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Use of Mycorrhizal Inoculum as a Soil Amendment During Prairie Restoration

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Introduction

First identified in 1840, mycorrhizae are symbiotic fungi found associated with the roots of a wide variety of plant species. Their role in the symbiotic partnership is providing a fine hyphal network capable of extending the range of root hairs (Allen, M.F. 1991). Decades of work with mycorrhizae associated with trees (ectomycorrhizae) revealed that most species of trees were obligatorily mycorrhizal (need mycorrhizae for survival) (Allen, M.F. 1991). Consequently, ectomycorrhizal inoculum has been commonly used during forestry plantings to enhance tree growth. Within the last 20 to 30 years, mycorrhizal associations (endomycorrhizae) have been found with many native prairie species (Table 1). These later findings open up the possibility that mycorrhizal inoculum could be used to aid the growth of prairie plant species. Experiments have shown that mycorrhizae enhance growth and fitness of native prairie plants . The benefits of mycorrhizal soil amendment could be used in restoring native prairies. As of yet, only small research trials using mycorrhizae have been done at prairie sites. This technique is therefore still relatively new. However, the success of trials done both in prairie restoration and other restoration fields is impressive and appears promising.

Benefits to native plants

Numerous studies on prairie plants have shown that many greatly benefit by symbiotic relationships with endomycorrhizal fungi (Hetrick 1995, Anderson 1994). Vesicular-arbuscular (VA) mycorrhizae, the predominant grass and forb endomycorrhizae, have been most thoroughly studied for their advantageous properties. Several prairie species (such as *Andropogon gerardii*, *Sorghastrum nutans*, and *Schizachyrium scoparium* [Wilson 1997, Anderson 1993]) have been shown to be obligatorily mycorrhizal . Many others are known to be facultatively mycorrhizal (may benefit from but not require mycorrhizae). Mycorrhizae likely play an integral role in prairie ecosystems.

Research on the symbiotic relationship has shown that mycorrhizal plants are aided by the fine hyphal network of the mycorrhizae. Mycorrhizae allow the fungal/plant symbiont to extract a greater amount of nutrients from the soil (Allen, M.F. 1991). Chief among the nutrients that are supplied by the fungi is phosphorus. Another nutrient that plants can utilize fungi to obtain is nitrogen (Sieverding 1991). Uptake of trace elements, such as zinc, copper, boron, and molybdenum, is also thought to be enhanced by VA mycorrhizae (Sieverding 1991).

A further benefit provided by the fungal relationship is increased drought tolerance. Several mechanisms have be theorized for the drought resistance provided by mycorrhizae, but research has not been done yet which conclusively accounts for the added drought tolerance. (Allen, M.F. 1991, Sieverding 1991, M. Smith per. comm.) Suggestions have also been made that the soil is benefited by mycorrhizal hyphae: hyphae bind soil, aiding in aggregation. Soil aggregation is thought to increase aeration, promote root development, and reduce wind erosion (Sieverding 1991).

As with all symbiotic relationships, plants must supply the fungi with something in exchange for nutrients. Plants fill their role in the relationship by providing the fungi with carbohydrates produced via photosynthesis. Approximately 3-20% of the total carbohydrates produced are translocated to the fungi under normal conditions (Sieverding 1991). Although 3-20% of the photosynthates is a rather large amount, the substantial benefit to the plant from added nutrients has been demonstrated in many situations (Allen, M.F. 1991).

The synergistic affects of added nutrient and water uptake are observable when mycorrhizal plant species are put in competition with non-mycorrhizal species. Work on *Andropogon gerardii* shows that it can not effectively compete with the cool-season grass *Koeleria pyramidata* in the absence of mycorrhizae. However, if colonized by mycorrhizal fungi, *A. gerardii* is able to outcompete the *K. pyramidata* (Hetrick 1994). Therefore, the added benefits of mycorrhizae are sufficient to alter the balance of fitness between competing prairie species. Research has also shown that although non-natives are the first to colonize disturbed areas, succession by mycorrhizal native prairie species replaces the invasive primary successional plants (Reeves 1979).

