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RESEARCH ARTICLE

# The Use of Commercial Bacterial Soil Inoculant Regime in an Urban Prairie Restoration

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**ABSTRACT**: For successful grassland restoration, commercial soil inoculants are often recommended to increase establishment success. In spring 2009, a 0.94-ha tract was targeted for restoration at Phil Hardberger Park, a 126-ha park in the heart of San Antonio, Texas. Woody species, mainly Texas persimmon (*Diospyros texana* Scheele), and Ashe juniper (*Juniperus asheii* Buchholz), were removed and the area was divided into 10 subplots measuring 911 $m^2$ on average. In September 2009, over 40,000 plugs of seven native grass species were planted. In addition, native prairie seed mixes, including various grass and forb seeds, were sown into the site at a rate of 11.26 kg/ha. Half of the native grass plants were treated with a soil bacteria inoculant plus additional nutrients (IN) (BioGensis IIITM DS Tainio Technology and Technique Inc.), and half were left as controls (C). Soil samples from the plots were taken in February 2010 and 2011 and analyzed for soil nutrients, bacteria, protozoa, and fungi. Vegetation data were collected October 2010 and May and October 2011 to assess differences in percent cover between the treatments. The IN treatment resulted in significantly higher percent cover in the second growing season of three native grasses, *Eriochloa sericea* (Scheele) Munro ex Vasey, *Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths, and *B. curtipendula* (Michx.) Torr; however, no significant differences were found between the IN and C plots for measures of overall native species abundance, soil resources, or the presence of soil microbes. We concluded that commercial soil inoculants may not have been necessary for the successful establishment of a native grassland community.

*Index terms:* inoculants, plant growth promoting bacteria, savanna restoration, soil microbes

## INTRODUCTION

Restoration ecologists often establish native species on disturbed or degraded sites without enhancing the soil organic matter and microbial activity (Potthoff et al. 2005). Addition of fertilizers, inocula, and conditioners to soil has been widely employed in agricultural settings but little has been done to study the manipulation or addition of soil inocula to improve restoration success (Henegham et al. 2008; Ohsowski et al. 2012). Recent studies show that amending soils with free-living bacteria can benefit plant diversity and productivity, and accelerate successional processes by tipping the competitive balance in favor of less dominant species (de Deyn et al. 2004; Middleton and Bever 2012).

Soil microbes and inoculants can increase biological activity, resulting in more productive and fertile systems (Potthoff et al. 2005). Indeed, there is evidence for a positive correlation between nutrients levels in microbial biomass, especially active nitrogen (N), and soil nutrient availability (Carter and MacLeod 1987; Dalal and Mayer 1987; Jenkinson and Parry 1989; Smith 1993; Saini et al. 2004). Nonetheless, increasing availability of soil nutrients may not have the intended effect of increasing the productivity of desirable species under the conditions where invasive species are present. Increasing N availability can favor early successional species while the opposite generally holds true for late successional species (Parrish and Bazzaz 1982; Heil and Bruggink 1987; McLendon and Redente 1992; Belnap and Sharpe 1993). Furthermore, high levels of soil available nutrients can slow succession progresses, again favoring aggressive ruderal species (McLendon and Redente 1992; Vasquez et al. 2008; James et al. 2011). Soil microbial biomass has also been shown to decline concomitant with declines in host plant species, suggesting that successful restoration may be contingent on restoration of mutualisms between the plant species and their associated microbes (Perry et al. 1989; Perry and Amaranthus 1990; Whisenant 1999; Renker et al. 2004; Harris et al. 2005; Young et al. 2005; Standish et al. 2007).

A number of studies have demonstrated that inocula of free-living soil bacteria and microorganisms can accelerate plant growth and enhance establishment success in restoration (Bever and Schultz 2003). Some of the most commonly used free-living bacterial soil inoculants in agriculture and restoration are species of the *Azospirillum* genus (Naiman et al. 2009). These bacteria are considered plant growth promoting bacteria (PGPB) as they have been shown to improve root growth and water and mineral uptake through production of phytohormones (Dobbelaere et al. 2001; Bashan et al. 2004). In agriculture, rice and soybeans inoculated with *Azospirillum brasilense* Tarrand have higher concentrations of micronutrients in their

tissues (Naiman et al. 2009). In a restoration study in degraded Sonoran Desert habitat, three species of cacti (*Pachycereus pringlei* Cardon, *Stenocereus thurberi* Engelm., and *Lophocereus schottii* (Engelm.) Hunt) had higher rates of establishment and survival following transplantation when first inoculated with *A. brasilense* (Bashan et al. 2004). Finally, strains of a native bacteria in Australia showed an increase of *Acacia* seedling establishment by 118% (Thrall et al. 2005; Middleton and Bever 2012).

