Influence of Soil Microorganisms on Plant Growth and Fitness

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INFLUENCE OF SOIL MICROORGANISMS ON PLANT GROWTH AND FITNESS

A Thesis

Presented to

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by

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Abstract

Most terrestrial plants benefit from symbiosis with soil microorganisms. Symbiotic bacteria and fungi have wide-ranging effects on host plants, including improved nutrition, disease resistance, and drought tolerance. Association with Arbuscular Mycorrhizal Fungi (AMF) can enhance growth and protect plants from environmental stressors while they share products of photosynthesis with the resident fungi. Scores of studies indicate that mycorrhizal plants are more resistant to drought stress than their non-mycorrhizal counterparts. Use of microbes as a plant and soil supplement in home gardens represents a sustainable alternative to resource-intensive inputs and may allow for reduced water use. I investigated the effects of commercially-available microbial products and a peat soil amendment on strawberry plants under water stress. In a greenhouse experiment, strawberry plants were grown across factorial treatments of two AMF mixtures, two types of soil, and two water treatments. Inoculated strawberry plants had greater total dry weight biomass and leaf surface area than un-inoculated plants but showed no increases in reproductive tissues. Plants grown in peat-amended soil had improved growth in all measures except number of fruits and flowers. This data shows that modification of urban and suburban soil with peat and soil microorganisms can improve plant biomass.

Despite a large body of literature that describes the effects of mycorrhizal colonization on plant resistance to water deficit, reviews of these works are only in narrative form and it is therefore difficult to quantify the magnitude of the effect. In a meta-analysis, we examined the
effect of mycorrhizal colonization on growth and yield of plants exposed to water-deficit stress. We found that, in terms of biomass measurements, mycorrhizal plants are more resistant to water stress compared to non-mycorrhizal plants. When variables such as habit, lifecycle, or water stress level are considered, differences in mycorrhizal effect on growth between variables are observed. For example, while growth of both annual and perennial plants is improved by symbiosis, perennials respond more favorably to colonization than annuals. Overall, meta-analysis reveals a quantifiable corroboration of the commonly held view that mycorrhizal symbiosis improves plant’s resistance to water-deficit conditions.

Efforts to restore native vegetation may benefit from treatment of native seeds and plants with AMF. Invasive plants can alter ecosystems by out-competing native species or changing the physicochemical properties of the local environment. Tamarisk (Tamarix spp.), a shrubby tree native to the Europe, Asia, and Africa, has now invaded 1.6 million acres of land in the western United States. Tamarisk exudes salt from its leaves that accumulates in surrounding soils and can affect the growth of native plant species. Here we examined the effect of AMF on the growth of an array of restoration species native to riparian areas of the southwest US under varied water and salinity regimes. AMF affected only one plant species with regard to root biomass and root:shoot ratio. Infection rates of inoculated plants varied from 0-61% and were limited by high salinity. We also found that salinity and water have strong effects on several native species, and that biomass decreases with increasing salinity or decreasing water.
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Chapter One: Influence of arbuscular mycorrhizae on growth of plants under water-deficit

Introduction

An estimated 80% of terrestrial plants have mycorrhizal associations during some or all of their life stages (Schussler et al. 2001). Thousands of studies have tested the physiological effect of mycorrhizae on plants, and many show a positive influence on plant function and fitness; it is commonly thought that symbiosis with vesicular arbuscular mycorrhizal fungi (VAM or AMF) is highly beneficial for plants (Ruiz-Lozano et al. 1995a, Clark and Zeto 2000). Mycorrhizal infection is shown to have wide-ranging effects on host plants, from improved nutrition (Marschner and Dell 1994) and stress tolerance (Marulanda et al. 2009) to herbivore defense (Gange and West 1994, Hempel et al. 2009) and disease resistance (Trotta et al. 1996, Liu et al. 2007). Fungal hyphae can even act as a means of resource sharing between two otherwise unconnected plants (Chiariello et al. 1982).

