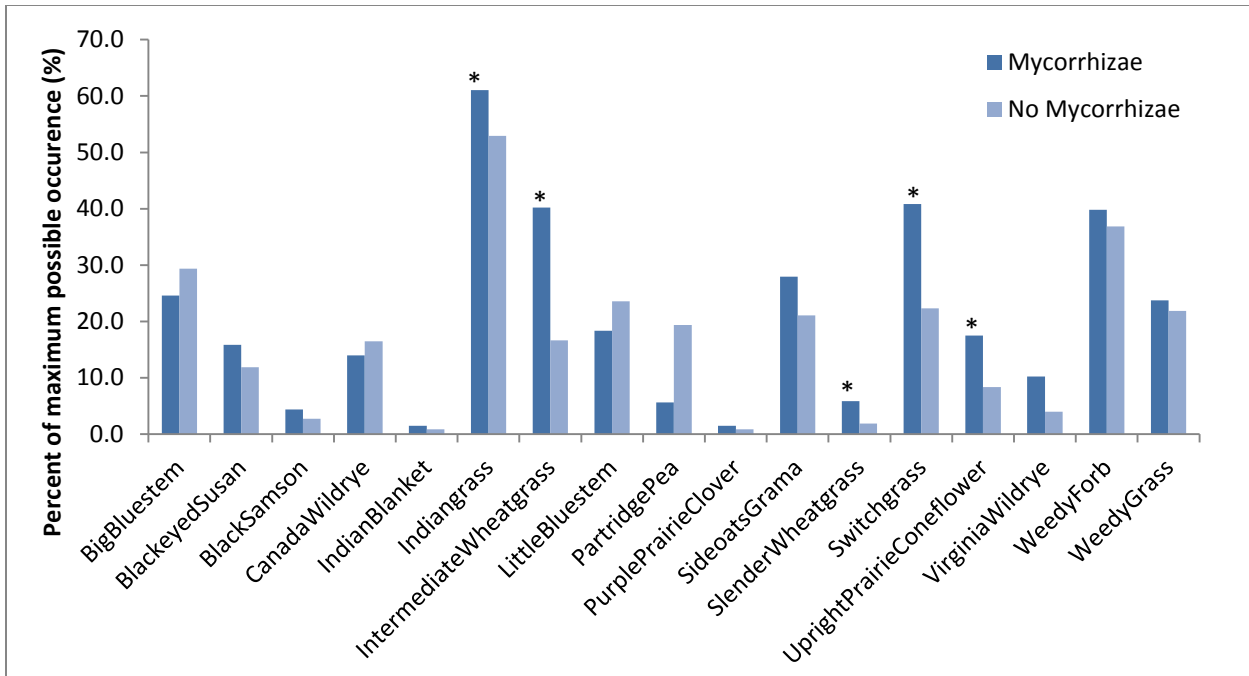
**Figure 2A and 2B: Cover of warm-season grasses in response to substrate type and fertilization.**

Warm-season grass cover in the third growing season is higher in the presence of topsoil regardless of nitrogen or phosphorus level. Results are averaged across phosphorus levels on the right and averaged across nitrogen levels on the right. Ashland results are in blue, SAC results are in green



**Figure 3A and 3B: Cover of bare ground in response to substrate type and fertilization.**

Bare ground cover in the third growing season is lower in the presence of topsoil regardless of nitrogen or phosphorus level. Results are averaged across phosphorus levels on the right and averaged across nitrogen levels on the right. Ashland results are in blue, SAC results are in green



**Figure 4: Species frequency (year 3) in the presence of mycorrhizae.**

\* indicates species with significantly higher frequency in mycorrhizae plots. No plots were significantly higher in the absence of mycorrhizae