Methods of inoculation

In restoration and reclamation settings, the absence or reduction of VA mycorrhizal fungal populations is common after a site has been highly degraded. Mycorrhzial fungi can be reintroduced via inoculation of the restoration site. However, two main obstacles exist in the re-introduction of mycorrhizae during prairie restorations. These include the application technology for and the limited supply of mycorrhizal inoculum.

Currently, inoculum is not commercially available for prairie restoration. Lack of commercial prairie inoculum means producing ones own mycorrhizal inoculum specifically for their prairie site prior to restoration. Unfortunetly, endo-mycorrhizae present in prairie soils require a living plant host during inoculum production making production difficult. In contrast, ecto-mycorrhizae, used in forestry, can be cultured easily without a host.

Endomycorrhizal inoculum can be produced by several methods. The most common method is by bulking up a small sample of soil isolated from a remnant site (Noyd 1995). For a prairie restoration, the first step is to identify a remnant prairie that contains sufficient numbers of mycorrhizae. Soil samples are taken and brought to a greenhouse. A small amount of the mycorrhizal soil is added to sterilized potted greenhouse soil. The pots are seeded with prairie plants which would be good hosts for the desired mycorrhizae. Potted plants are grown to maturity, allowing mycorrhizae to colonize the plants and reproduce via spores. The pots are then left unwatered to senesce and thoroughly dry. At this point, the soil in the pots can be used for inoculum. The soil will contain a mixture of mycorrhizal spores, fungal hyphae and root pieces which are colonized by mycorrhizae. All of these are thought to be effective in inoculation.

Once inoculum is produced, a variety of methods can be used to inoculate the restoration site. These methods were developed for agricultural use or have been derived from other areas of restoration and as a result are not fully amenable to prairie restoration (Hayman 1981)**.** Most field research has used the trench method of inoculation (Sieverding 1994). In this method, a small trench is made (just under seed planting depth) and inoculum is placed directly in the trench. Seeds are placed over the inoculum and covered with soil. The seeds germinate and their roots penetrate the inoculum layer. Hyphae can then colonize the young roots as they enter the inoculum layer. The trenching method works very well and produces high levels of mycorrhizal colonization.

Although the most commonly used method of inoculation, trench inoculation has a few drawbacks. The first limitation is that only plants in the trench line will become colonized. Therefore, if trenches are widely spaced, only a small part of the restoration site will be colonized. In time, both the plants and mycorrhizae will spread through the restoration site. Therefore, the trenching technique increases the time required for complete restoration. The extra time may be a factor in considering the feasibility of a restoration project. A second weakness is that this method is very labor intensive. Trench inoculation is impractical for large scale prairie restorations. The amount of labor required for trenching make these restorations totally unfeasible.

Another inoculation technique is broadcasting. In the broadcasting method, seeds and inoculum are broadcast spread over an area. The seeds and inoculum are then mixed in with the top few centimeters of soil. Inoculum growing in the immediate vicinity of germinating seeds will colonize the seedling’s roots. By using broadcast spreading of inoculum, one can cover a large area with seed and inoculum quickly. The weakness of this system is the amount of inoculum required: Since only inoculum immediately adjacent to seeds will colonize the seeds, a great deal of inoculum must be spread with the seeds to ensure their colonization (Hayman 1991).

Various inoculum pellet techniques, some patented, have been studied and shown to be effective in conveying live inoculum to the field. This technique is a hybrid of the broadcast and the trenching method. In typical pellets, inoculum is mixed with moist clay to form a small pellets, which are then rolled in seeds. The seed pellets can then be dried and planted over large areas using a seed drill. The advantage of this method is that inoculum can readily colonize the germinating seed via direct contact. Less inoculum is required and the labor requirements are minimal. (Sieverding 1991). Inoculum pellets work well when restorations involve small seeds that can be made into manageable pellets. However, many prairie grasses have large fluffy seeds with bracts on them that might limit their ability to form pellets usable in a seed drill. Inoculum pelleting is still more of an experiment than a proven field technique.