Arbuscular mycorrhizae are in the fungal phylum Glomeromycota, separated from other fungal groups by morphological and molecular traits (Schussler et al. 2001). These organisms develop arbuscules and vesicles, structures that distinguish them from other filamentous fungi, inside of plant roots. Hyphae are fungal filaments (Figures 1 and 2),
which make up the vegetative portion of a fruiting body, collectively known as mycelium. Arbuscules (Figure 1) are highly branched fungal hyphae that act as the point of bidirectional transport of nutrients (from the fungus to the plant) and photosynthate (from the plant to the fungus) between the symbiotic partners (Parniske 2008). Vesicles (Figure 2) are thought to be storage organs which, when found in plant roots, contain very high concentrations of neutral lipids and phospholipids (Jabajihare et al. 1984).

Infection of plant roots is preceded by recognition of a host by fungal hyphae or spores. It is believed that root exudates and associated signaling molecules emitted by a plant are responsible for “inviting” infection at specific locations along the root, especially during times of stress. For example, under water-stress conditions, Bahia grass (Paspalum notatum) exudes high concentrations of tryptophan dimer compounds that are not seen when plants are sufficiently watered; these polymers induce hyphal growth and branching of Gigaspora margarita and Glomus caledonium toward roots (Horii et al. 2009). Strigolactones are other widely studied plant signaling molecules that also stimulate germination and hyphal branching of AMF (Akiyama et al. 2005). Besserer et al. (2006) showed that a possible molecular mechanism of branching factors was induction of mitochondrial activity in the fungus.

Interactions between microbial organisms and their hosts range from mutualism to parasitism. Most agronomically important fungal organisms are classified as disease-causing crop pests, parasites that kill or injure their hosts while consuming tissues or resources. In contrast, there are mutualistic microbes that improve host fitness or
resource acquisition in exchange for other resources or habitat. Commensal relationships, in which one partner benefits while the other is left unimproved but unharmed, occupy the center of the range. AMF may span the continuum from parasite to mutualist depending upon environmental and biological conditions (Kogel et al. 2006). While symbiosis yields increased access to and solubilization of phosphorous (P), in high-P soil conditions mycorrhizal infection may actually result in a carbon deficit to the plant resulting in a negative growth response (Graham and Eissenstat 1998, Mortimer et al. 2005). Although entirely non-mycorrhizal plants are rare in nature, we know that certain soil disturbances, such as tillage, not only break up large water-stable aggregates (Beare et al. 1994) but can also reduce microbial abundance (Drijber et al. 2000). Urban and suburban development includes top soil removal and compaction, depressing the local soil biota as well as disfiguring soil structures. Manipulative studies using non-mycorrhizal controls therefore can illustrate the benefits of inoculating disturbed and depleted soils.

Presence of mycorrhizae in soil has some influence on plant fitness independent of root colonization. Auge et al. (2001) showed soil that had mycorrhizal cowpea (*Vigna unguiculata*) grown in it for seven months had more water-stable aggregates and extraradical hyphal densities than soil with non-mycorrhizal cowpea, even when root mass, length, surface area, and density were similar. A later experiment (Auge 2004), in which wild-type and non-colonizing mutant bean plants (*Phaseolus vulgaris*) were grown in soil colonized for 12 months with *Glomus intraradices* and *Gigaspora margarita* on
Sorghum (*Sorghum bicolor*), demonstrated that about half of the increase in stomatal conductance \((g_s)\) was derived from soil colonization by AMF, illustrated by the increased \(g_s\) in non-colonizing bean relative to the wild-type. In other words, mycorrhizal soil affected non-mycorrhizal plants growing in that soil.

This work is intended to establish a statistical quantification of the effect of mycorrhizae on water-stressed plants. This is not an examination of the mechanistic or molecular means of AMF influence, rather the frequency and degree of that influence on plants. Collectively, the studies included in this meta-analysis examine 36 measures of plant growth of 43 host species in 41 plant genera and at least 18 species of AMF within five genera. We framed our analysis to address the question of whether symbiosis has a real effect on plant resistance to water-deficit and, if so, what is the overall effect size? Although vote-counting would indicate that mycorrhizal symbiosis does improve plant performance, reporting effect size is a better way to illustrate the magnitude of the effect. Other questions can subsequently be answered, dependent upon the volume and quality of the collected data. For example:

1) Is the size of the effect different between plant growth variables? In other words do variables such as lifecycle (annual v. perennial) or tissue (herbaceous v. woody) affect response to symbiosis?

2) Is the effect of symbiosis different among study sites, such as field or nursery v. greenhouse or growth chamber? Interpretations of study site data vary. One meta-analysis shows greenhouse plants significantly more improved by
mycorrhizae than field plants (Lekberg and Koide 2005) while another calls the effect “relatively unimportant” when other variables are controlled (Hoeksema et al. 2010).

