Sources of inocula influence mycorrhizal colonization of plants in restoration projects: a meta-analysis

Mia R. Maltz1,2, Kathleen K. Treseder1

Inoculation may influence mycorrhizal colonization and provide benefits to plants in restoration projects. However, it is unclear whether inoculation has consistent effects across ecosystem types, if it has long-term effects on colonization, and whether sources of inocula differ in their effectiveness. To address these issues, we performed a meta-analysis of published restoration studies across a variety of ecosystems to examine the effects of mycorrhizal inoculation on mycorrhizal establishment and plant growth under field conditions. Although we included trials from a variety of geographic locations, disturbance types, and ecosystem types, the majority were based in temperate ecosystems in the Northern Hemisphere, and fewer trials were from tropical ecosystems. Across ecosystem types, we found that inoculation consistently increased the abundance of mycorrhizal fungi in degraded ecosystems, and thus improved the establishment of plants. These benefits did not significantly attenuate over time. Moreover, inocula from different sources varied in their effects on mycorrhizal colonization. Inocula sourced from reference ecosystems and inocula with specific fungal species yielded higher increases in mycorrhizal colonization than did inocula from commercial sources. These results suggest that inocula source matters, and that an initial investment into mycorrhizal inoculation could provide lasting benefits for facilitating the establishment of the below- and aboveground components of restored ecosystems.

Key words: arbuscular, ectomycorrhizal, Fisher’s z-transform, fungi, reference ecosystem

Implications for Practice
• Mycorrhizal restoration via inoculation is a simple means to improve mycorrhizal abundance and plant performance in restoration projects.
• If whole inoculum from reference ecosystems is easily accessible, it may be more efficient and cost-effective than the use of commercial inoculum.
• An initial investment in mycorrhizal inoculation could provide benefits for up to several years in the field.

Introduction
Individual studies have indicated that mycorrhizal fungi can improve the success of native plant establishment in restored ecosystems (e.g. Stahl et al. 1988; Sylvia et al. 1993; Allen et al. 2003a; Graham et al. 2013). Indeed, mycorrhizal fungi provide nutrients to plants, usually leading to increased photosynthetic rates and improved plant productivity (Mosse 1957; Allen et al. 1981; McGonigle & Fitter 1988; Lekberg & Koide 2005; Hoeksema et al. 2010). As other inhabitants rely on vegetation for food and cover, restoration of mycorrhizal fungi may have far-reaching benefits within the ecosystem. Nevertheless, little is known about the extent to which mycorrhizal fungi can be directly manipulated in restoration projects, and which sources of mycorrhizal inocula are most effective.

A number of human activities can reduce the abundance of mycorrhizal fungi in degraded ecosystems (Allen & McMahon 1985; McGonigle & Miller 1996; Boddington & Dodd 2000; Egerton-Warburton & Allen 2000; Lekberg & Koide 2005; Sheng et al. 2012; Kohl et al. 2014). For instance, soil erosion, excessive irrigation, and agriculture practices can diminish the viability of fungal spores due to increased exposure to solar radiation, salinity, or fungicides (Hirrell & Gerdemann 1980; Saif 1981; Trappe 1981; Hayman 1982; Juniper & Abbott 2006; Tian et al. 2009; Homma et al. 2012). In addition, overgrazing can compact soils and reduce soil pore sizes, inhibiting mycorrhizal activities. Moreover, reduced vegetative cover and exotic plant invasions can alter the degree to which mycorrhizal fungi are supported by their host plants (Ehrenfeld et al. 2005; Wolfe et al. 2005; Palenzuela & Barea 2006). Furthermore, anthropogenic introductions of salt, nitrogen, or mine tailings can often have toxic effects on mycorrhizal fungi (Juwarkar & Jambhulkar 2008; de Souza et al. 2012; Estrada et al. 2013). Thus, direct intervention may be required to restore the abundance and function of mycorrhizal fungi in degraded ecosystems (Thrall et al. 2005; Bozzolo & Lipson 2013).
A relatively small portion of restoration projects have attempted to manipulate mycorrhizal fungi directly (Sylvia 1989; Greipsson 2010). Those who have addressed this issue typically apply inoculum (i.e. material containing spores or hyphal fragments) to soil in the field or to growth media in nursery pots prior to outplanting. The rationale for this practice is that the addition of mycorrhizal propagules should improve the likelihood that roots will encounter mycorrhizal spores or hyphae and become colonized by the fungus (Christensen & Allen 1980). Generally, plants that are more extensively colonized by arbuscular mycorrhizal (AM) fungi tend to grow better (Treseder 2013). In a recent meta-analysis, Piñeiro et al. (2013) reported that mycorrhizal inoculation was particularly effective in improving the survival and growth of planted seedlings in arid lands. Currently, it is unclear whether this technique is broadly successful across a range of ecosystem types.

Indeed, additions of mycorrhizal inoculum need not necessarily improve mycorrhizal colonization of plant roots in a given ecosystem. In some cases, mycorrhizal inoculation may have little effect on colonization because adequate inoculum is already naturally present in the ecosystem. The availability of mycorrhizal propagules is only one determinant of mycorrhizal abundance—a number of other factors may inhibit mycorrhizal fungi in ecosystems marked for restoration (Skujins & Allen 1986). For instance, harsh environmental conditions in degraded landscapes could reduce the viability of inoculum in the soil, or could inhibit the extension of hyphae from the roots of nursery-inoculated plants (Plett et al. 2015; van der Heijden et al. 2015). Thus, it is worth examining whether enhanced root colonization is sustained over the long term (i.e. months to years) in field-established plants.