The final and most labor intensive technique is that of using seedlings which have been colonized by mycorrhizae. Seedlings can easily be grown in nursery beds containing mycorrhizae. They are colonized by mycorrhizae with high efficiency. The seedlings are then transplanted into the restoration site. The labor involved in transplanting the seedlings would make this an impractical method for prairie restorations. However, if target species were important at a restoration site, this method could be utilized to benefit a limited number of species which need help with establishment at a site.

The ability of each of these techniques to establish adequate soil mycorrhizae has been studied under various conditions (Hayman 1981, Sieverding 1991). Yet many of these experiments were done in agricultural settings or during restoration/reclamation projects of a different nature (mine reclamation or arid land restoration). The potential for restoration of a large-scale prairie site using mycorrhizae is still experimental. The central reason that prairie restoration/reclamations have not been done using mycorrhizae is the cost of both labor and mycorrhizal inoculum**.**

Inoculum limitations

Currently small scale inoculum production is expensive due to overhead and labor costs. Most researchers doing prairie restoration use inoculum they have produced in pots in their own greenhouses. Research production provides them with only enough inoculum to treat a small research plot. A restoration site requires a great deal more inoculum than can be produced in pots. Depending on the application technique mentioned above and literature values, one would likely need between .01 and 11 tons of high quality inoculum per hectare (Seiverding 1991, Hayman 1981, Noyd 1995). Quantities of prairie inoculum this large can not be produced using typical research inoculum production methods. However, with heightened interest in restoration, it is likely that large scale production of inoculum will begin in the near future. Also, with the refinement of application techniques for inoculum, the future of prairie restoration using mycorrhizal inoculum shows promise.

Ecotype variation is another limitation of mycorrhizal inoculation. The extent of ecotype variation is not known, but allelic diversity and species composition of mycorrhizae probably vary greatly over distances. At present, those interested in using mycorrhizal inoculum in prairie restoration make their own inoculum from mycorrhizae found at nearby remnant sites. Current small scale local production thus avoids problems with ecotype variation over distances. However, if cheaper commercial inoculum was produced, the producers would be less inclined to supply inoculum that is site specific. For example, a common tree inoculum (with both endo- and ectomycorrhizae) is produced in Philadelphia, Pennsylvania. The origin of the mycorrhizae in the commercial product is likely not from a forest in Minnesota, yet the product is used in Minnesota to enhance tree growth. The efficacy of using spores from a vastly different location is not known. Site differences in factors such as resistance to cold, heat, pathogens and soil conditions may mean that mycorrhizae from a distant location would not survive at a local restoration. These products could also conceivably contain invasive species. A restoration 'purist' would be unlikely to use a commercial inoculum that has not originated from a remnant site close to the restoration site. If research indicates that inoculum should be ecotype (site) specific, the price of inoculum is likely to be much higher.

Priorities for Mycorrhizal Amendment

Unfortunately, cost limitations will limit the role of mycorrhizae to severely disturbed sites. These sites may need mycorrhizae for any vegetative success . Sites that have no remnant populations of mycorrhizae and/or lacking vegetation for many years will be the most likely candidates for this amendment. Sites that may have been growing a monoculture of a non-mycorrhizal species for a long time might also benefit from mycorrhizal amendment during prairie restoration. In short, only in sites that have a near total lack of mycorrhizae would it be economically feasible.