3) Are certain plant functional groups more improved by colonization? Response to infection of some grasses vary (Hartnett et al. 1994) and there may not be a synergistic effect of co-inoculation of mycorrhizae and the N-fixing bacteria associated with legumes despite improving access to different macronutrients (Larimer et al. 2010).

4) Does the effect correlate to the stress level experienced by the plant? Are more stressed plants improved to a greater degree than less stressed plants? Plants can prevent mycorrhizal infection, in non-limiting conditions, and the carbon-costs of infection can be classified as parasitic if plants can independently obtain sufficient resources.

5) Does effect size differ among treatment species? Studies in our analysis examined single species of mycorrhizae, combinations of species, and consortiums of mycorrhizae from different origins however the large majority of experiments (89.7%) investigated the effects of treatments of species of Glomus. Are certain species of Glomus more effective at improving plant-water relations?

6) Are regions of plant growth affected differently by mycorrhizae? Depending on the plant species and the variable being analyzed, we might expect better response to infection expressed in specific areas of growth. If we group measures into
categories that represent aboveground, belowground, reproductive, or whole plant
growth, do we see improvements in some of these categories more than others
either between or within a variable such as lifecycle or study site?

Meta-analyses we conducted to answer these questions included measures of all plant
growth parameters using the log-transformed response ratio, or log response ratio (LRR).

LRR measures the proportionate change between treatment and control groups (Hedges
et al. 1999), log-transformed for variability. In our analysis, this is mycorrhizal (M) to
non-mycorrhizal (NM) plant growth, or ln(XM/XNM), transformed due to the high
variation in values of plant growth parameters collected.

Methods

Eligibility Criteria for Included Studies

We found studies through searches of Web of Science (Institute for Scientific
Information), ProQuest Dissertations & Theses (PQDT) and references therein, using
keywords *arbusc*, *mycorrhiz*, *water*, and *stress*. We used the Boolean-truncation (*)
to include variations of the primary terms of interest. These searches yielded 285
published and unpublished studies dating from 1983; however, works had to meet certain
eligibility criteria to be included. For instance, the analysis had to be a manipulative
study comparing drought-stressed plants with mycorrhizal treatment to a non-
mycorrhizal, drought-stressed sample. After reading abstracts of these 285 articles, we
rejected 221 papers based on these criteria and refined the list to 64 eligible studies. Still
more were excluded because data was only reported graphically and authors were unresponsive to requests for raw data, ultimately resulting in 54 eligible studies. For any study to be eligible, results had to be reported as tabular, numerical data. We omitted research with data that was not a measure of morphological growth or yield (e.g. biomass) and defined growth as a change in size or numbers of observable plant tissues between treatment and control groups after the study period. Parameters such as nutrient content, gene expression, photosynthetic rate, or other biochemical or metabolic effects, were not included in the meta-analysis.

**Study Coding**

From each of the 54 eligible studies (Appendix 1), we collected information on mycorrhizal classification, host plant classification, life-cycle, habit, test site, and growth measure as well as statistical data including sample size, mean effect, and standard deviation/error. In some cases, a mix or consortium of inoculum were used so we used substitute or collective terms when specific identification was not available. For example, we used a default label of ‘AMF’ if mycorrhizal genera were mixed or not identified and ‘spp’ if species were mixed or not identified within a genus. Additionally, we collected any relevant notes that were important to the treatment or result such as plant age, water content data, or drought period/severity (Appendix 2). Plant growth parameters had a broad range but followed a common motif, typically 2-3 words beginning with the plant part, tissue type, or collective term followed by the metric and units, such as *root dry weight (g)*. We considered 36 different measures of plant growth
in the analysis. When it was clear that the authors used terms synonymous with common plant terms, for example *foliar area* versus *leaf area*, we substituted the more common terminology. In some cases, similar measures were reported in different units; when this occurred, we converted the less commonly reported units to the most common. Shoot height, for instance, was most often reported in centimeters (cm) so if a study reported shoot height as 0.6 meters (m), we changed this to 60 cm. We also categorized nursery studies as ‘field’ and growth chamber experiments as ‘greenhouse’. Most of these variables were included in the selected studies or could be deduced from information provided (i.e. tissue type could be determined from the plant species) however even studies with some information missing could be included in subset analyses of the variables they did report. We coded studies to include the following variables: lifecycle (annual or perennial), habit (herbaceous or woody), study site (field or greenhouse), functional group (tree/shrub, forb, grass, or legume), water regime (water-stressed or well-watered), and water stress level (low, moderate, or severe).