*Pseudomonas*, another PGPB, can produce phosphatase that can aide in solubilizing phosphorus and other nutrients (de Freitas et al. 1997; Rodriguez et al. 2006; Naiman et al. 2009). Some strains of *Pseudomonas* have been linked to producing cytokinins (Garcia de Salamone et al. 2007; Naiman et al. 2009), a plant hormone that can promote cell division, shoot initiation and development, bud formation, and help with a plant's response to pathogens. A study conducted by Wang et al. (1996) found that *Pseudomonas fluorescens* Flugge was effective at controlling diseases in cotton seedlings (Wang et al. 2004). This could be a result of *P. fluorescens* binding to plant roots and competing with pathogens (Schroth and Hancock 1982; Weller 1988). Free-living bacteria can also be added as seed inocula. In a study conducted by Shrivastava et al. (2000), addition of *Azotobacter* to *Brassica juncea* (Linnaeus) Czern. seeds increased yield by as much as 11% (Shrivastava et al. 1989; Shrivastava et al. 2000; Saini et al. 2004). Finally, there is evidence that suggests native plant diversity within the prairie ecosystem can be increased by the addition of arbuscular mycorrhizal (AM) fungi (Bever and Schultz 2003). AM fungi can increase growth and can give native species a competitive advantage, resulting in accelerated restoration (Smith et al. 1998; Bever and Schultz 2003).

In Texas, a number of local companies recommend the use of free-living, bacterial soil inoculants to improve grassland species establishment. The claim is that these inoculants improve soil microbial status and favor native species. The goal of this study was to assess the effects of the addition of a commercially available, free-living bacteria soil inoculant regimen on the success of grassland restoration (defined here as an increase in native vegetation cover) in a savannoid ecosystem in central Texas. We also aimed to assess the effect of soil inoculant on soil nutrient status and soil foodweb composition and abundance. The experiment was conducted as part of a City of San Antonio restoration project in an inner-city park, and was also aimed to determine if future grassland restoration efforts should include soil inoculant treatments.

## METHODS

The City of San Antonio owns approximately 1725 ha of natural areas, which are managed by its Natural Areas program. It is the goal of Natural Areas staff to restore appropriate areas to native grassland, determine best practices for restoring grassland, and explore possible agents that could facilitate this restoration process. This study was conducted at Phil Hardberger Park, a city-owned property dominated by mixed oak-elm-juniper vegetation in a savannoid ecosystem. Based on recommendations of a local soil inoculant specialist (Wendy Leonard, pers. comm. with G. Freeborg, biologist, BioDiversity, Inc.), we hypothesized that the use of a commercial inoculant would facilitate restoration of grasses and forbs by increasing available soil nutrients.

In the spring and summer of 2009, City of San Antonio staff used chainsaws to remove woody vegetation from a 0.94-ha tract targeted for grassland restoration. The wood was chipped and removed from the site. The area was then planted with approximately 40,000 native grass plugs including big bluestem (*Andropogon gerardii* Vitman), little bluestem (*Schizachyrium scoparium* (Michx.) Nash.), Indiangrass (*Sorghastrum nutans* (L.) Nash.), sideoats grama (*Bouteloua curtipendula* (Michx.) Torr.), Upland switch grass (*Panicum virgatum* L.), Eastern gama grass (*Tripsacum dactyloides* (L.) L.), and Inland seaoats (*Chasmanthium latifolium* (Michx.) Yates) in September 2009.

All species planted as plugs were established in recycled "Pine cell" tubes (Stuewe & Sons) that measured 16 cm deep, 2.5 cm in diameter, and 66 ml in volume. Osmocote was added as a plus treatment to all plants while in the greenhouse as part of their standard operating procedures for plants grown in small containers. Per the recommendation of our local consultants (Wendy Leonard, pers. comm. with G. Freeborg, biologist, BioDiversity, Inc.), half of these plugs were inoculated with microbes and treated with nutrients (IN). The other half served as controls (C). The IN seeds were treated with a "slurry" seed coating material containing five strains of *Arthrobacter*, *Azosprillum lipoferum* Beijerinck, two strains of *Azobacter*, five strains of *Bacillus*, two strains of *Bacteroides*, *Kurtha zopfil* (Tainio Technology and Technique, Inc.), and three strains of *Pseudomonas,* at an application rate of 1 g of slurry to 1600 g of seed. This soil inoculant recipe is sold under the trade name BioGensis IIITM SD and was developed by Tainio Technology and Technique, Inc. It is recommended as a promoter of healthy seedling emergence and vigor.

Per the consultant recommendation, once established (at one month), IN grasses were sprayed with a foliar spray containing 1.56% Ammoniacal nitrogen, 0.18% Nitrate nitrogen, 0.26% Urea nitrogen, 13% phosphoric acid, and 2% soluble potash (Pepzyme G 1A) at a rate of 60 ml per plant (Tainio Technology and Technique, Inc.). Pepzyme G 1A was recommended as a soil microbe stimulant.

The site was divided in half by a 3-m-wide mulched path to designate A and B plots. All IN treatments were assigned to B (northern) plots, and C treatments were assigned to all A (southern) plots. Each of the C and IN plots was further divided into five subplots separated by a 3-m-wide mulched path. We were unable to randomize the treatment assignments between A and B subplots within a block because the planting of the 40,000 plugs was accomplished through a San Antonio community volunteer effort on a single Saturday morning. In order to maintain the treatment assignments, all plugs grown under IN treatment were placed on the northern portion of the site and vice versa for the C treatments. This ensured that there was no confusion in the midst of unloading

container trays and managing more than 400 volunteers. Subplots measured 911 $m^2$ on average. Grass plugs were sown randomly (by species) across all plots at a density of $4/m^2$. To facilitate plug planting, an Echo® Gas Drill (Forestry Supply) with a 2.5-cm steel auger drill bit (Irwin Tools) was used to make 2.5-cm diameter holes 16 cm into the ground. The site was also seeded with a native prairie mix (Native American Seed) at an average rate of 0.001 kg of seed per $m^2$ both before and after the planting of these grasses. To increase the potential for germination success over winter and the following spring, all plots were mowed in December 2010.