## Appendices

### ***Appendix 1: Type A complex seeding mixture used in research plots.***

| Type "A"   | Minimum Purity | Application Rate<br>(Lbs PLS/acre)* |
|--|----------------|-------------------------------------|
| Virginia wildrye – NE, IA                                  | 85             | 6                                   |
| Canada wildrye – Mandan                                    | 85             | 4                                   |
| Slender wheatgrass   | 85             | 4                                   |
| Intermediate wheatgrass – Slate, Oahe, Mandan              | 85             | 4                                   |
| Western wheatgrass – Flintlock, Barton                     | 85             | 4                                   |
| Switchgrass – Pathfinder, Blackwell, Trailblazer           | 90             | 1.5                                 |
| Indiangrass – Oto, NE-54, Holt                             | 75             | 3                                   |
| Big bluestem – Pawnee, Roundtree                           | 60             | 3                                   |
| Sideoats grama – Butte, Trailway, El Reno                  | 75             | 3                                   |
| Little bluestem – Aldous, Blaze, Camper                    | 60             | 2                                   |
| Illinois bundleflower – inoculated                         | 90             | 0.5                                 |
| Purple prairie clover – inoculated, Kanab                  | 90             | 0.5                                 |
| Upright prairie coneflower ( <i>Ratibida columnifera</i> ) | 90             | 0.5                                 |
| Mexican red hat ( <i>Ratibida columnifera</i> , red)       | 90             | 0.75                                |
| New England aster ( <i>Aster novae-angliae</i> )           | 90             | 0.1                                 |
| Indian blanket ( <i>Gaillardia pulchella</i> )             | 90             | 1                                   |
| Black-eyed Susan ( <i>Rudbeckia hirta</i> )                | 90             | 0.5                                 |
| Black samson ( <i>Echinacea angustifolia</i> )             | 90             | 0.25                                |
| Oats/Wheat**   | 90             | 10                                  |

\* Approved mechanical drill application rate in pounds of pure live seed (PLS) per acre.

\*\* Wheat in the fall.

All seed shall be origin Nebraska, adjoining states, or as specified. A contractor proposing to use a substitute variety, or origin shall submit for the engineer's consideration a seed tag representing the seed which shows the variety, origin and analysis of the seed.

**Appendix 2: Frequency of occurrence of common species (%) in response to treatments**

**Frequency of occurrence of common species (%) in response to soil type.**

| Year                       | Species                    | Ashland        |         | SAC            |         |
|----------------------------|----------------------------|----------------|---------|----------------|---------|
|                            |                            | Cut-slope soil | Topsoil | Cut-slope soil | Topsoil |
| 2010*                      | Big bluestem               | 55.3           | 50.5    | 10.0           | 11.4    |
|                            | Black-eyed Susan           | 23.8           | 16.2    | 30.5           | 29.7    |
|                            | Illinois bundleflower      | 17.1           | 17.3    |                |         |
|                            | Indian blanket             | 25.2           | 17.3    |                |         |
|                            | Indiangrass                | 49.4           | 50.5    | 21.2           | 18.1    |
|                            | Intermediate wheatgrass    |                |         | 54.1           | 66.0    |
|                            | Partridge pea              |                |         | 31.6           | 1.0     |
|                            | Sideoats grama             | 38.7           | 39.9    |                |         |
|                            | Slender wheatgrass         |                |         | 34.0           | 50.3    |
|                            | Switchgrass                | 41.3           | 43.5    | 21.9           | 17.9    |
|                            | Upright prairie coneflower | 24.7           | 18.3    | 25.1           | 37.6    |
|                            | Weedy forb                 | 55.6           | 53.2    | 66.2           | 76.5    |
|                            | Weedy grass                | 73.8           | 70.7    | 41.9           | 57.1    |
|                            | 2011                       | Big bluestem   | 47.2    | 48.4           | 22.6    |
| Black-eyed Susan           |                            | 26.4           | 23.1    | 28.8           | 18.9    |
| Illinois bundleflower      |                            | 8.6            | 16.2    |                |         |
| Indian blanket             |                            | 19.6           | 22.2    |                |         |
| Indiangrass                |                            | 51.6           | 66.7    | 40.2           | 46.9    |
| Intermediate wheatgrass    |                            |                |         | 43.7           | 54.3    |
| Partridge pea              |                            |                |         | 8.6            | 3.0     |
| Sideoats grama             |                            | 38.0           | 40.2    |                |         |
| Slender wheatgrass         |                            |                |         | 4.7            | 10.3    |
| Switchgrass                |                            | 31.4           | 40.5    | 26.9           | 24.4    |
| Upright prairie coneflower |                            | 23.5           | 21.8    | 23.8           | 33.1    |
| Weedy forb                 |                            | 57.0           | 57.2    | 60.5           | 38.0    |
| Weedy grass                |                            | 43.4           | 43.9    | 35.3           | 43.5    |
| 2012                       |                            | Big bluestem   | 48.5    | 40.9           | 24.1    |
|                            | Black-eyed Susan           | 12.2           | 10.9    | 15.1           | 5.0     |
|                            | Illinois bundleflower      | 9.2            | 18.1    |                |         |
|                            | Indian blanket             | 4.2            | 1.7     |                |         |
|                            | Indiangrass                | 65.7           | 68.1    | 52.4           | 60.1    |
|                            | Intermediate wheatgrass    |                |         | 22.8           | 29.0    |
|                            | Partridge pea              |                |         | 23.8           | 4.4     |
|                            | Sideoats grama             | 34.0           | 40.8    |                |         |
|                            | Slender wheatgrass         |                |         | 1.3            | 1.6     |
|                            | Switchgrass                | 31.6           | 39.9    | 24.7           | 28.7    |
|                            | Upright prairie coneflower | 8.3            | 12.0    | 9.6            | 13.4    |
|                            | Weedy forb                 | 20.1           | 23.8    | 43.2           | 25.2    |
|                            | Weedy grass                | 2.4            | 4.0     | 36.9           | 16.4    |