**Calculating Effect Sizes**

Each unique assemblage of variables was coded as a single observation for calculating effect size. For example, a study might examine the effect of *Glomus mosseae* on leaf area (cm$^2$), root dry weight (g), and flower count of pepper and cucumber. These variables yield six effect sizes in combination: 1 mycorrhizal treatment x 2 plant species x 3 growth parameters. Complex, multi-factorial studies can, therefore, produce a large number of effect sizes.
Few studies examining water relations of plant-mycorrhizal ecology reported data sufficient for all meta-analytical methods. Many excellent papers were rejected from consideration simply for presenting data exclusively in a graphical format. Most researchers who did, in fact, report tabular, numerical data failed to include any measures of variation. Only 14 of the 54 experiments cited here provided adequate detail to calculate Hedges’ $d$, a commonly reported effect size. A few studies even failed to explicitly indicate sample size in their methods. One reason why many of these studies needed to be removed could be that their publication date, from the 1980s and 1990s, was before it was widely advocated to include effect size in published data. In graphically reported data, measures of variation are usually illustrated using error bars and although it is possible to digitize graphs to determine means and variation (Borowicz 2001), we elected to exclude them from meta-analysis. To broaden the analysis to all studies that minimally included mean effect and sample size, we used the natural log-transformed response ratio or Log Response Ratio (LRR). We calculated the effect size using MetaWin (Sinauer Associates, Sunderland, MA). Effect size values are positive if treatment yields an increase in the plant growth measure compared to untreated controls and negative if treatment is deleterious to plant growth.

**Results Analysis**

Tests for significance were conducted in JMP version 9 (SAS Institute Inc., Cary, NC) using one-way analysis of variance (ANOVA). Most tests were between binary variables such as annual versus perennial or herbaceous versus woody. Some tests,
however, were on summary statistics of certain treatments or measures that included more than two factors. If a significant difference was discovered between three or more variables, we followed with post-hoc tests using Tukey-Kramer HSD to determine which means were different. Statistical tests were conducted on effect size of water-stressed treatments unless otherwise indicated. We analyzed the overall effect of infection, the overall effect of infection between two or more variables, the improvement of categorical regions of plants growth, i.e. aboveground (AG), belowground (BG), reproductive (RP), or whole plant (WP) between variables, and the improvement of categorical regions of plants growth within a variable.

Results

Overall Effects

Under water-deficit conditions, mycorrhizal plants outperform non-mycorrhizal plants in most measures of growth and yield. The overall log response ratio of 0.324±0.020 (Figure 3) was determined from 602 effect size calculations of water-stressed plants. Only measures with an effect size n>1 were included in the calculations. The positive mean value indicates an overall plant growth promoting effect of mycorrhizae under water stress. Pod dry weight and grain head count (n = 20 and n = 4, respectively) were not significantly changed while shoot:root ratio (n = 3) and shoot growth (n = 2) were negatively affected by infection. Total calculations for some of these parameters may be inadequate. Indeed, grain head count, shoot:root ratio, and shoot
growth were measured only in single studies. When growth measures are organized into categories that represent the primary region of plant growth, i.e. AG, BG, RP, or WP, mean effect sizes are all positive. A difference in the overall effect of colonization is evident between regions of growth (F_s = 3.50, df = 3, 601, P = 0.015). Whole plant measures showed the most improvement from mycorrhizae. Significant differences are between WP and BG (P = 0.013) and AG and BG (P = 0.044). There are no differences between any other groups (Figure 4).

**Effects of Plant Habit Variables**

In response to drought stress, mycorrhizal annuals and perennials perform differently (F_s = 13.14, df = 1, 601, P = 0.0003) (Figure 5). Both respond favorably to symbiosis; however, perennial species show more growth overall. Mean LRR is 0.272±0.024 for annuals and 0.426±0.036 for perennials. WP growth in perennial plants is higher than annual plants (F_s = 17.52, df = 1, 139, P < 0.0001) but AG, BG, and RP growth is the same. Mycorrhizae have no effect on annual plants’ shoot dry weight (n=57), pod dry weight (n = 16), total dry weight (n = 26), or shoot:root ratio (n = 3). Among perennials, root:shoot ratio (n = 8) is unaffected and shoot growth (n = 2) is negatively influenced.