In February of 2010 and 2011, 10 soil samples were collected from each subplot and composited into a single sample. Collections were made to 15 cm with a stainless steel hand trowel. After each collection from each plot, hand trowels were rinsed with water and soaked in warm bleach bath for approximately five minutes. Hand trowels were then removed and dried using paper towels. From the composite sample, two samples of 470 ml of soil were placed into zip lock bags. One sample was sent to Soil Food Web (Oregon, LLC) where they were run through a Full Foodweb Analysis to assess the presence and abundance of soil bacteria, fungi, and protozoa. For active bacteria and fungi, samples were prepared and stained with fluorescein diacetate and quantified using microscopy (Soderstrom 1977; Schnurer and Roswall 1982; Ingham and Klein 1984a; Ingham and Klein 1984b; Stamatiadis et al. 1990). To analyze total bacteria, the laboratory prepared samples by using the fluorescein isothiocyanate method and direct enumeration of samples was completed by microscopy (Babiuk and Paul 1970; Van Veen and Paul 1979; Ingham and Horton 1987; Ingham 1994). Total fungi was prepared by using microscopy, and the width and length was measured and converted to biomass (Van Veen and Paul 1979; Lodge and Ingham 1991; Ingham 1995). Protozoa including ciliates, flagellates, and amoeba were estimated by direct counting of the sample using microscopy. The second soil sample was sent to Soil Testing and Consulting Services at Crop Services International (CSI) who tested for nitrate ($NO_3$-) and ammonium ($NH_4$+) through a Cation Exchange (CEC) and LaMotte soil test. The CEC test is an extraction test that measures the nutrient holding capacity of soil. The LaMotte test is a mild extraction test that is used to approximate the extractability of these nutrients by plant root extrudates. A Mehlich III Extractable Elements procedure was used for these two tests (Logan Lab) (Mehlich 1984).

In October 2010 and May and October 2011, trained citizen scientist volunteers completed vegetation surveys. In each subplot, along a randomly placed 50-m transect, vegetation data were collected from 1 × 1 m quadrats every 5 m. Transects ran the long orientation of the plots and the location of the quadrat on either side of the transect was chosen randomly. Percent cover of woody vegetation, herbaceous vegetation, individual species, bare ground, and litter was assessed.

A nonparametric Wilcoxon rank-sum test was used to test the differences in the central tendencies of the two main groups (IN and C) in terms of medians at each of the time intervals for overall herbaceous cover, the overall bare ground cover, woody species, litter, and the percent coverage of the individual species. The Wilcoxon rank-sum test was also used to test the differences in the central tendencies of the two main groups (IN and C) in terms of medians at each of the two time intervals for the number of soil microbes and nitrogen content between the IN and C plots. A nonparametric Wilcoxon each pair test was used to correct for multiple comparisons.

## RESULTS

We found no significant differences between C and IN treatments for the following measures: bare ground total cover, total herbaceous cover, woody species cover, and total litter cover (Table 1). Likewise, percent cover for most species was not enhanced by IN treatment, but we did find significant positive effects for Texas cupgrass (*Eriochloa sericea* (Scheele) Munro ex Vasey), bluegrama (*Boutouloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths), and sideoats grama (*B. curtipendula* (Michx.) Torr.) in October 2011, the last time interval measured (Table 1). Our soil food web analysis demonstrated no effect of inocula on the number of soil microbes (Table 2). This was true for all species of bacteria, fungi, and protozoa.

Measures of soil $NO_3$- and $NH_4$+ were significantly lower in the second year of data collection, while there was no significant effect of treatment on soil nutrients (Figure 1). In the first year of data collection it appeared that IN treatment had an overall net negative effect on herbaceous plant cover. Nonetheless, by the third data collection date (~year 1.5), total herbaceous cover declined and was equivalent between treatments (Figure 2). This is supported by the data on bare ground, which increased over the three data collection intervals (Figure 3) with no significant differences between treatments. Total year to date precipitation for 31 October substantially decreased from 2010 (92.7 cm) to 2011 (32.8 cm), according to data available by Weather Underground from a location near the site (http://www.wunderground.com).

## DISCUSSION

Our aim was to test the hypothesis that the addition of a commercially available, free-living bacteria soil inoculant regimen would improve the success of grassland restoration in a central Texas savannoid ecosystem. We found that a commercial soil inoculant regime did not alter the overall productivity of our system or the native species' establishment, for either grasses or forbs. The inoculant treatment also did not change the soil nutrient or food web composition. Our only significant result was in a higher abundance of three native grass species, *Eriochloa sericea* (Texas cupgrass), *Bouteloua gracilis* (bluegrama), and *B. curtipendula* (sideoats grama), all of which were planted by seed except *B. curtipendula,* which was both seeded and planted via plugs.