In addition, the mycorrhizal species represented in a given inoculum may not always be beneficial for the revegetated plant community. In fact, many mycorrhizal species may exhibit host preferences, host selectivity, or some degree of host specificity (Helgason et al. 1998; Smith & Read 2008; Sánchez-Castro et al. 2012; van der Heijden et al. 2015), which may not allow them to form relationships with certain restored plants. Even species of arbuscular mycorrhizal fungi—which are thought to be relative generalists—can vary in the degree to which they colonize plant roots (Powell et al. 2009) and in their effects on plant growth (Hoeksema et al. 2010). Mycorrhizal fungi from neighboring reference ecosystems (i.e. ecosystems that exhibit characteristics intended of the restored ecosystem) may be better-suited for the local environment. Indeed, in a recent review of restoration studies from semiarid ecosystems, Barea et al. (2011) noted that inocula derived from exotic mycorrhizal fungi may not be as effective as inocula from indigenous fungi. Common sources of mycorrhizal inocula for restoration work include commercially available inocula, whole inocula derived from soil or roots collected from reference ecosystems, or species-specific inocula isolated in the laboratory. If we can identify sources of inocula that best improve mycorrhizal abundance, this information can be used by restoration ecologists to develop best practices.

Toward this end, we performed a meta-analysis of published restoration studies that examined effects of mycorrhizal inoculation on mycorrhizal establishment (as percent root length colonized, PRLC) and plant growth under field conditions. We tested the hypotheses that additions of mycorrhizal inocula would result in sustained increases in PRLC (hypothesis 1) and improved plant growth (hypothesis 2) in the field, and that whole inocula from neighboring reference ecosystems would elicit larger increases in PRLC than would commercial inocula (hypothesis 3).

**Methods**

We surveyed articles published in the literature and found 28 manipulative field-based restoration trials from 22 publications that addressed the influence of mycorrhizal fungal inoculation on percent colonization of plant roots. We used the Google Scholar (scholar.google.com) and Web of Science (webofknowledge.com) search engines, and also directly searched the archives of the Restoration Ecology journal (link.springer.com/journal/572). Our search terms were (restor* and mycorrhiz*), (restor* and inocul*), or (restor* and fung*). We used the following “decision rules” to select trials: the projects must have (1) used active ecological restoration of a degraded or constructed ecosystem, (2) incorporated an inoculated treatment and an uninoculated control, and (3) measured PRLC on (4) field-grown plants. We included trials from a variety of ecosystem types, geographic locations, and disturbance types. The landscapes in the selected trials were degraded primarily by human activities (Table 1).

In each trial, mycorrhizal colonization was directly manipulated via the addition of mycorrhizal inoculum as an active restoration technique. The fungal inoculum was sourced from reference ecosystems, commercial sources, or specific fungal isolates (Table 1). Commercial sources included mycorrhizal fungi mixed with unspecified/proprietary granular materials (e.g. Nutri-Link from Schenck & Smith, Native Plants Inc.) and pre-inoculated seedlings that were prepared by commercial nurseries using proprietary methods (Sylvia et al. 1993; de Aragón et al. 2013). Some of the species-specific inocula were prepared from soil originating from the experimental site, mycelial cultures obtained from curated collections, or sporocarps originating from either the experimental site or other ecosystems (Rincón et al. 2007; Alguacil et al. 2011). Some mycorrhizal species were selected based on their desired ecological traits, such as production of abundant fruit bodies or facilitation of plant establishment. Every selected trial included an inoculated treatment group compared with an uninoculated control group. Fungal inoculum was applied directly to soils in the field study site or to plants in the greenhouse prior to outplanting in the field site.

Plants grew in the field for 4 months (Sylvia et al. 1993) to 54 months (de Aragón et al. 2013). Next, the plants were harvested and roots were analyzed for mycorrhizal colonization. In all cases, microscopy was used to evaluate
Table 1. Studies included in meta-analysis.