Percent frequency is the average frequency across frame sizes.

\* Ashland was planted in June of 2010, so 2010 has only one observation date. All other observations are the average of the June and September observations.

## Frequency of occurrence of common species (%) in response to nitrogen.

| Year  | Species                    | Ashland         |                  |                  | SAC             |                  |                  |
|-------|----------------------------|-----------------|------------------|------------------|-----------------|------------------|------------------|
|       |                            | 0 lbs<br>N/acre | 18 lbs<br>N/acre | 36 lbs<br>N/acre | 0 lbs<br>N/acre | 18 lbs<br>N/acre | 36 lbs<br>N/acre |
| 2010* | Big bluestem               | 55.7            | 49.2             | 53.8             | 12.0            | 7.8              | 12.3             |
|       | Black-eyed Susan           | 17.9            | 17.1             | 24.6             | 28.3            | 29.6             | 32.4             |
|       | Illinois bundleflower      | 21.2            | 14.9             | 15.8             |                 |                  |                  |
|       | Indian blanket             | 21.6            | 21.4             | 20.6             |                 |                  |                  |
|       | Indiangrass                | 52.1            | 51.5             | 46.4             | 21.9            | 18.8             | 18.3             |
|       | Intermediate wheatgrass    |                 |                  |                  | 56.9            | 63.0             | 60.2             |
|       | Partridge pea              |                 |                  |                  | 16.9            | 15.7             | 16.3             |
|       | Sideoats grama             | 40.4            | 33.3             | 44.2             |                 |                  |                  |
|       | Slender wheatgrass         |                 |                  |                  | 41.3            | 43.0             | 42.3             |
|       | Switchgrass                | 40.9            | 42.9             | 43.5             | 21.3            | 21.1             | 17.4             |
|       | Upright prairie coneflower | 22.5            | 19.2             | 22.8             | 35.1            | 27.2             | 31.9             |
|       | Weedy forb                 | 50.6            | 55.4             | 56.9             | 72.6            | 71.3             | 70.1             |
|       | Weedy grass                | 72.4            | 74.6             | 69.7             | 51.0            | 49.1             | 48.3             |
| 2011  | Big bluestem               | 48.6            | 46.0             | 51.5             | 29.6            | 27.4             | 21.0             |
|       | Black-eyed Susan           | 27.8            | 26.9             | 21.2             | 23.3            | 27.3             | 20.9             |
|       | Illinois bundleflower      | 13.5            | 11.9             | 12.6             |                 |                  |                  |
|       | Indian blanket             | 23.5            | 22.4             | 18.1             |                 |                  |                  |
|       | Indiangrass                | 63.5            | 57.3             | 60.2             | 44.2            | 44.8             | 41.7             |
|       | Intermediate wheatgrass    |                 |                  |                  | 45.3            | 59.5             | 42.2             |
|       | Partridge pea              |                 |                  |                  | 4.7             | 6.6              | 6.0              |
|       | Sideoats grama             | 41.8            | 37.2             | 40.6             |                 |                  |                  |
|       | Slender wheatgrass         |                 |                  |                  | 6.7             | 5.3              | 10.4             |
|       | Switchgrass                | 44.9            | 31.0             | 34.5             | 20.8            | 30.9             | 25.2             |
|       | Upright prairie coneflower | 27.2            | 21.6             | 20.6             | 28.4            | 29.0             | 27.8             |
|       | Weedy forb                 | 62.1            | 56.1             | 56.5             | 45.3            | 55.6             | 46.7             |
|       | Weedy grass                | 43.9            | 42.7             | 46.8             | 35.5            | 45.8             | 36.8             |
| 2012  | Big bluestem               | 47.1            | 43.1             | 46.6             | 31.3            | 30.1             | 27.5             |
|       | Black-eyed Susan           | 12.9            | 13.3             | 9.1              | 12.8            | 9.5              | 7.8              |
|       | Illinois bundleflower      | 16.3            | 12.9             | 12.6             |                 |                  |                  |
|       | Indian blanket             | 3.9             | 3.5              | 3.3              |                 |                  |                  |
|       | Indiangrass                | 73.4            | 68.7             | 62.6             | 58.1            | 56.4             | 54.2             |
|       | Intermediate wheatgrass    |                 |                  |                  | 23.9            | 28.8             | 25.1             |
|       | Partridge pea              |                 |                  |                  | 11.7            | 15.9             | 14.9             |
|       | Sideoats grama             | 41.5            | 33.3             | 39.7             |                 |                  |                  |
|       | Slender wheatgrass         |                 |                  |                  | 3.1             | 1.4              | 2.9              |
|       | Switchgrass                | 32.9            | 36.5             | 39.7             | 21.5            | 31.3             | 27.3             |
|       | Upright prairie coneflower | 12.3            | 9.0              | 9.9              | 10.8            | 11.9             | 11.8             |
|       | Weedy forb                 | 22.1            | 23.7             | 21.3             | 34.7            | 34.4             | 33.6             |
|       | Weedy grass                | 3.2             | 3.3              | 3.2              | 25.8            | 26.8             | 27.4             |