The goal of this and other grassland restoration projects within the city-owned Natural Areas is to increase diversity indices and vegetation structures where appropriate (Ohsowski et al. 2012; Ruiz-Jaen and Aide 2005). Many sites within these natural

**Table 1.** ***P*** **values for the differences in herbaceous cover, bare ground total cover, woody species cover, total litter cover, and individual species cover over three time intervals, October 2010, May 2011, and October 2011, between plots inoculated with soil microbes plus nutrients (IN) and plots not inoculated with soil microbes (C) in a grassland restoration project at Phil Hardberger Park in San Antonio, Texas. Included are** ***P*** **values, means (indicated by C or IN for each time interval), and standard deviations (SD). * indicates significant differences at the 0.05 level.**

| | Oct-10 | | | May-11 | | | Oct-11 | | |
|---|---|---|---|---|---|---|---|---|---|
| **Variable** | ***P* value** | **C±SD** | **IN±SD** | ***P* value** | **C±SD** | **IN±SD** | ***P* value** | **C±SD** | **IN±SD** |
| **Ground cover** | | | | | | | | | |
| Herbaceous cover | 0.6071 | 76.4 ± 4.1 | 75.4 ± 1.8 | 0.2738 | 53.1 ± 6.7 | 56.2 ± 6.0 | 0.2738 | 55.6 ± 14.9 | 53.1 ± 7.1 |
| Bare ground total | 0.8492 | 3 ± 2.8 | 4.4 ± 4.3 | 0.7897 | 3.3 ± 2.1 | 5.0 ± 2.7 | 0.5000 | 6.9 ± 5.0 | 6.4 ± 5.4 |
| Woody species cover | 0.8571 | 11.2 ± 5.5 | 9.0 ± 5.2 | 0.7897 | 8.0 ± 4.9 | 5.9 ± 1.5 | 0.3651 | 8.6 ± 6.1 | 9.6 ± 3.9 |
| Litter total | 0.8889 | 66.8 ± 9.1 | 56.4 ± 21.1 | 0.2738 | 70.4 ± 12.8 | 75.8 ± 8.7 | 0.1548 | 45.5 ± 12.7 | 53.0 ± 6.3 |
| Woody litter | 0.0714 | 8.2 ± 5.6 | 17.6 ± 10.6 | 0.3452 | 8.0 ± 7.1 | 10.5 ± 7.8 | 0.0754 | 14.9 ± 14.9 | 29.3 ± 14.7 |
| Herbaceous litter | 0.9246 | 58.8 ± 3.8 | 42.2 ± 25.4 | 0.3452 | 62.4 ± 9.3 | 65.2 ± 11.3 | 0.3452 | 27.8 ± 6.6 | 33.0 ± 8.2 |
| **Individual species cover** | | | | | | | | | |
| *Simsa calva* | 1.0000 | 0 | 0 | 0.5000 | 0 | 0.2 ± 0.4 | 0.5000 | 0 | 0.2 ± 0.4 |
| *Rudbeckia hirta* | 0.5000 | 1.6 ± 3.6 | 2.2 ± 3.5 | 1.0000 | 0 | 0 | 1.0000 | 0 | 0 |
| *Xanthocephalum* | 0.3333 | 7.2 ± 13.4 | 2.8 ± 1.9 | 1.0000 | 0 | 0 | 1.0000 | 0.4 ± 0.6 | 0 |
| *Croton* spp. | 0.5833 | 12.9 ± 14.2 | 11.2 ± 8.2 | 0.2222 | 0 | 0.4 ± 0.6 | 0.5000 | 0.2 ± 0.5 | 0.4 ± 0.6 |
| *Gilia incisum* | 0.5000 | 0 | 0.2 ± 0.4 | 1.0000 | 0 | 0 | 1.0000 | 0 | 0 |
| *Engelmannia pinnatifida* | 0.1587 | 0.7 ± 0.4 | 1.6 ± 1.1 | 0.1190 | 0.64 ± 0.5 | 1.4 ± 1.0 | 1.0000 | 0 | 0 |
| *Verbena canescens* | 0.3611 | 0.2 ± 0.4 | 0.8 ± 1.3 | 0.2222 | 0 | 0.6 ± 1.0 | 1.0000 | 0.4 ± 0.5 | 0 |
| *Gaillardia pulchella* | 1.0000 | 5.3 ± 5.7 | 0.2 ± 0.5 | 0.1111 | 9.0 ± 6.0 | 14 ± 5.9 | 0.5000 | 0.8 ± 1.8 | 0.6 ± 1.0 |
| *Abutilon fruticosa* | 0.6746 | 9.7 ± 6.9 | 7.8 ± 5.9 | 0.6825 | 4.8 ± 5.1 | 3.2 ± 2.8 | 0.2302 | 5.8 ± 6.4 | 9.2 ± 7.1 |
| *Coreopsis lanceolata* | 0.2976 | 3.4 ± 2.9 | 4.6 ± 3.2 | 1.0000 | 0 | 0 | 1.0000 | 0 | 0 |
| *Helianthus maximiliani* | 0.7381 | 1.8 ± 2.7 | 0.4 ± 0.6 | 1.0000 | 0 | 0 | 0.5000 | 0 | 0.2 ± 0.5 |
| *Salvia farinacea* | 0.6825 | 5.7 ± 5.7 | 4.4 ± 3.8 | 0.7143 | 2.9 ± 3.5 | 1.6 ± 1.5 | 0.7222 | 4.1 ± 4.0 | 2. 6± 1.1 |
| *Acalyphs lindheimeri* | 1.0000 | 0 | 0 | 0.9762 | 0.8 ± 0.8 | 0.2 ± 0.5 | 0.8770 | 1.3 ± 1.0 | 0.8 ± 0.8 |
| *Ratibida columnifera* | 0.1587 | 0.7 ± 0.5 | 2.6 ± 3.1 | 0.0833 | 0.4 ± 1.0 | 1.8 ± 1.5 | 0.6587 | 0.6 ± 0.6 | 0.8 ± 1.3 |
| *Oenothera speciosa* | 0.3214 | 2.8 ± 1.9 | 3.4 ± 2.2 | 1.0000 | 0 | 0 | 1.0000 | 0.1 ± 0.1 | 0 |
| *Glandularia bipinnatifida* | 0.3611 | 0.3 ± 0.4 | 0.6 ± 1.0 | 1.0000 | 0.2 ± 0.4 | 0 | 1.0000 | 0.1 ± 0.1 | 0 |
| *Sida* spp. | 0.1230 | 3.2 ± 2.4 | 6 ± 3.6 | 1.0000 | 1.4 ± 1.2 | 0 | 0.7302 | 4.6 ± 1.9 | 4 ± 1.6 |
| *Siphonoglossa pilosella* | 1.0000 | 0 | 0 | 1.0000 | 0 | 0 | 0.7778 | 0.2 ± 0.5 | 0.2 ± 0.5 |
| *Maurandya antiriniflora* | 0.6032 | 6.2 ± 6.0 | 6 ± 6.6 | 1.0000 | 3.1 ± 2.8 | 0 | 0.7183 | 3.6 ± 54.9 | 0.2 ± 0.5 |