Sites that would not be candidates for mycorrhizal amendment during restoration are sites that have had vegetation growing on them, such as former agricultural or lightly polluted land. These sites should have remnant mycorrhizal populations and therefore not need amendment with costly mycorrhizae although fungal populations might be significantly reduced and may lack species diversity. Soil tests could be performed prior to restoration to determine the level of remnant mycorrhizae. Although this type of testing would be expensive and would not indicate whether mycorrhizal species present would effectively colonize one's intended prairie species. A simpler way to determine whether mycorrhizae were present would be to look at the species of plants present on a site. If any species of plants known to be mycorrhizal (including weeds and/or crops) are present, then there is likely to be a remnant mycorrhizal population.

Evaluation of Inoculation

To date, little data is available on the success of prairie restorations using mycorrhizae, because costs of inoculating the sites have been prohibitive. Therefore, researchers studying mycorrhizal amendments for prairie restoration have used a variety of simulations to study these amendments. Several pot culture studies have been performed. Many of these used mycorrhizal soil taken from native prairie and placed in pots (Hetrick 1994, Anderson 1994, and Allen E.B. 1984a). Various seeds are put in pots with mycorrhizae or in pots with sterilized soil (no living mycorrhizae). These studies all show similar results. Mycorrhizal plants increase biomass if they are colonized by mycorrhizae. Non-mycorrhizal plants show no gain in biomass if grown in pots containing mycorrhizae. If both types of plants are together in the presence of mycorrhizae, the mycorrhizal plants predominate. Several studies have shown that without mycorrhizae, the non-mycorrhizal plants predominate

A more recent study used microcosms to simulate the affect of mycorrhizae in a prairie environment (Wilson 1997). The microcosm was a simulated prairie measuring 40 x 52 x 32 cm which contained several grass species. Several such microcosms with or without mycorrhizae were prepared. The mycorrhizal microcosms showed larger amounts of above ground plant biomass and a greater ratio of C4 grasses to C3 grasses. Two legume species (*Amorpha canescens* and *Dalea purpurea*) showed a significantly higher survivorship when mycorrhizae were present. These results suggest that some native prairies require the presence of mycorrhizae to conform to their true 'native' state.

Correlative evidence from field studies finds that disturbed sites with non-native vegetation often have a greatly reduced amount of mycorrhizae (Reeves 1979). These studies concluded that the loss of mycorrhizae was likely a factor in the reduction of native vegetation. Although these studies merely correlated mycorrhizae with native vegetation, the correlation can be used as supporting evidence as to the need to include mycorrhizal amendments in restoration efforts.

Conclusion

The potential benefits of using mycorrhizal inoculum during prairie restoration are high. Yet at present, mycorrhizal amendments for prairie restorations still are in the experimental stages. Research to date has shown great promise. In order for mycorrhizal amendments to become fully accepted, they must demonstrate that they are an effective use of money in a restoration setting. However at present, mycorrhizal soil amendments remain mainly in the realm of research on prairie restoration sites.

Future advances in inoculum production should significantly increase the number of prairie restorations using mycorrhizae and their scope. Refinement of inoculum production will also likely be concurrent with increases in inoculation technology. In the future, one may be able to buy bags of prairie mycorrhizal inoculum for restoration/reclamation purposes as is the case for rhizobium in agricultural legume production and ectomycorrhizae for forestry purposes. As restoration specialist begin to become more acquainted with mycorrhizal amendments for prairie species, they will most likely want to use these amendments to enhance their restoration efforts.