Percent frequency is the average frequency across frame sizes.

\* Ashland was planted in June of 2010, so 2010 has only one observation date. All other observations are the average of the June and September observations.

## Frequency of occurrence of common species (%) in response to phosphorus.

| Year  | Species                    | Ashland         |                  |                  | SAC             |                  |                  |
|-------|----------------------------|-----------------|------------------|------------------|-----------------|------------------|------------------|
|       |                            | 0 lbs<br>P/acre | 20 lbs<br>P/acre | 40 lbs<br>P/acre | 0 lbs<br>P/acre | 20 lbs<br>P/acre | 40 lbs<br>P/acre |
| 2010* | Big bluestem               | 50.1            | 53.3             | 55.1             | 11.4            | 8.1              | 12.6             |
|       | Black-eyed Susan           | 18.1            | 23.9             | 17.6             | 31.4            | 27.4             | 31.5             |
|       | Illinois bundleflower      | 14.6            | 18.1             | 19.1             |                 |                  |                  |
|       | Indian blanket             | 22.8            | 19.0             | 21.8             |                 |                  |                  |
|       | Indiangrass                | 48.1            | 49.6             | 52.4             | 19.9            | 20.7             | 18.3             |
|       | Intermediate wheatgrass    |                 |                  |                  | 62.0            | 60.6             | 57.5             |
|       | Partridge pea              |                 |                  |                  | 16.7            | 16.0             | 16.1             |
|       | Sideoats grama             | 41.4            | 40.1             | 34.2             |                 |                  |                  |
|       | Slender wheatgrass         |                 |                  |                  | 42.0            | 43.9             | 40.6             |
|       | Switchgrass                | 41.9            | 41.4             | 41.7             | 20.8            | 20.8             | 18.1             |
|       | Upright prairie coneflower | 19.3            | 23.8             | 20.1             | 35.7            | 27.1             | 31.4             |
|       | Weedy forb                 | 54.4            | 54.2             | 51.5             | 69.9            | 72.8             | 71.2             |
|       | Weedy grass                | 75.6            | 71.5             | 65.6             | 49.8            | 48.6             | 50.1             |
| 2011  | Big bluestem               | 48.7            | 51.5             | 43.3             | 23.1            | 27.3             | 27.7             |
|       | Black-eyed Susan           | 24.5            | 25.1             | 24.7             | 26.9            | 21.8             | 22.7             |
|       | Illinois bundleflower      | 11.5            | 13.9             | 11.9             |                 |                  |                  |
|       | Indian blanket             | 20.1            | 21.3             | 21.3             |                 |                  |                  |
|       | Indiangrass                | 58.8            | 61.0             | 57.6             | 40.0            | 44.2             | 46.5             |
|       | Intermediate wheatgrass    |                 |                  |                  | 52.0            | 43.0             | 51.9             |
|       | Partridge pea              |                 |                  |                  | 4.6             | 7.0              | 5.7              |
|       | Sideoats grama             | 38.8            | 37.9             | 40.6             |                 |                  |                  |
|       | Slender wheatgrass         |                 |                  |                  | 7.4             | 7.5              | 7.6              |
|       | Switchgrass                | 37.9            | 33.3             | 36.6             | 27.3            | 25.6             | 24.1             |
|       | Upright prairie coneflower | 22.4            | 24.4             | 21.0             | 32.6            | 28.9             | 23.7             |
|       | Weedy forb                 | 57.7            | 57.4             | 56.1             | 46.0            | 50.3             | 51.3             |
|       | Weedy grass                | 45.3            | 43.0             | 42.7             | 43.1            | 33.7             | 41.3             |
| 2012  | Big bluestem               | 43.6            | 43.3             | 47.2             | 31.7            | 28.8             | 28.3             |
|       | Black-eyed Susan           | 11.3            | 12.6             | 10.8             | 10.7            | 8.1              | 11.3             |
|       | Illinois bundleflower      | 11.3            | 16.3             | 13.3             |                 |                  |                  |
|       | Indian blanket             | 3.1             | 3.1              | 2.6              |                 |                  |                  |
|       | Indiangrass                | 67.7            | 67.9             | 65.0             | 56.7            | 55.3             | 56.7             |
|       | Intermediate wheatgrass    |                 |                  |                  | 25.6            | 20.7             | 31.5             |
|       | Partridge pea              |                 |                  |                  | 9.4             | 15.3             | 17.7             |
|       | Sideoats grama             | 38.5            | 40.8             | 32.9             |                 |                  |                  |
|       | Slender wheatgrass         |                 |                  |                  | 1.4             | 1.7              | 1.3              |
|       | Switchgrass                | 37.8            | 37.0             | 32.5             | 26.6            | 24.8             | 28.8             |
|       | Upright prairie coneflower | 10.1            | 10.5             | 9.9              | 11.4            | 10.8             | 12.4             |
|       | Weedy forb                 | 23.1            | 19.4             | 23.4             | 35.3            | 29.7             | 37.6             |
|       | Weedy grass                | 4.0             | 2.4              | 3.2              | 22.6            | 23.6             | 33.8             |