| Trial | Location | Ecosystem Type | Disturbance Type | Inoculum Type | Inoculation Method | Mycorrhizal Type | Duration in Field (Months) | Fisher’s z_{PRLC} | Var (z_{PRLC}) | Plant Parameter | Fisher’s z_{plant} | Var (z_{plant}) |
|-------|----------|----------------|-----------------|---------------|-------------------|-----------------|--------------------------|-----------------|---------------|-----------------|-----------------|---------------|
| Al Agely and Sylvia (2008) | 29°53′N, 81°17′W | Coastal dunes | — | From reference ecosystem | Inoculation in nursery | AM | 24 | 1.00 | 0.05 | Shoot dry mass | 1.13 | 0.05 |
| Al Agely and Sylvia (2008) | 29°39′N, 84°52′W | Coastal dunes | — | From reference ecosystem | Inoculation in nursery | AM | 24 | 0.78 | 0.05 | Shoot dry mass | 1.66 | 0.05 |
| Alguacil et al. (2011) | 38°12′N, 1°13′W | Shrubland | Agriculture | Single species | Inoculation in nursery | AM | 14 | 0.01 | 0.11 | Shoot dry mass | 1.02 | 0.11 |
| Allen et al. (2005) | 21°12′N, 87°10′W | Tropical forest | Fire | From reference ecosystem | Inoculation in nursery | AM | 26 | 0.00 | 0.14 | Seedling height | 0.16 | 0.14 |
| Caravaca et al. (2003) | 38°22′N, 1°10′W | Shrubland | Desertification | Single species | Inoculation in nursery | AM | 18 | 2.65 | 0.20 | Shoot dry mass | 3.45 | 0.20 |
| Cook et al. (2011) | 47°58′N, 123°35′W | Temperate forest | Dam | From reference ecosystem | Inoculation in nursery | AM | 20 | 0.00 (AM) | 0.33 (AM) | Shoot dry mass | −0.45 | 0.33 |
| Cuenca et al. (1998) | 5°40′N, 61°32′W | Tropical savanna | Road construction | Natural inoculum not from reference ecosystem | Inoculation in field | AM | 5 | 1.33 | 0.06 | Aboveground biomass per unit ground area | 0.37 | 0.06 |
| de Araúz et al. (2010) | 41°35′N, 1°31′E | Temperate forest | Fire | Commercial | Inoculation in nursery | EM | 54 | 0.10 | 0.03 | Seedling height | 0.17 | 0.03 |
| de Souza et al. (2010) | 6°28′S, 34°55′W | Coastal dunes | Mining | Single species | Inoculation in nursery | AM | 13 | 0.60 | 0.09 | Seedling height | 0.48 | 0.09 |
| Duponnois et al. (2011) | 31°54′N, 8°17′W | Shrubland | Desertification | From reference ecosystem | Inoculation in nursery | AM | 36 | 0.38 | 0.33 | Seedling height | 1.07 | 0.33 |
| Gemma and Koske (1997) | 42°4′N, 70°9′W | Coastal dunes | Grazing | From reference ecosystem | Inoculation in nursery | AM | 11 | −0.02 | 0.33 | Culms height | 1.13 | 0.33 |
| Graham et al. (2013) | 2°18′S, 113°50′E | Tropical forest | Logging | Single species | Inoculation in nursery | AM and EM | 6 | 0.40 (AM) and 0.04 (AM) | — | Plant dry mass | 0.40 | 0.04 |
| Pagano et al. (2009) | 15°9′S, 43°49′W | Tropical forest | Logging | Three species | Inoculation in nursery | AM and EM | 16 | 0.66 (AM) | 0.11 (AM) | — | — | — |
| Palenzenzuela et al. (2002) | 38°23′N, 1°10′W | Shrubland | Desertification | Single species | Inoculation in nursery | AM | 8 | 1.42 | 0.20 | Shoot dry mass | 0.28 | 0.20 |
| Quezada et al. (2003) | 3°25′S, 1°10′W | Shrubland | — | Single species | Inoculation in nursery | AM | 8 | 2.30 | 0.03 | Shoot dry mass | 1.02 | 0.03 |
| Requena et al. (2001) | 36°50′N, 2°27′W | Shrubland | Desertification | Single species | Inoculation in nursery | AM | 10 | 0.97 | 0.20 | Shoot dry mass | 0.34 | 0.20 |
| Richter and Stutz (2002) | 31°32′N, 110°4′W | Temperate grassland | Agriculture | From reference ecosystem | Inoculation in nursery | AM | 10 | 0.52 | 0.11 | Seedling height | 0.54 | 0.11 |
| Rincón et al. (2007) | 40°25′N, 3°42′W | Temperate forest | Industrial activities | Single species | From reference ecosystem | AM | 24 | 0.48 | 0.14 | Seedling height | 0.48 | 0.14 |
| Roldán et al. (1996) | 37°59′N, 1°7.8′W | Temperate forest | Topsoil clearing | Single species | Inoculation in field | EM | 14 | 0.97 | 0.14 | Seedling height | 0.05 | 0.01 |
| Smith et al. (1998) | 45°60′N, 93°21′W | Temperate grassland | Road construction | From reference ecosystem | Inoculation in nursery | AM | 15 | 0.62 | 0.20 | % cover | 0.12 | 0.20 |
| Stahl et al. (1988) | 43°4.5′N, 10°7′17′W | Shrubland | Mining | From reference ecosystem | Inoculation in nursery | AM | 12 | −0.06 | 0.11 | Plant height | 0.28 | 0.11 |
| Sylvia et al. (1993) | 25°47′N, 80°7.8′W | Coastal dunes | Constructed beaches | From reference ecosystem | Inoculation in nursery | AM | 6 | 1.13 | 0.33 | Shoot dry mass | 1.13 | 0.33 |
the mycorrhizal structures in plant roots. Four trials quantified ectomycorrhizal (ECM) fungi on unstained roots. The remaining examined arbuscular mycorrhizal fungi, following staining with trypan blue. Likewise, all of the trials examined fungal structures and measured the PRLC by using the gridline intersect method (Phillips & Hayman 1970; Ambler & Young 1977; Giovannetti & Mosse 1980; McGonigle et al. 1990; Brundrett et al. 1994).

We excluded studies that lacked a direct field component or measured mycorrhizal abundance using spore density or other metrics besides PRLC. To maintain independence of trials, in the case of longitudinal studies that used several time points to measure PRLC, we included only the final PRLC measurement recorded (i.e., representing the longest duration of time). If a study reported multiple sets of results (e.g., geographic location or inoculum type) in which an independent untreated control group was compared with an inoculated treatment group, then each system was designated as a different trial (Sylvia et al. 1993; Al Agely & Sylvia 2008).

For each trial, we obtained the mean PRLCs and numbers of replicates for the inoculated treatment and un inoculated controls. We used these data along with reports of standard error, standard deviation, or summary statistics to calculate a product–moment correlation for each study as in Rosenthal (1991). We then calculated Fisher’s $z$-transform as an effect size for each study, using the formula:

$$z_{PRLC} = \frac{1}{2} \ln \left( \frac{1 + r}{1 - r} \right)$$

In addition, the variance of $z$ was calculated as:

$$v_z = \left( \frac{1}{n - 3} \right)$$

For all but one trial, the investigators also measured some aspect of plant performance, usually as shoot dry mass or seeding height (Table 1). We calculated Fisher’s $z_{plant}$ and its variance from these data as described for Fisher’s $z_{PRLC}$, above.

To test hypothesis 1, we used a random-effects model to estimate a cumulative Fisher’s $z_{PRLC}$ of all 28 trials (Rosenberg et al. 2007). Each trial was weighted by the reciprocal of the variance of $z$ ($v_z$). In addition, 95% confidence intervals were calculated via bias-corrected bootstrapping with 999 iterations (Rosenberg et al. 2007). We compared the cumulative Fisher’s $z_{PRLC}$ against a mean value of zero. Hypothesis 1 would be supported if the cumulative Fisher’s $z_{PRLC}$ were significantly greater than zero. Likewise, hypothesis 2 would be supported if the cumulative Fisher’s $z_{plant}$ of the 27 trials were significantly greater than zero.

To test hypothesis 3, and check for other aspects of mycorrhizal restoration methods that might influence outcomes, we performed a series of categorical model meta-analyses on grouped data (Rosenberg et al. 2007). Specifically, for hypothesis 3, we tested for differences in cumulative Fisher’s $z$ between trials that used various inoculum sources (e.g., whole inocula from reference ecosystem, single species inoculum, or commercial inoculum). Hypothesis 3 would be supported if the cumulative Fisher’s $z$ were significantly higher in trials that used...
whole inocula from references ecosystems than in those that used commercial inocula. We also tested for significant differences among trials that inoculated seedlings in the nursery (followed by outplanting) versus those that applied inoculum in the field. Likewise, we compared studies that measured AM versus ECM colonization of plant roots, and checked for differences among ecosystem types.