*Continued*

Table 1. (*Continued*)

| | Oct-10 | | | May-11 | | | Oct-11 | | |
|---|---|---|---|---|---|---|---|---|---|
| **Variable** | ***P* value** | **C±SD** | **IN±SD** | ***P* value** | **C±SD** | **IN±SD** | ***P* value** | **C±SD** | **IN±SD** |
| **Individual species cover** | | | | | | | | | |
| *Euphorbia* spp. | 1.0000 | 1.6 ± 3.1 | 0 | 0.2222 | 0 | 0.4 ± 0.6 | 0.7778 | 0.2 ± 0.4 | 0 |
| *Ipomopsis rubra* | 0.7381 | 0.4 ± 0.6 | 0.4 ± 0.6 | 1.0000 | 0 | 0 | 1.0000 | 0.1 ± 0.1 | 0 |
| *Clematis drummondii* | 0.5000 | 0 | 0.8 ± 1.8 | 1.0000 | 0 | 0 | 1.0000 | 0.1 ± 0.1 | 0 |
| *Hymenopappus* | 0.9087 | 7.4 ± 5.7 | 2.8 ± 3.3 | 1.0000 | 0.6 ± 0.9 | 0 | 1.0000 | 0.1 ± 0.1 | 0 |
| *Zexmania hispida* | 0.9167 | 1.0 ± 1.7 | 0.2 ± 0.5 | 1.0000 | 0.1 ± 0.1 | 0 | 1.0000 | 0.1 ± 0.1 | 0 |
| *Andropogon gerardii* | 0.7024 | 1.8 ± 1.8 | 1.4 ± 1.0 | 0.7778 | 0.2 ± 0.5 | 0.2 ± 0.5 | 1.0000 | 1.2 ± 1.3 | 0 |
| *Bouteloua gracilis* | 0.7778 | 0.2 ± 0.5 | 0.2 ± 0.5 | 0.6389 | 0.4 ± 0.6 | 1 ± 2.2 | 0.0397* | 0.6 ± 0.6 | 2.2 ± 1.6 |
| *Bouteloua curtipendula* | 0.1190 | 5.1 ± 4.1 | 11.2 ± 7.5 | 0.3254 | 8 ± 6.4 | 8.4 ± 3.8 | 0.0516* | 6.9 ± 4.2 | 11.8 ± 4.3 |
| *Buchloe dactyloides* | 1.0000 | 0.3 ± 0.7 | 0 | 0.5000 | 1.1 ± 1.7 | 0.6 ± 0.6 | 0.2222 | 0.1 ± 0.3 | 1 ± 1.4 |
| *Carex planostachys* | 0.5238 | 11.1 ± 4.0 | 10.6 ± 7.9 | 0.2817 | 10.5 ± 3.3 | 12.8 ± 5.9 | 0.1944 | 6.5 ± 2.2 | 8.4 ± 2.9 |
| *Eragrostis intermedia* | 0.7778 | 0.2 ± 0.4 | 0.2 ± 0.5 | 1.0000 | 0 | 0 | 0.1032 | 0.4 ± 0.9 | 1.2 ± 0.8 |
| *Eragrostis trichodes* | 0.7778 | 2.4 ± 3.4 | 1.6 ± 3.1 | 1.0000 | 0 | 0 | 0.5000 | 0.2 ± 0.5 | 0.4 ± 0.6 |
| *Eriochloa sericea* | 0.7381 | 0.8 ± 1.1 | 0.4 ± 0.6 | 0.7976 | 1.1 ± 1.3 | 0.8 ± 1.8 | 0.0437* | 1.2 ± 0.5 | 3.4 ± 2.7 |
| *Nassella leucotrichsa* | 0.6865 | 10.1 ± 10.9 | 3.8 ± 2.8 | 0.7778 | 7.3 ± 6.2 | 5.2 ± 2.2 | 0.6825 | 7.0 ± 7.0 | 4.6 ± 3.1 |
| *Panicum hallii* | 0.9802 | 2.7 ± 3.1 | 0.6 ± 1.0 | 0.2222 | 0.1 ± 0.1 | 0.4 ± 0.6 | 0.5000 | 1.2 ± 1.3 | 1.6 ± 2.1 |
| *Panicum virgatum* | 0.6587 | 0.8 ± 1.3 | 0.6 ± 1.0 | 1.0000 | 0 | 0 | 0.2222 | 0.4 ± 0.5 | 1.6 ± 1.8 |
| *Schizachyrium scoparium* | 0.6468 | 3.4 ± 2.1 | 3.8 ± 3.3 | 0.5595 | 1.4 ± 0.9 | 1.2 ± 0.8 | 0.7976 | 0.9 ± 0.7 | 0.8 ± 0.8 |
| *Sorghastrum nutans* | 0.8968 | 11.8 ± 8.6 | 5.2 ± 4.9 | 0.8095 | 11.1 ± 7.6 | 6.8 ± 6.5 | 0.7817 | 6.4 ± 3.4 | 4.8 ± 4.0 |
| *Tripsacum dactyloides* | 0.9167 | 0.4 ± 0.6 | 0.2 ± 0.5 | 0.8571 | 1.5 ± 1.7 | 0.6 ± 1.0 | 0.5000 | 0.6 ± 1.3 | 0.8 ± 1.8 |