There is also a difference between herbaceous and woody plant response to mycorrhizal symbiosis (F_s = 9.07, df = 1, 601, P = 0.003) (Figure 6). BG growth is much greater in woody plants than herbaceous ones (F_s = 6.47, df = 1, 117, P = 0.012) but AG and WP growth is the same between the tissue types. Both WP and AG growth of
herbaceous species is more than BG (P = 0.028 and P = 0.044, respectively). Pod dry weight (n = 20), grain head count (n = 4), and shoot:root ratio (n = 3) of the herbaceous samples are not influenced. In woody species, root:shoot ratio (n = 6) was unaffected and shoot growth (n = 2) was reduced by infection. No growth region of woody plants was favored significantly more than another by infection and no reproductive structures of woody species were measured for comparison.

Effects of Study Site

We found that field and greenhouse results are the same overall (F_s = 0.98, df = 1, 592, P = 0.324) (Figure 7). Increase in RP biomass of greenhouse plants is greater than those in the field (F_s = 4.80, df = 1, 67, P = 0.032) and large WP gains are observed in field-grown plants compared to greenhouse-grown (F_s = 8.64, df = 1, 135, P < 0.0001). WP growth is strongly favored over BG (P = 0.001), RP (P = 0.0003), and AG (P < 0.0002) within field plants. No particular area of growth benefits more than another among greenhouse-grown plants. Plant growth in greenhouse studies is generally positively affected by mycorrhizae save for pod dry weight (n = 20), root:shoot ratio (n = 14), and shoot:root ratio (n = 3). In the field, grain head count (n = 4) and root dry weight (n = 7) are unchanged while shoot growth (n = 2) was weakened by infection.

Effects of Functional Group

Response of four functional groups to mycorrhizae varies significantly when drought-stressed (F_s = 14.08, df = 3, 601, P < 0.0001) (Figure 8). Trees and shrubs benefit most overall from colonization. Post-hoc tests reveal differences between all
functional groups except grasses and legumes. AG growth of trees and shrubs is higher than legumes (P = 0.002) but not forbs or grasses. The tree/shrub group also has the highest BG growth, significantly higher than legumes (P = 0.0009). No reproductive structures were measured on trees and shrubs but there are RP growth differences between the other functional groups (F$_s$ = 14.43, df = 2, 68, P < 0.0001). Forbs have the largest increase in biomass of RP parts and grew much more than legumes (P < 0.0001) and grasses (P = 0.0005). RP growth of grasses and legumes is not different. Still more differences exist in WP growth measures (F$_s$ = 6.18, df = 3, 92, P = 0.0007). Trees and shrubs are, again, the most improved, growing significantly better than grasses (P = 0.002). Forbs also grew better than grasses in response to mycorrhizae (P = 0.007).

Forbs show the most improvement in RP growth, significantly better than BG growth (P = 0.034). WP growth of forbs also benefits from infection, also significantly more than BG measures (P = 0.050). WP growth of legumes is increased more than BG growth (P = 0.009) and RP structures (P = 0.039). Growth regions of grasses and tree/shrubs are not improved more than any other.

**Effects of *Glomus* Species**

Species analysis (Figure 9) examined the effects of the seven most applied species of *Glomus* inoculum among all studies. These are, in order of most to least often used, *G. mosseae* (n = 133), *G. intraradices* (n = 127), *G. fasciculatum* (n = 59), *G. versiforme* (n = 53), *G. deserticola* (n = 49), mixtures of two or more *Glomus* species (n = 39), and *G. etunicatum* (n = 35). No difference is evident between any of these seven inoculum
treatments on overall plant improvement ($F_s = 1.92$, $df = 6, 490$, $P = 0.076$). Some mycorrhizae are more beneficial to some regions of plant growth than other species. Mixed inoculum improved RP growth more than all other species and *G. versiforme* increased WP growth more than *G. fasciculatum* ($P = 0.023$).