Finally, we performed two continuous model meta-analyses (Rosenberg et al. 2007) to examine whether inoculum effects decreased over time under field conditions. For each test, the continuous variable was the amount of time that inoculated plants grew in the field before being assayed for PRLC or plant growth (i.e. "duration in field"). Fisher’s $z_{\text{PRLC}}$ was the effect size for one test; Fisher’s $z_{\text{plant}}$, the other.

We checked for "file-drawer" biases, by which failure to publish null effects of inoculation would influence our results. We used two tests: Kendall’s tau test for rank correlations between effect size and sample size, and Orwin’s fail-safe $N$ test. We used MetaWin 2.0 for all analyses, and effects were considered significant when $p < 0.05$.

Results

Characteristics of Selected Studies

The selected trials were conducted on landscapes degraded primarily by anthropogenic activities such as agriculture, logging, construction, desertification, grazing, and mining (Table 1). The majority were based in the Northern Hemisphere, with 15 studies from North America and nine from Western Europe. The remaining studies were located in Morocco, Indonesia, and South America. Coastal dunes, shrublands, and temperate forests were the dominant ecosystem types, followed by tropical forests, temperate grasslands, and tropical savanna. Inoculation of seedlings in the nursery, followed by outplanting in the field site, was more common than the application of inoculum directly to soil in the field.

General Responses to Inoculation

Across all 28 trials, inoculation with mycorrhizal fungi increased PRLC, as indicated by a cumulative Fisher’s $z_{\text{PRLC}}$ of 0.65 (0.40–0.94, 95% confidence interval), which was significantly greater than the null value of zero (Fig. 1, $p < 0.001$). The improvement in mycorrhizal abundance was accompanied by a significant increase in plant growth in the inoculated treatments, with a cumulative Fisher’s $z_{\text{plant}}$ of 0.57 (0.36–0.91, 95% CI) across all 27 trials that measured plant responses (Fig. 2, $p < 0.001$). These results supported hypotheses 1 and 2, respectively. Moreover, the inoculation effects did not notably decline with longer exposure to field conditions (Fig. 3). Specifically, the effects of inoculation on PRLC ($Q = 0.332, p = 0.498$) and plant growth ($Q = 0.695, p = 0.565$) did not vary significantly with the duration of the field component.

The meta-analysis did not appear to be particularly sensitive to publication bias. Specifically, Kendall’s tau tests were not significant for mycorrhizal colonization ($r = 0.009, p = 0.949$) or plant responses ($r = 0.099, p = 0.469$). Moreover, fail-safe tests indicated that 76 mycorrhizal colonization trials with null results would need to be added to render the inoculation effect non-significant. For plant responses, 42 null-results trials would need to be added.

Variation Among Sources of Inocula

Sources of inocula elicited significantly different effects on PRLC (Fig. 1, $p = 0.047$). In particular, inocula from reference ecosystems and single species yielded higher increases in PRLC than did inoculum from commercial sources (Fig. 1). The pairwise differences between inocula from reference ecosystems and commercial sources supported hypothesis 3. We note that values of Fisher’s $z_{\text{plant}}$ associated with the different inocula sources tended to display the same pattern as for Fisher’s $z_{\text{PRLC}}$, but in this case, differences among sources were not significant (Fig. 2, $p = 0.317$).

Other Factors

We observed no significant differences in Fisher’s $z_{\text{PRLC}}$ between nursery-inoculated versus field-inoculated trials (Fig. 1, $p = 0.573$), AM versus ECM fungi ($H = 0.369, p = 0.458$), or ecosystem types ($p = 0.372$). Moreover, Fisher’s $z_{\text{plant}}$ did not differ significantly between inoculation methods.
Discussion

Our findings suggest that restoration ecologists can intentionally increase the abundance of mycorrhizal fungi in degraded ecosystems, and thus improve the establishment of native plants. Moreover, these benefits can last up to several years. Specifically, mycorrhizal colonization of plant roots in field-based restoration projects significantly increased, on average, when mycorrhizal inocula was added. At the same time, plant performance in the field significantly improved. Neither effect declined significantly with time. These responses are consistent with our understanding of the ecology of mycorrhizal fungi—they are important plant mutualists (Allen et al. 2003b; Smith & Read 2008; Hoeksema et al. 2010) that are sensitive to anthropogenic disturbance (Cudlin et al. 2007; Compant et al. 2010; Pickles et al. 2012; Mohan et al. 2014) and may require restoration in their own right.

Moreover, certain sources of inocula were more effective than others. When fungal inoculum from a reference ecosystem or a single fungal taxon was used to inoculate field restoration projects, PRLC increased significantly more than when commercial inoculum was used. Likewise, Sylvia et al. (1993) examined the effects of different inoculum types in Florida dune restorations and found that native mycorrhizal inocula consistently yielded greater mycorrhizal colonization than commercial inocula. Higher root colonization from reference inocula could result from complementary interactions between the fungi and host plants that have developed over time under comparable conditions. In contrast, when a commercial source of inoculum is used, inoculation may shift the composition of the mycorrhizal community away from specialist native mycorrhizal fungi toward generalist “weedy” mycorrhizal fungi that might be less effective mutualists for native plants (Barea et al. 2011). Indeed, Koch et al. (2011) found that incorporating exotic mycorrhizal fungi into a degraded site substantially changed mycorrhizal communities—even more than plant invasions did. Mycorrhizal community composition may influence restoration outcomes because mycorrhizal species differ in their ability to form relationships with a given plant species, and in their responses to environmental conditions (van der Heidjin 2002; Klironomos 2003; Morris et al. 2007; Hoeksema et al. 2010). The difference observed here in effectiveness of inoculum sources is consistent with this notion.