areas landscapes have undergone severe degradation and changes in vegetation structure either by encroachment of woody species such as Ashe juniper or by prior overgrazing. Such sites can no longer support their pre-existing habitat and human intervention is necessary in order to establish native plant species and build the soil thus further recovering the natural successional processes (Ohsowski et al. 2012). Practitioners who use inoculums containing soil microbes and soil conditioners containing nutrients hope to enhance belowground food webs to create a more diverse and healthy plant community (van der Heijden et al. 2008; Kardol and Wardle 2010; Ohsowski et al. 2012).

However, the addition of both soil microbes and nutrients may not be ideal for grassland restoration. Soil microbes have been found to take up a greater amount of N than plants and the majority of the active N in the soil is from the microbial biomass (Jackson et al. 1988; Saini et al. 2004; Vasquez et al. 2008). Increasing soil microbial biomass can lead to greater N availability, which has been found to influence species composition (Belnap and Sharpe 1993). Limiting N availability can favor late successional species, while increasing N availability can favor early successional species (Parrish and Bazzaz 1982; Heil and Bruggink 1987; McLendon and Redente 1992; Belnap and Sharpe 1993). Although no significant differences were found between the IN and C plots with respect to individual species, late successional grasses like *S. nutans* had greater overall cover in C verses IN plots over all three time intervals. Other species like *A. gerardii* showed a higher percent cover in the first and third time interval only while *P. virgatum* cover was higher for the first time interval only.

In this study, a number of the grass species planted and sown in the savanna were late-seral grassland species, therefore, adding nutrients, especially N, could have negatively affected their abundance and distribution. As succession progresses, plant available nutrients tend to decrease (McLendon and Redente 1992; Vasquez et al. 2008). This decrease can be seen with respect to $NO_3$- and $NH_4$+ readings over the two time intervals. When $NH_4$+ begins

Table 2. *P* values for differences in soil microbes, bacteria, protozoa, fungi, and nutrients over two time intervals, February 2010 and 2011, between plots inoculated with soil microbes plus nutrients (IN) and plots not inoculated with soil microbes (C) in a grassland restoration project at Phil Hardberger Park in San Antonio, Texas. Included are *P* values, means (indicated by C or IN for each time interval), and standard deviations (SD). There were no significant differences at the 0.05 level.