Most species also did not benefit any particular growth area over another area, including *G. deserticola*, *G. etunicatum*, *G. fasciculatum*, or *G. mosseae*. *G. intraradices* increases WP growth more than BG ($P = 0.006$) and AG ($P = 0.008$). Mixed species of *Glomus* augment RP biomass better than BG growth ($P = 0.021$). *G. versiforme* promotes WP growth more than RP ($P = 0.029$), BG ($P = 0.001$), and AG ($P = 0.008$).

**Effects of Water Regime and Water-stress Level**

The overall difference between well-watered and water-stressed plants that were both treated with mycorrhizae is not significant ($F_s = 3.74$, $df = 1, 869$, $P = 0.053$) (Figure 10). AG, BG, RP, and WP growth are all statistically similar whether well-watered or water-stressed. Furthermore, mycorrhizal association had no effect on response to low, medium, or high levels of stress experienced by plants ($F_s = 0.07$, $df = 2, 601$, $P = 0.932$) (Figure 11). Mycorrhizae had no effect on any particular growth region when well-watered. Water-stressed samples, however, do show differences in the effect on growth region ($F_s = 3.50$, $df = 3, 601$, $P = 0.015$). When water-stressed, BG growth was least improved. Both WP ($P = 0.013$) and AG ($P = 0.044$) benefitted more from infection than BG growth.
Some levels of stress do influence which area of growth is improved. Low stress ($F_s = 3.86, \text{ df } = 3, 64, P = 0.014$) favors WP over AG growth ($P = 0.047$) and moderate stress ($F_s = 2.91, \text{ df } = 3, 492, P = 0.034$) improves AG growth more than BG ($P = 0.036$). Severe stress does not change the effect on a growth area ($F_s = 2.69, \text{ df } = 3, 43, P = 0.059$).

**Discussion**

Our analysis included data from 54 published articles that explored the effects of AMF on growth of water-stressed plants. The experiments included 19 species of vesicular arbuscular mycorrhizal fungi in five genera, 41 different host plant species, 36 plant growth measurements, and four water-stress treatments (high-, medium-, or low-stress, and well-watered). We examined effects between lifecycle, habit, study site, functional group, water regime and level of stress, and mycorrhizal treatment species. The majority of experiments (55.6%) examined herbaceous annuals, followed by woody perennials including tree species (32.7%), and herbaceous perennials (11.7%). Most experiments (79%) took place in greenhouses or growth chambers. Field studies investigated either large woody species or traditional field crops such as maize (*Zea*), wheat (*Triticum*), and sorghum (*Sorghum*). Forbs were tested most often (31.5%) followed closely by legumes (29.6%). Tree and shrub experiments accounted for 20.4% and grasses 18.5% of the research. Eight studies (14.8%) compared varying levels of water-stress on plant growth but most examined only a single level stress treatment.
Overall plant growth is strongly improved by mycorrhizal colonization. Perennial plants respond more positively under drought-like conditions than annual species. We would assume that this was due, perhaps, to a greater investment in persistent roots and recurring shoots, however, perennial growth improvements were only observed in the whole plant measures of total dry weight even though root dry weight, root length, and root:shoot were recorded for perennial plants. Also counter to our assumptions is that growth of reproductive structures was not improved by colonization of annual plants. Semelparous species would seem to benefit most from developing tissues concerned with continuing a genetic presence. Mean log response ratio of reproductive growth was the highest of all area categories in annual plants but it was not statistically significant.

Responses observed between plants of different tissue types were not the same, with woody plants more improved than herbaceous species. Woody species are generally more likely to be perennial and, in this meta-analysis, all the woody plants examined were perennial, primarily trees and shrubs. Enduring, woody shoots require year-round nutritional support, and it may be because of this that belowground growth was significantly enhanced in woody species.

In a recent meta-analysis, Hoeksema et al. (2010) suggest that site location had only a small effect on plant response to mycorrhizal symbiosis; however we found no difference in the outcomes between plants grown in greenhouse or field. Water shortage likely has an effect on mycorrhizal growth (Bolgiano et al. 1983) but is also among the most limiting influences on plant growth. Faber et al. (1991) demonstrated the improved
transport of water by mycorrhizal hyphae. We found that the effect of mycorrhizae on well-watered plants was no different than on water-stressed plants. Although the statistical difference is not significant, the result is notable in that it shows that water-stressed plants grow as well as those that are watered adequately when both are mycorrhizal. Likewise, varying levels of water-stress are equally ameliorated by AMF. It is likely that certain plant-mycorrhizae relationships express more synergy than other combinations as reported by several studies but our analysis found that, among the most studied species of Glomus, there were no differences in the effect on water-stressed plants.