Percent frequency is the average frequency across frame sizes.

\* Ashland was planted in June of 2010, so 2010 has only one observation date. All other observations are the average of the June and September observations.

### ***Appendix 3: Summary of frequency results***

Species frequency did not respond predictably to any of the treatments. Frequency of any given species was different between sites, between data collection periods, and in response to treatments. In general, most species either did not respond to treatments, or responses to treatment were part of complex 2-way or 3-way interactions. Most significant differences between treatments were seen only when examining the smallest and most specific treatment comparisons, i.e., comparing the effects of 0, 20, or 40 lbs phosphorus on the June 2011 frequency of Indiangrass in topsoil plots at Ashland that received no nitrogen fertilizer. Under these circumstances, generalizing about species responses to treatments becomes problematic.

Species frequency rarely responded to treatments at either site, but species were more likely to respond to treatments at SAC than at Ashland. In addition, there was no consistency in treatment responses within a functional group (grasses, forbs, legumes, etc).

#### **First year (2010) frequency.**

Overall, there were few significant effects of treatment on frequency results, and all instances of significant response to treatment occurred as part of a 2-way or 3-way interaction.

At Ashland, Indiangrass in September 2010 had higher frequency in plots with 40 lbs/acre phosphorus than with 20 lbs/acre ( $p = 0.05$ ). Neither soil nor nitrogen had any significant impact on any species at Ashland, and phosphorus did not significantly affect any species besides Indiangrass.

At SAC, significant responses to soil type (5 species in June 2010, 3 species in September 2010) and nitrogen were widespread (4 species in June 2010, 5 species in September 2010), but almost all occurred within 3-way interactions. All instances of significant differences between soils found species frequency in topsoil plots was greater than in existing cut-slope soils. There was no discernible pattern in responses to nitrogen fertilizer. No rate of nitrogen addition was consistently higher or lower than others. No functional group was more or less likely to respond to nitrogen, and there was no level of phosphorus or type of soil in which a significant response to nitrogen was more likely. Responses to phosphorus were less common (3 species in June 2010, 2 species in September 2010), and there were no discernible patterns found among the responses.

Overall, there were few clear patterns of response to the treatments. When species responded to soil type, topsoil was clearly favored over existing cut-slope soils, but these differences were present for fewer than half of the species.

#### **Second year (2011) frequency**

In Ashland, there were few significant effects of treatments, and all were involved in interactions. There were two instances of significantly higher species frequency in topsoil plots (switchgrass in June 2011, Indiangrass in September 2011), one instance of responses to nitrogen (sideoats grama in September 2011, interaction resulted in conflicting patterns), and two instances of responses to phosphorus (Indiangrass and sideoats grama, both in September 2011, interactions resulted in conflicting patterns).