Researchers have long investigated the role of mycorrhizal fungi in ecological restoration (Ramos-Zapata et al. 2006; Siguenza et al. 2006; van der Wal et al. 2006; White et al. 2008;...
Vargas et al. 2010). For example, Skujins and Allen (1986) recommended facilitating mycorrhizal growth in degraded sites via inoculation and soil organic retention. Recently, Piñeiro et al. (2013) synthesized data from restoration projects in degraded drylands to compare effectiveness of various restoration techniques. They reported that inoculation with mycorrhizal fungi was generally better than tree shelters, organic amendments, and hydrogels in improving the growth and survival of seedlings. Likewise, Barea et al. (2011) reviewed the use of mycorrhizal inoculation in revegetation projects on degraded semiarid lands in Southeast Spain, finding that inoculation improved both plant development and soil quality. Barea et al. (2011) also compared native inoculum from reference ecosystems versus exotic commercial inoculum and found that in dry environments, native, drought-adapted mycorrhizal fungi improve plant performance more than non-native mycorrhizal fungi. Our meta-analysis extends these observations beyond drylands, and suggests that inoculation could improve colonization and plant growth in a variety of ecosystems.

Similar findings have been documented in studies that did not involve restoration. For example, Lekberg and Koide (2005) conducted a meta-analysis on agricultural systems, and reported that mycorrhizal colonization and plant biomass was augmented by inoculation. Moreover, Hoeksema et al. (2010) conducted a broad meta-analysis of the effects of mycorrhizal inoculation on plant growth in studies based in laboratories, agricultural fields, plantations, and natural ecosystems (including one restoration project). They observed increases in plant growth in response to inoculation in the field as well as the laboratory. None of these other meta-analyses compared inoculation methods in the nursery to those in the field.

In our meta-analysis, inoculation of mycorrhizal fungi increased plant performance, on average. Plant responses to inoculation treatments largely mirrored those of mycorrhizal colonization. If inoculation increases PRLC, then this in turn increases the surface area of mycorrhizal fungi interacting with plant roots and accessing nutrients in the soil environment. Plants with greater PRLC would then benefit from receiving more nutrients from mycorrhizal fungi than would uninoculated plants (Fitter 1985; Read 1999; Varma & Hock 1999; Allen et al. 2003b). In fact, Barea et al. (2011) demonstrated that inoculation increases outplanting performance, survival, and plant biomass in restoration projects in semiarid lands, ostensibly because mycorrhizal fungi improve plant resistance to the drought stress, nutrient deficiency, and soil degradation that are common in degraded ecosystems.

Our findings can be useful in developing best practices in restoration ecology. Intentional inoculation could improve the growth and establishment of a mycorrhizal community in restored ecosystems, which may benefit aboveground vegetation communities. In addition, using inoculum from a reference ecosystem or a species-specific inoculum may increase PRLC more than a commercial inoculum. The use of inocula from reference ecosystems could also be economically advantageous for practitioners who otherwise might purchase commercial inocula. In large-scale projects, the cost of commercial inocula would not be trivial. There was no significant difference between PRLC in species-specific inoculum and from reference ecosystems, yet it is more technically challenging and time consuming to isolate mycorrhizal species than it is to obtain inoculum from a reference ecosystem.

When obtaining inocula from undisturbed sites, practices that might harm reference ecosystems should be avoided. To reduce disturbance, sourcing small volumes of soil from reference sites to inoculate plants in the greenhouse prior to outplanting in the field may be preferable to transferring large volumes of native soil (Cairns 1995). Furthermore, sourcing inoculum from edges of reference ecosystems could decrease disturbance in untrammeled sites (Mitsch & Jørgensen 2004). Sourcing inocula from restored sites with similar historical disturbances could be advantageous, because fungal propagules from these restored sites may be adapted to the special conditions associated with a particular disturbance (Greipsson 2010; Orlowska et al. 2011). In cases where reference sites are slated for development, topsoil containing fungal propagules could be salvaged from the reference site prior to construction or development (Rowe et al. 2007). Nevertheless, it is worth considering that after several months, soil stockpiling may reduce mycorrhizal abundance and compromise the effectiveness of this inoculum source (Galatowitsch 2012). The most appropriate inoculum choice depends on several factors, but altogether, it may be more practical and economically feasible for restoration ecologists to integrate reference inoculum into their restoration protocols, instead of species-specific or commercial inoculum.

The number of trials represented in this meta-analysis is somewhat small, because we restricted our selection to restoration projects that assessed PRLC. Nevertheless, we were able to detect significant effects of inoculation across trials. Furthermore, inoculation effects were positive in the majority of trials—only four of 28 trials reported a decline in PRLC, and three of 27 in plant growth. Nevertheless, we note that even though a variety of ecosystem types were represented, the trials were mostly based in temperate ecosystems in the Northern Hemisphere. Additional research in tropical ecosystems and the Southern Hemisphere would be valuable.

Although mycorrhizal fungi are just one component of a broader belowground community, they are the only component of the microbial community we examined in this meta-analysis. This focus is deliberate; O’Neill et al. (1991) posited that mycorrhizal fungi are “keystone mutualists” in terrestrial ecosystems, and therefore may exert a disproportionate influence on other soil microbes on the site. If mycorrhizal fungi affect community structure and ecosystem processes, and thus restoration outcomes, then they may influence other members of the soil microbial community. Moreover, mycorrhizal inoculation may increase plant cover through added plant biomass, which could lead to increased protection of exposed soil surfaces from solar radiation and other harsh environmental conditions. This added plant biomass would also provide substrate for decomposer microbes that may provide additional benefits to restored ecosystems, such as erosion control or increased soil organic matter. Future studies exploring the role of mycorrhizal...
inoculation in influencing the total microbial community and ecosystem processes at restoration sites would clarify the direct and indirect roles of these fungi (Brunson et al. 2010; Kulmatiski 2011; Binet et al. 2013).

In conclusion, this meta-analysis indicates that mycorrhizal fungi can be directly manipulated in many restoration sites via inoculation. In turn, inoculation generally improved restoration success by increasing plant performance. Moreover, sources of inocula varied in their effects on mycorrhizal colonization. In particular, the use of inoculum from reference ecosystems may be particularly effective and practical, compared with species-specific and commercial inocula. Land managers may wish to consider incorporating mycorrhizal fungi in their restoration efforts to better facilitate the establishment of below- and aboveground components of ecosystems.