There is no dearth of studies investigating the physiological improvement of plants by mycorrhizal symbiosis and earlier meta-analyses have been conducted on the effects and interactions of some of these factors (Borowicz 2001, Morris et al. 2007, Koricheva et al. 2009), however this meta-analysis is the first to quantitatively affirm the view that arbuscular mycorrhizal symbiosis benefits plants in terms of morphological growth when exposed to low-water conditions and the variations on those effects within differing contexts. We recognize that AMF can provide a range of benefits to their hosts. It is worth noting that other factors, such as improved P-uptake, may have interacting effects on plant growth when less water is available.

In conclusion, meta-analysis of the literature supports the assertion that mycorrhizal plants show better tolerance to water-deficit than non-mycorrhizal plants, as shown by increased biomass. Most measures of growth are augmented by the symbiosis,
when plants are subjected to water-stress; however, aboveground biomass such as leaf area, plant height, and stem diameters are significantly more improved than belowground measures like root length or root dry weight.
Chapter Two: Influence of Peat and Soil Microorganisms on Plant Growth in Semi-arid Gardens

Introduction

AMF are one of the most abundant groups of organisms on Earth and form a biotrophic relationship with more than two-thirds of terrestrial plants (Helgason and Fitter 2009). This relationship is obligate for the fungus and facultative for the host plant. For plants, the benefits of mycorrhizal colonization can include enhanced uptake of nutrients (Marshner, 1994), resistance to pathogens (Borowicz 2001, Wehner et al. 2010), improved access to water, and resistance to water stress (Auge 2004). The effects of AMF can also be harmful, suppressing growth of some non-host plant species (Rinaudo et al. 2010) and parasitizing carbon in non-limiting conditions (Kogel et al. 2006).

Mutualisms exist in nature in a variety of species. Indeed, some mutualistic relationships result in emergent organisms, such as lichens and corals. Symbioses with bacteria are known to contribute to the success of plant growth (e.g. Glick 1995, Vessey 2003, Marulanda et al. 2009) with N-fixation as one of the most studied benefits. Another fungal symbiont, the ectomycorrhizae, do not enter into plant cells like the endomycorrhizal AMF but still provide similar nutritional (Bending and Read 1995) and
stress-resistance benefits (Parke et al. 1983), mostly to temperate tree species (Marks and Kozlowski 1973). Mycorrhiza Helper Bacteria interact with both ectomycorrhizae and AMF to help establish and improve mycorrhizal infection possibly by helping the symbionts communicate with each other chemically or facilitating interactions between mycorrhizae and plants (Garbaye 1994). While bacterial and ectomycorrhizal benefits to plants are extensive, AMF are far less host-specific, with colonization occurring in 60% (Helgason and Fitter 2009) to 80% (Schussler et al. 2001) of vascular plant species including most crops. AMF symbioses are usually mutualistic, with both organisms receiving some benefit from the relationship. Where plant growth is limited by water or nutrients, plants can benefit from better access via the fungi’s extensive hyphal network which interacts directly with plant roots. In exchange, AMF receive plant-fixed carbon, in the form of carbohydrates, and a habitat from which they can propagate.

Prior to colonization of a plant, roots must be discovered by the fungal hyphae. Infection can be initiated by spores or external hyphae and AMF are known to recognize certain compounds at or near the rhizoplane which induce spore germination and hyphal branching. These root exudates are released by plants to stimulate AMF (Akiyama et al. 2005). Upon contact with the root epidermis, the hyphae form a structure called the appressorium, a modification of the hyphal tip. Appressoria adhere to the root surface and produce enzymes that digest the cell wall, facilitating penetration (Kubo 2005). Breach of the cell wall then initiates formation of a cytoplasmic column in the root cell, through which the hyphae grow within and between cells (Siciliano et al. 2007),
eventually differentiating into the eponymous arbuscule (Lima et al. 2009). Hyphae can also grow out into the soil where they may acquire additional nutrients, form spores, or colonize other roots (Sanders 2002).