In SAC, there were more frequent significant responses, but a similar pattern to that seen at Ashland. There were five instances of significant responses to soil (4 species in June 2011, 1 species in

September 2011). All responses were part of interactions and in all but one instance, species frequency was greater in topsoil plots. There were six instances of significant response to nitrogen (5 species in June 2011, 1 species in September 2011), and in most cases, plots with higher level of nitrogen had higher species frequency than plots with lower nitrogen levels. All cases in which higher nitrogen resulted in lower species frequency were found on existing cut-slope soils. This suggests that while responses to nitrogen were sometimes found, added nitrogen may have been more beneficial when applied to topsoil treated plots than when applied to existing cut-slope soil. Responses to phosphorus were both less frequent and more ambiguous than nitrogen. Three species responded to phosphorus in June 2011 and 2 species in September 2011. In June 2011, species responses to phosphorus are conflicting, with some species having greater species frequency with no phosphorus and others having greater species frequency with 40 lbs/acre phosphorus. In September 2011, plots receiving 40 lbs/acre phosphorus had greater species frequency than plots receiving no phosphorus.

Overall, responses to soils are consistent with the second year. Although soil type is the least likely of the treatments to result in a significant difference, species frequency is nearly always higher in topsoil plots than in standard soils when significant differences are identified. In contrast, both nitrogen and phosphorus responses can vary, and results suggest that differences in soils could lead to differences in response to fertilization.

### **Third year (2012) frequency**

Overall, there were fewer significant effects of treatments in the third year, but significant differences continue to be most commonly found within 2-way and 3-way interactions. There was a substantial decrease in responses to treatments in the September 2012 data collection period, likely related to the extreme drought in the region during that time.

At Ashland, the number of species responding to soil type increased slightly from previous years. Three species in June and two species in July responded significantly to soil type, and topsoil plots had greater species frequency than existing cut-slope soils. Nitrogen responses were slightly more common than year two (4 species in June 2012, two species in September 2012), but as seen previously, results were conflicting and no rate of nitrogen fertilization clearly favored species frequency. Phosphorus effects were even more reduced in year three than nitrogen. Only two species responded to phosphorus in June, and none in September 2012. However despite the small number of involved species, interactions between treatments still resulted in conflicting results both within and between species.

At SAC, three species responded significantly to soil type in June 2012, 1 species responded in September 2012). All species with significant responses to soil type in June 2012 had higher species frequency in topsoil plots than in existing cut-slope soil plots. In contrast, the only species that responded to soil type in September 2012 demonstrated greater species frequency in existing cut-slope soil plots than topsoil plots. However, that “species” was weedy grass, a non-desirable group of annual grasses. There were 5 species at SAC that responded positively to nitrogen (4 species in June 2012, 1 species September 2012). As in other years, results are conflicting and there is no clear pattern showing higher species frequency in response to any rate of nitrogen fertilization. Five species responded to phosphorus rates in June 2012, and 1 species in September 2012. As in other years, results are conflicting and there is no clear pattern showing higher species frequency in response to any rate of



phosphorus fertilization.

Analysis using main effects identified two instances where soil had a significant effect (topsoil higher), two instances where nitrogen had an overall effect (conflicting results), and no instances where phosphorus had an overall effect. Overall, none of the factors tested have a strong overall effect on the third year sum of frequency.

Phosphorus was the least likely of the treatments to have a significant effect. Phosphorus effects were never found in the main effects analysis - there were only significant effects of phosphorus in simple effects. When phosphorus did have a significant effect, it was most often P92 having higher sum of frequency than P0 or P46. When P92 was found to be less than P0 or P46, it was most likely to be in N36 plots.

Nitrogen levels affected frequency of a few species, but did not do so consistently. None of the nitrogen levels were consistently higher than the others, and there was no clear pattern of response to interactions with soil or phosphorus. Nitrogen was the factor most likely to be involved in interactions. Soil type\*N was the most common interaction, accounting for 4 of 6 interactions in Ashland (out of 19 possible) and 2 of 5 interactions (out of 20 possible) in SAC. There were only two instances where the main effects of nitrogen were significant, but the results are exact opposites.

Soil type had relatively little impact on frequency of each species. When there were significant impacts of soil types, topsoil tended to result in higher frequency than existing cut-slope soils (2 of 2 main effects, 4 of 5 simple effects).