Acknowledgments
We thank the authors of all studies included in this meta-analysis. We thank S. Allison, A. Bruce, L. Cat, J. Chan, S. Davis, T. Huxman, C. Looby, Y. Marusenko, J. Martiny, C. Nguyen, A. Romero, and P. Tang for technical assistance and intellectual feedback. This study was funded by the Center for Environmental Biology grant to K. Treseder and M. Maltz, entitled Fungal Facilitators of Ecosystem Services. Neither of the authors of the above manuscript has declared any conflict of interest, which may arise as being named as an author on the manuscript.

LITERATURE CITED
Al Agely A, Sylvia DM (2008) Compatible host/mycorrhizal fungus combinations for micropropagated sea oats: II. Field evaluation. Mycorrhiza 18:257–261
Alguacil MD, Torrecillas E, Kohler J, Roldán A (2011) A molecular approach to ascertain the success of “in situ” AM fungi inoculation in the revegetation of a semi-arid, degraded land. Science of the Total Environment 409:2874–2880
Allen EB, Allen ME, Egerton-Warburton LM, Corkidi L, Gomez-Pompa A (2003a) Impacts of early- and late-seral mycorrhizae during restoration in seasonal tropical forest, Mexico. Ecological Applications 13:1701–1717
Allen MF, Allen EB, Egerton-Warburton LM, Gomez-Pompa A (2005) Effects of mycorrhizae and nontarget organisms on restoration of a seasonal tropical forest in Quintana Roo, Mexico: factors limiting tree establishment. Restoration Ecology 13:325–333
Allen MF, MacMahon JA (1985) Impact of disturbance on cold desert fungi: comparative microscale dispersion patterns. Pedobiologia 28:215–224
Allen MF, Smith WK, Moore TS, Christensen M (1981) Comparative water relations and photosynthesis. New Phytologist 88:683–693
Allen MF, Swenson W, Querejeta JI, Egerton-Warburton LM, Treseder KK (2003b) Ecology of mycorrhizae: a conceptual framework for complex interactions among plants and fungi. Annual Review of Phytopathology 41:271–303
Ambler JR, Young JL (1977) Techniques for determining root length infected by vesicular-arbuscular mycorrhizae. Soil Science Society of America Journal 41:551–556
Barea JM, Palenzuela J, Cornejo P, Sánchez-Castro I, Navarro-Fernández C, López-García A, Estrada B, Aizcón R, Ferrol N, Aizcón-Aguilar C (2011) Ecological and functional roles of mycorrhizas in semi-arid ecosystems of Southeast Spain. Journal of Arid Environments 75:1292–1301
Binet MN, Sage L, Malan C, Clement JC, Redeker D, Wipf D, Geremia RA, Lavorel S, Mouhamadou B (2015) Effects of mowing on fungal endophytes and arbuscular mycorrhizal fungi in subalpine grasslands. Fungal Ecology 6:248–255
Boddington CL, Dodd JC (2000) The effect of agricultural practices on the development of indigenous arbuscular mycorrhizal fungi. I. Field studies in an Indonesian ultisol. Plant and Soil 218:137–144
Bozzolo FH, Lipson DA (2013) Differential responses of native and exotic coastal sage scrub plant species to N additions and the soil microbial community. Plant and Soil 371:37–51
Brundrett ML, Melville L, Peterson L (1994) Practical methods in mycorrhiza research: based on a workshop organized in conjunction with the Ninth North American Conference on Mycorrhizae, University of Guelph, Ontario, Canada. Mycologue Publications, Waterloo, Ontario
Brunson JL, Pyke DA, Perakis SS (2010) Yield responses of ruderal plants to sucrose in invasive-dominated sage steppe of the Northern Great Basin. Restoration Ecology 18:304–312
Cairns J (1995) Rehabilitating damaged ecosystems. Lewis Publishers, Boca Raton, Florida
Caravaca F, Alguacil MM, Figuerroa D, Barea JM, Roldán A (2003) Re-establishment of Retama sphaerocarpa as a target species for reclamation of soil physical and biological properties in a semi-arid Mediterranean area. Forest Ecology and Management 182:49–58
Christensen M, Allen MF (1980) Effect of VA mycorrhizae on water stress tolerance and hormone balance in native western plant species. University of Wyoming, Laramie
Compant S, van der Heijden MG, Sesitisch A (2010) Climate change effects on beneficial plant-microorganism interactions. FEMS Microbiology Ecology 73:197–214
Cook KL, Wallender WW, Bledsoe CS, Pasternack G, Upadhyaya SK (2011) Effects of native plant species, mycorrhizal inoculum, and mulch on restoration of reservoir sediment following dam removal, Elwha River, Olympic Peninsula, Washington. Restoration Ecology 19:251–260
Cudlin P, Kieliszewska-Rojucka B, Rudawksa M, Grebenc T, Alberton O, Leotto T, et al. (2007) Fine roots and ectomycorrhizas as indicators of environmental change. Plant Biosystems 141:406–425
Cuenca G, De Andrade Z, Escalante G (1998) Arbuscular mycorrhizae in the rehabilitation of fragile degraded tropical lands. Biology and Fertility of Soils 26:107–111
de Aragón JM, Fischer C, Bonet JA, Olivera A, Olíach D, Colinas C (2012) Economically profitable post fire restoration with black truffle (Tuber melanosporum) producing plantations. New Forests 43:615–630
de Souza RG, da Silva DKA, de Mello CMA, Goto BT, da Silva FSB, Sampiao EVSP, Maia LC (2013) Arbuscular mycorrhizal fungus in revegetated mined dunes. Land Degradation & Development 24:147–155
de Souza RG, Goto BT, da Silva DKA, da Silva FSB, Sampiao EVSP, Maia LC (2010) The role of arbuscular mycorrhizal fungi and cattle manure in the establishment of Tocoyena selloana Schum. in mined dune area. European Journal of Soil Biology 46:237–242
Dupponnois R, Ouahmane L, Kane A, Thioulouse J, Hafidi M, Boumezough A, Prin Y, Baudoin E, Galiana A, Dreyfus B (2011) Nurse shrubs increased the early growth of Cupressus seedlings by enhancing belowground mutualism and soil microbial activity. Soil Biology & Biochemistry 43:2160–2168
Egerton-Warburton LM, Allen EB (2000) Shifts in arbuscular mycorrhizal communities along an anthropogenic nitrogen deposition gradient. Ecological Applications 10:484–496
Ehrenfeld JG, Ravit B, Elgersma K (2005) Feedback in the plant-soil system. Annual Review of Environment and Resources 30:75–115
Estrada B, Arroca R, Maathuis FJ, Barea JM, Ruiz-Lorazo JM (2013) Arbuscular mycorrhizal fungi native from a Mediterranean saline area enhance maize tolerance to salinity through improved ion homeostasis. Plant, Cell & Environment 36:1771–1782
Fitter AH (1985) Functioning of vesicular-arbuscular mycorrhizas under field conditions. New Phytologist 99:257–265
Galatowitsch SM (2012) Ecological restoration. Sinauer Associates, Sunderland, Massachusetts