Table 1 Mycorrhizal Plants

Grasses Thought to Be Mycorrhizal*		
Common name	Scientific Name	Reference
Big bluestem	*Andropogon gerardii*	Hetrick 1988
Blue grama	*Buteloua gracilis*	Allen, E.B. 1984 b
Canada wild rye	*Elymus candensis*	Harnett 1993
Switch grass	*Panacium vergatum*	Hetrick 1988
Little blue Stem	*Schizachyrium scoparium*	Anderson 1993
Western wheat grass	*Agropyron smithii*	Allen, E.B. 1984 a
Indian grass	*Sorgastrum nutans*	Wilson 1997
Forbs Thought to Be Mycorrhizal*		
Common name	Scientific Name	Reference
Purple prairie clover	*Dalea purpurea*	Wilson 1997
Leadplant	*Amorpha canescens*	Wilson 1997

Rough gray feather	*Liatris aspera*	Hetrick 1988
Sage species	*Artemisia tridentata*	Allen, E.B. 1984 b
Prairie False Indigo	*Babtisia Lecantha*	Hetrick 1988

* These are species which research indicates are mycorrhizal. Many research papers also list other species that are believed to be mycorrhizal, but have yet to be studied. This partial list is only drawn from a small portion of the literature.

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References

Allen, M.F. 1991. *Ecology of Mycorrhizae,* Cambridge University Press, New York

Allen, E.B. and M.F. Allen. 1984a. Competition between plants of different successional stages: Mycorrhizae as regulators. Canadian Journal of Botany 62:2625-2629

Allen, E.B. 1984b. VA Mycorrhizae and colonizing annuals: Implications for Growth, Competition, and Succession In *VA Mycorrhizae and reclamation of arid and semi-arid lands*. S.E. Williams and M.F. Allen editors. Scientific Report SA1261 Wyoming Agicultural Experimental Station. Laramie, Wyoming.

Anderson, R.C. and K.J. Roberts. 1993. Mycorrhizae in Prairie Restoration: Response of Three Little Bluestem (*Schizachryrium scoparium*) Populations to Mycorrhizal Inoculum From a Single Source. Restoration Ecology 1(June):83-87

Anderson R.C., B.A.D. Hetrick, and G.W.T. Wilson. 1984. Mycorrhizal Dependance of *Andropongn gerardii* and *Schizachyrium scoparium* in Two Prairie Soils. American Midland Naturalist 132:366-376

Harnett, D.C., B.A.D. Hetrick, G.W.T. Wilson, and D.J. Gibson. 1993. Mycorrhizal influence on intra- and interspeicific neighbour interations amoung co-occurring prairie grasses. Journal of Ecology. 81:787-795

Hayman D.S., E. J. Morris, and R. J. Page. 1981. Methods for inoculating Field Crops with Mycorrhizal Fungi. Annals of Applied Biology 99:247-253

Hetrick, B.A.D., D.C. Hartnett, G.W.T. Wilson, and D.J. Gibson. 1994. Effects Of Mycorrhizae, Phosphorus Availability, And Plant Density On Yield Relationships Amount Competing Tallgrass Prairie grasses. Canadian Journal of Botany 72:168-176

Hetrick, B.A.D., D.G. Kitt, and G.T. Wilson. 1988. Mycorrhizal dependence and growth havit of warm-season and cool-season tallgrass prairie plants. Can. J. Bot. 66:1376-1380

Noyd, R.K. 1995. Ch. 2 in *Ecological Interactions between native prairie grasses and AMF in the reclamation of ore tailings*, Native Praire Grasses And Microbial Community Responses To Reclamation Of Taconite Iron Ore Tailing. Doctorial Thesis, University of Minnesota, St. Paul, MN, USA

Reeves F.B., D. Wagner, T. Moorman, and J. Kiel. 1979. The role of endomycorrhizae in revegetation practices in the semi-arid West. I A comparison of incidence of mycorrhae in severely disturved vs. natural enviorments. Amer. J. Bot. 66:6-13

Sieverding, E. 1991.*Vesicular-Arbuscular Mycorrhiza Managemment,* Technical Cooperation-Federal Republik of German, Eschborn.

Smith, Michael. personal communication, University of Minnesota, Graduate student

Wilson, G.W.T. and D.C. Hartnett. 1997. Effects of Mycorrhizae on Plant Growth and Dynamics in Experimental Tallgrass Prairie Microcosms. American Journal of Botany 84(4):478-482