| | February 2010 | | | February 2011 | | |
|---|---|---|---|---|---|---|
| **Variable** | ***P* value** | **C±SD** | **IN±SD** | ***P* value** | **C±SD** | **IN±SD** |
| Active bacteria | 0.9524 | 61.0 ± 2.2 | 56.9 ± 6.6 | 0.5000 | 32.7 ± 7.9 | 22.2 ± 10.0 |
| Total bacteria | 0.7262 | 582.2 ± 214.4 | 525.4 ± 131.2 | 0.7897 | 353.6 ± 2.2 | 323.8 ± 47.7 |
| Active fungi | 0.9524 | 23.6 ± 10.7 | 14.6 ± 3.6 | 0.5714 | 1.9 ± 2.2 | 1.6 ± 1.9 |
| Total fungi | 0.5794 | 939.2 ± 67.3 | 900.8 ± 131.3 | 0.5317 | 535.2 ± 93.4 | 532.8 ± 183.0 |
| Hyphal diameter | 0.5000 | 2.82 ± 0.1 | 2.83 ± 0.03 | 0.3571 | 2.8 ± 0.06 | 2.81 ± 0.04 |
| Flagellates | 0.4206 | 845.4 ± 603.4 | 2788.2 ± 3261.5 | 0.4444 | 374 ± 261.7 | 1421.6 ± 2355.3 |
| Amoebae | 0.4206 | 3867.6 ± 63.2 | 6462.8 ± 7513.8 | 0.6667 | 2496.4 ± 1392 | 4866.8 ± 6798.7 |
| Ciliates | 0.2698 | 34.4 ± 50.2 | 62.2 ± 48.2 | 0.2619 | 7.8 ± 17.4 | 6 ± 6.6 |
| Total nematodes | 0.5794 | 2.51 ± 2.1 | 2.4 ± 1.4 | 0.5794 | 6.2 ± 2.7 | 6.2 ± 4.9 |
| Total fungi to bacteria | 0.5794 | 1.8 ± 0.6 | 1.8 ± 0.4 | 0.5794 | 1.5 ± 0.2 | 1.6 ± 0.5 |
| Active to total fungi | 0.8810 | 0.1 ± 0.2 | 0.02 ± 0.01 | 0.6587 | 0.004 ± 0.004 | 0.003 ± 0.003 |
| Active to total bacteria | 0.4921 | 0.11 ± 0.04 | 0.12 ± 0.04 | 0.5635 | 0.09 ± 0.03 | 0.01 ± 0.04 |
| Active fungi to active | 0.9286 | 0.4 ± 0.2 | 0.3 ± 0.1 | 0.5952 | 0.06 ± 0.08 | 0.04 ± 0.05 |
| Actino bacteria (µg/g) | 0.2738 | 6.4 ± 3.8 | 7.6 ± 4.1 | 0.9325 | 7.6 ± 5.3 | 4.3 ± 1.9 |
| $NO_3$- | 0.3175 | 14.2 ± 17.5 | 20 ± 15.2 | 1.0000 | 2 ± 0 | 2 ± 0 |
| $NH_4$+ | 0.2381 | 49.6 ± 8.3 | 53.6 ± 9.2 | 0.0635 | 32.8 ± 1.8 | 36.8 ± 3.4 |

to decline in the soil, microbes switch to using $NO_3$- and competition for available $NO_3$- between soil microbes and grasses is intense (Jackson et al. 1988; Vasquez et al. 2008). However, $NH_4$+ remained higher than $NO_3$- for both time intervals and for both C and IN plots. This suggests that soil microbes may not have switched to using $NO_3$- and thus competition between grasses and microbes for available $NO_3$- might not have been a factor. Despite the need for N by soil microbes, increased fertility and N in the soil can favor early successional species like invasive annual grasses and can suppress target species like late-seral native grasses (Carbajo et al. 2011; James et al. 2011). With respect to this restoration project, *Bothriochloa ischaemum* (L.) Keng, an invasive exotic grass, was observed but at an estimated cover of less than one percent. Also, herbaceous cover was greater for the first time interval than for the last time interval, and the opposite was true for bare ground cover, which steadily increased over all three time intervals. This decrease in herbaceous cover and increase in bare ground may have been a result of decreased precipitation from 2010 to 2011. However, the success of re-establishing a native grassland community is accomplished by promoting the establishment of native grass species in a grassland restoration project while keeping exotic annuals and grasses suppressed (Kulmatski et al. 2006; Kulmatski 2011; Middleton and Bever 2012).

The use of inocula in the restoration process has played an important role in establishing the soil microbial-association with plants and thus the long-term success of restoration projects. Agricultural practices, for example, have been found to alter the AM fungi community by favoring less mutualistic AM fungi species, which has been shown to increase the invasion of abandoned agriculture fields by exotic plant species (Helgason et al. 1998; Johnson et al. 2003; Kulmatski et al. 2006; Middleton and Bever 2012). Studies have shown that late successional plants can be promoted by free-living bacterial soil inocula, and late successional plant species are the targets of most restoration projects. However, including nutrients in addition to soil inocula may only be necessary in nutrient poor sites

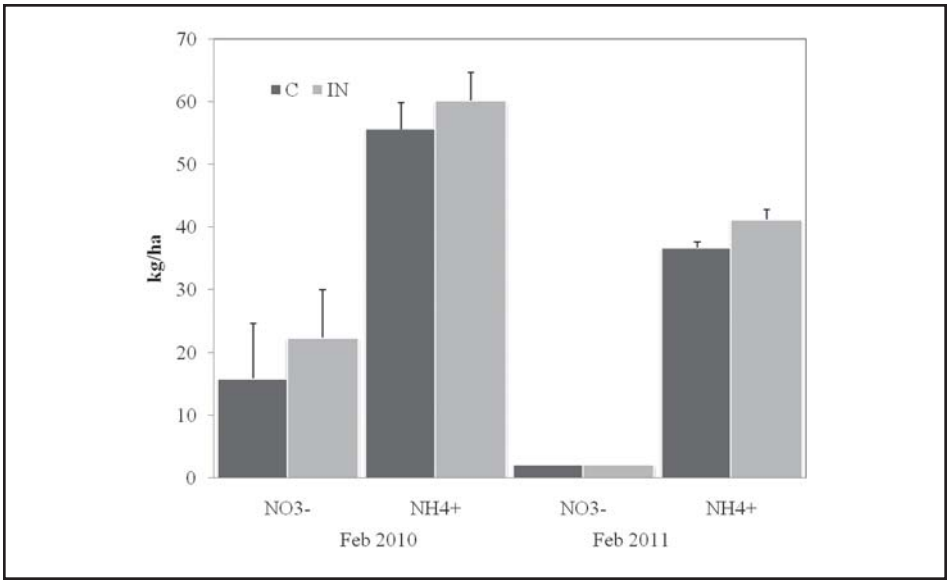


**Figure 1. $NO_3$- and $NH_4$+ levels for plots inoculated with soil microbes plus nutrients (IN) and plots not inoculated with soil microbes (C) (February 2010 and 2011) in a grassland restoration project at Phil Hardberger Park in San Antonio, Texas. Standard error bars are also depicted.**