Restoration Ecology September 2015
Shorea balangeran

Graham LLB, Turjaman M, Page SE (2013) Shorea balangeran and Dyera polyphylla (syn. Dyera lowii) as tropical pea swamp forest restoration transplant species: effects of mycorrhizae and level of disturbance. Wetlands Ecology and Management 21:307–321

Greipsson S (2010) Restoration ecology. Jones and Bartlett Learning, Burlington, Massachusetts

Hayman DS (1982) Influence of soils and fertility on activity and survival of vesicular-arbuscular mycorrhizal fungi. Phytopathology 72: 1119–1125

Helgason T, Daniell TJ, Husband R, Fitter AH, Young JP (1998) Ploughing up the wood-wide web? Nature 394:431

Hirrell MC, Gerdemann JW (2011) The effect of elevated carbon dioxide on the interaction between Eucalyptus grandis and diverse isolates of Pisolithus sp. is associated with a complex shift in the root transcriptome. New Phytologist 206:1423–1436

Powell JR, Parrent JL, Hart MM, Klironomos JN, Rillig MC, Maherali H (2009) Phylogenetic trait conservatism and the evolution of functional trade-offs in arbuscular mycorrhizal fungi. Proceedings of the Biological Sciences 276:4237–4245

Picketts BJ, Egger KN, Massicotte HB, Green DS (2012) Ectomycorrhizas and climate change. Fungal Ecology 5:73–84

Piñeiro J, Maestre FT, Bartolome L, Valdecaros A (2013) Ecotechnology as a tool for restoring degraded drylands: a meta-analysis of field experiments. Ecological Engineering 61:133–144

Plett JM, Kohler A, Khachane A, Keniry K, Plett KL, Martin F, Anderson JC (2015) The effect of elevated carbon dioxide on the interaction between Eucalyptus grandis and diverse isolates of Pisolithus sp. is associated with a complex shift in the root transcriptome. New Phytologist 206:1423–1436

Powell JR, Parrent JL, Hart MM, Klironomos JN, Rillig MC, Maherali H (2009) Phylogenetic trait conservatism and the evolution of functional trade-offs in arbuscular mycorrhizal fungi. Proceedings of the Biological Sciences 276:4237–4245

Querejeta JI, Barea JM, Allen MF, Caravaca F, Roldán A (2003) Differential response of delta C-13 and water use efficiency to arbuscular mycorrhizal fungal activity. Ecology Letters 13:1627–1639

Kohl L, Oehl F, van der Heijden MGA (2014) Agricultural practices indirectly influence plant productivity and ecosystem services through effects on soil biota. Ecological Applications 24:1842–1853

Kulmatiski A (2011) Changing soils to manage plant communities: activated carbon as a restoration tool in ex-arable fields. Restoration Ecology 19:102–110

Lekberg Y, Koide RT (2005) Is plant performance limited by abundance of arbuscular mycorrhizal fungi? A meta-analysis of studies published between 1988 and 2003. New Phytologist 168:189–204

McGonigle TP, Fitter AH (1988) Growth and phosphorus inflows of Trifolium repens L with a range of indigenous vesicular-arbuscular mycorrhizal infection levels under field conditions. New Phytologist 108: 59–65

McGonigle TP, Miller MH (1996) Development of fungi below ground in association with plants growing in disturbed and undisturbed soils. Soil Biology & Biochemistry 28:263–269

McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA (1990) A new method which gives an objective-measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytologist 115:495–501

Mitsch WJ, Jørgensen SE (2004) Ecological engineering and ecosystem restoration. Wiley, Hoboken, New Jersey

Mohan JE, Cowden CC, Baas P, Dawadi A, Frankston PT, Helmick K, et al. (2014) Mycorrhizal fungi mediation of terrestrial ecosystem responses to global change: mini-review. Fungal Ecology 10:3–19

Morris WF, Hufbauer RA, Agrawal AA, Bever JD, Borowicz VA, Gilbert GS, et al. (2007) Direct and interactive effects of enemies and mutualists on plant performance: a meta-analysis. Ecology 88:1021–1029

Moss B (1957) Growth and chemical composition of mycorrhizal and non-mycorrhizal apples. Nature 179:923–924

O’Neill EG, O’Neill RV, Norby RJ (1991) Hierarchy theory as a guide to mycorrhizal research on large-scale problems. Environmental Pollution 73:271–284

Orlowska E, Orlowski D, Mesiasz-Przybyłowicz J, Turnau K (2011) Role of mycorrhizal colonization in plant establishment on an alkaline gold mine tailing. International Journal of Phytoremediation 13:185–205

Pagano MC, Scotti MR, Caballo MN (2009) Effect of the inoculation and distribution of mycorrhizae in Phaltymenia reticulata Benth under monoculture and mixed plantation in Brazil. New Forests 38:197–214

Palenzuela J, Azcón-Aguilar C, Figueroa D, Caravaca F, Roldán A, Barea JM (2002) Effects of mycorrhizal inoculation of shrubs from Mediterranean ecosystems and composted residue application on transplant performance and mycorrhizal developments in a desertified soil. Biology and Fertility of Soils 36:170–175

Palenzuela J, Barea JM (2006) The impact of ecosystem degradation on arbuscular mycorrhizal fungal diversity in the rhizosphere of plant species of singular ecological value. Proceedings of the 5th International Conference on Mycorrhiza. Granada, Spain

Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society 55:158

Ramos-Zapata JA, Orellana R, Allen EB (2006) Establishment of Desmoncus orthocharanus martius (Arecalesae): effect of inoculation with arbuscular mycorrhizae. Revista De Biologia Tropical 54:65–72

Richter BS, Stutz JC (2002) Mycorrhizal inoculation of big sacaton: implications for grassland restoration of abandoned agricultural fields. Restoration Ecology 10:607–616

Rincón A, de Felipe MR, Fernández-Pascual M (2007) Inoculation of Pinus halepensis with selected ectomycorrhizal fungi improves seedling establishment two years after planting in a degraded gypsium soil. Mycorrhiza 18:102–110

Roldán A, Querejeta JI, Albaladejo J, Castillo V (1996) Growth response of Pinus halepensis to inoculation with Pisolithus arhizus in a terraced rangeland amended with urban refuse. Plant and Soil 179:35–43

Rosenberg MS, Adams DC, Gurevitch J (2007) MetaWin: statistical software for meta-analysis (Version 2.1). Sinauer Associates, Sunderland, Massachusetts
Mycorrhizal inoculation in restoration

Rosenthal R (1991) Meta-analytic procedures for social research. Revised ed. Sage Publications, Newbury Park, California

Rowe HL, Brown CS, Claassen VP (2007) Comparisons of mycorrhizal responsiveness with field soil and commercial inoculum for six native montane species and Bromus tectorum. Restoration Ecology 15:44–52

Saif SR (1981) The influence of soil aeration on the efficiency of vesicular-arbuscular mycorrhizae: effect of soil oxygen on the growth and mineral uptake of Eupatorium-odoratum L inoculated with Glomus-macrocarpus. New Phytophys 88:649–659

Sánchez-Castro I, Ferrol N, Cornejo P, Barea JM (2012) Temporal dynamics of arbuscular mycorrhizal fungi colonizing roots of representative shrub species in a semi-arid Mediterranean ecosystem. Mycorrhiza 22:449–460

Sheng M, Lalande R, Hamel C, Ziadi N, Shi YC (2012) Growth of corn roots and associated arbuscular mycorrhizae are affected by long-term tillage and phosphorus fertilization. Agronomy Journal 104:1672–1678

Siguenza C, Corkidi L, Allen EB (2006) Feedbacks of soil inoculum of mycorrhizal fungi altered by N deposition on the growth of a native shrub and an invasive annual grass. Plant and Soil 286:153–165

Skujins J, Allen MF (1986) Use of mycorrhizal for land rehabilitation. MIRCEN Journal 2:161–176

Smith MR, Charvat I, Jacobson RL (1998) Arbuscular mycorrhizae promote establishment of prairie species in a tallgrass prairie restoration. Canadian Journal of Botany-Revue Canadienne De Botanique 76:1947–1954

Smith SE, Read DJ (2008) Mycorrhizal symbiosis. Academic Press, San Diego, California

Stahl PD, Williams SE, Christensen M (1988) Efficacy of native vesicular arbuscular mycorrhizal fungi after severe soil disturbance. New Phytophys 110:347–354

Sylvia DM (1989) Nursery inoculation of sea oats with vesicular-arbuscular mycorrhizal fungi and outplanting performance on Florida beaches. Journal of Coastal Research 5:747–754

Sylvia DM, Jarstfer AG, Vosatka M (1993) Comparisons of vesicular-arbuscular mycorrhizal species and inocula formulations in a commercial nursery and on diverse florida beaches. Biology and Fertility of Soils 16:139–144

Thrall PH, Millsom DA, Jeavons AC, Waayers M, Harvey GR, Bagnall DI, Brockwell J (2005) Seed inoculation with effective root-nodule bacteria enhances revegetation success. Journal of Applied Ecology 42:740–751

Tian H, Gai JP, Zhang JL, Christie P, Li XL (2009) Arbuscular mycorrhizal fungi in degraded typical steppe of inner Mongolia. Land Degradation & Development 20:41–54

Trappe JM (1981) Mycorrhizae and productivity of arid and semiarid range-lands. Pages 581–599. In: Manassah JT, Briske EJ (eds) Advances in food producing systems for arid and semiarid lands. Academic Press, New York

Treseder KK (2013) The extent of mycorrhizal colonization of roots and its influence on plant growth and phosphorus content. Plant and Soil 371:1–13

van der Heijden MGA (2002) Arbuscular mycorrhizal fungi as a determinant of plant diversity: in search of underlying mechanisms and general principles. Springer-Verlag, Berlin, Germany

van der Heijden MGA, Martin FM, Selosse MA, Sanders IR (2015) Mycorrhizal ecology and evolution: the past, the present, and the future. New Phytologist 205:1406–1423

van der Wal A, van Veen JA, Pijl AS, Summerbell RC, de Boer W (2006) Constraints on development of fungal biomass and decomposition processes during restoration of arable sandy soils. Soil Biology & Biochemistry 38:2890–2902

Vargas R, Hasselquist N, Allen EB, Allen MF (2010) Effects of a hurricane disturbance on aboveground forest structure, arbuscular mycorrhizae and belowground carbon in a restored tropical forest. Ecosystems 13:118–128

Varma A, Hock B (1999) Mycorrhiza: structure, function, molecular biology, and biotechnology. Springer-Verlag, Berlin, Germany

Walker RF (2005) Comparison of organic and chemical soil amendments used in the reforestation of a harsh Sierra Nevada site. Restoration Ecology 11:466–474

White JA, Tallaksen J, Charvat I (2008) The effects of arbuscular mycorrhizal fungal inoculation at a roadside prairie restoration site. Mycologia 100:6–11

Wolfe BE, Husbands BC, Klironomos JN (2005) Effects of a belowground mutualism on an aboveground mutualism. Ecology Letters 8:218–223

Coordinating Editor: Christine Hawkes

Received: 28 January, 2015; First decision: 12 March, 2015; Revised: 23 April, 2015; Accepted: 24 April, 2015; First published online: 1 June, 2015

Restoration Ecology September 2015