where the sole purpose would be supporting soil microbes rather than adding nutrients to the soil. According to data from CSI, throughout the entire site $NO_3$- remained lower than the recommended range of 45 kg to 90 kg per ha, while $NH_4$+ remained within that same recommended range. If establishing late successional plants is the goal of the restoration project, then promoting plant growth through the use of soil inocula will most likely occur in nutrient poor soils (Carbajo et al. 2011).

This study implies that the use of inocula did not make a significant difference in the overall productivity and establishment of native grasses and forbs at Phil Hardberger Park. Significant differences, however, were found with respect to three grass species, *Eriochloa sericea* (Texas cupgrass), *Bouteloua gracilis* (bluegrama), and *B. curtipendula* (sideoats grama) in the last time interval. Unfortunately, we cannot say if this was due to inocula, nutrients, or location of planting. There were also numerous factors that could have confounded the results. This restoration project was on a small scale and was to occur in one specific location, thus we were unable to properly randomize the treatments. Also, the overall topography of the site could have washed nutrients and inoculum from the IN plots into the C plots. However, restoring soil microbial associations with plants in restoration sites remains of great concern if restoration projects are to be successful. Additional studies should be conducted not only on the benefits of inocula on grassland restoration but perhaps also on the effects of adding nutrients along with soil inocula.

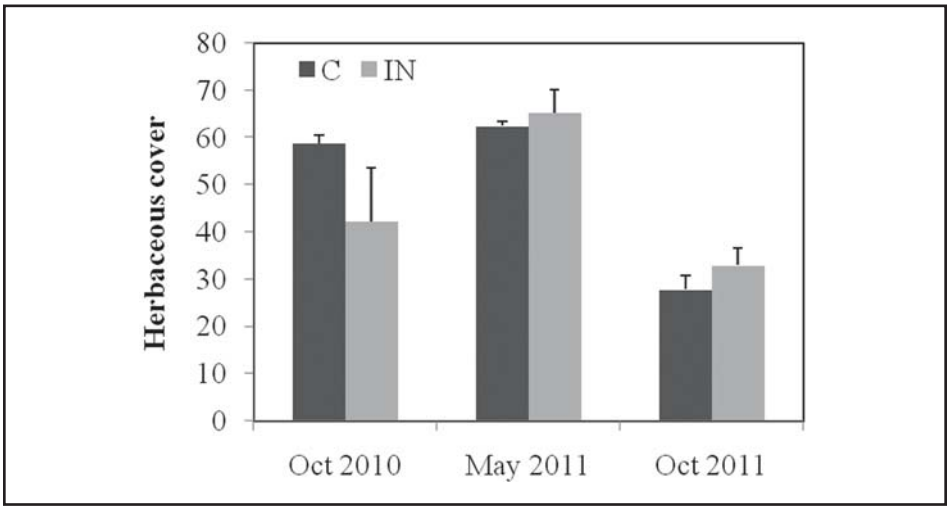


**Figure 2. Total herbaceous cover levels over three individual time intervals (October 2010 and May and October 2011) for plots inoculated with soil microbes plus nutrients (IN) and plots not inoculated with soil microbes (C) in a grassland restoration project at Phil Hardberger Park in San Antonio, Texas. Standard error bars are also depicted.**

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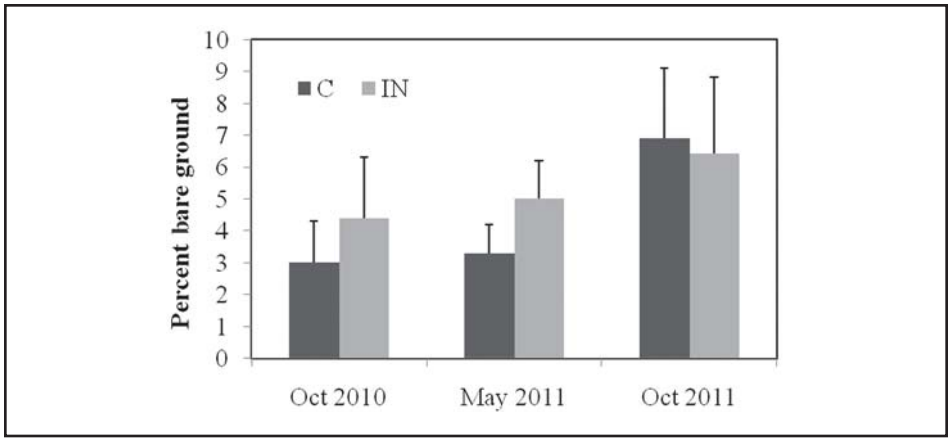


**Figure 3. Percent bare ground levels over three individual time intervals (October 2010 and May and October 2011) for plots inoculated with soil microbes plus nutrients (IN) and plots not inoculated with soil microbes (C) in a grassland restoration project at Phil Hardberger Park in San Antonio, Texas. Standard error bars are also depicted.**


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