The arbuscule acts as the interface between the two symbionts and the site of bi-directional movement of substances across the fungal membrane. Arbuscules typically form in the inner layers of the root cortex; suberin lamellae in the endodermal wall prevent penetration into the stele (Holley and Peterson 1979). Once the arbuscule is formed and the interface established, the exchange of organic compounds can take place. The most commonly studied nutrient flux is that of carbon, from the plant to the fungus, and phosphorus, from the fungus to the plant (Hodge et al. 2010). Photosynthates are released into the peri-arbuscular space between the plant cytoplasm and the fungal membrane, and converted into hexose sugars that are moved across the fungal membrane by a transporter protein (Hahn and Mendgen 2001). The carbon cost to the plant varies by species but is worth the mycorrhizal benefits, including solubilized P and other resources. In a study using $^{14}$C-labeled CO$_2$ in cucumber plants, Jakobsen and Rosendahl (1990) found that *Glomus fasciculatum* consumed 20% of photo-assimilated carbon. Similar experiments have found results ranging from 4-10% (Kucey and Paul 1982, Snellgrove et al. 1982, Koch and Johnson 1984), and estimates of 10-20% are often cited in the literature.

Non-nutritional benefits are also conferred to plants by AMF. One of these is increased resistance to microbial pathogens (Wehner et al. 2010). Some soil microbes
are disease organisms that cause wilts, rots, blights, and rusts, for example. Manifestation of these conditions can result in decreased production or death of the affected plant. A meta-analysis examining mycorrhizal and non-mycorrhizal plants infected with root pathogens or soil invertebrates found that inoculation with AMF had a strong negative effect on pathogen growth and even harmed some sedentary endoparasitic nematodes (Borowicz 2001). Resistance to pathogens is increased by chemical as well as physical means. Depression of disease organisms corresponds to increased phenolics in infected root cells or cell wall thickenings in reaction to pathogenic hyphae and by the formation of encasement structures around hyphae that do penetrate root cells; none of these reactions are seen in infected, non-mycorrhizal root systems (Cordier, 1998). These observations invite the idea that stimulating microbial diversity is a viable alternative to broad-spectrum chemical fungicides for disease suppression.

AMF sometimes have a deleterious effect. Rinaudo et al. (2010) showed that the total biomass of several non-host weed species grown in conjunction with mycorrhizal sunflower was negatively affected by presence of AMF. Total weed biomass in plots with sunflower was 47% lower, on average, in plots with AMF compared to those without. Francis and Read (1995) developed a system that modeled establishment of pioneer weed species using a mesh filter to exclude plant roots while allowing hyphae. Germination, growth, and survival were sharply reduced by the presence of AMF in non-host species, including several agronomically important weeds. AMF hyphae had
penetrated the roots of the non-host species, disrupting development of the roots. This antagonistic effect on growth suggests potential of AMF as a biological weed control. Depression of host-plant growth in non-limiting conditions (e.g. high N or P availability) may be attributed to plant carbon-cost for mycorrhizal colonization (Buwalda and Goh 1982, Peng et al. 1993). Crush (1976) posited that AMF symbiosis becomes parasitic with increases in P availability. Their study showed 3-16% reduction in growth of white clover (*Trifolium repens*) and alfalfa (*Medicago sativa*). These studies indicate that mycelium produced by some mycorrhizae compete enough for carbon resources to curtail plant growth.

Water acquisition is highly improved by mycorrhizal inoculation. Hyphae hold soil particles together to physically and chemically stabilize aggregate structures which is important to water retention and penetration (Jastrow et al. 1998). Hyphae also function as an extension of plant root surface area by increasing soil contact. Water-stressed plants that are colonized by mycorrhizae have higher biomass and relative water use efficiency than stressed non-mycorrhizal plants (Al-Karaki et al. 2004, Wu et al. 2006a). Hyphal exploration of soil results in better acquisition of water by plants. Faber et al. (1991) designed an apparatus that excluded plant roots from an adjacent soil chamber but allowed mycorrhizal hyphae to penetrate and explore that soil. They compared mycorrhizal cowpea (*Vigna unguiculata*) with hyphae in the exclusion chamber to plants that had the hyphae severed. Gravimetric measurements of the soil indicated that plants with intact hyphae transpired 35% more water than plants with severed hyphae. Besides
improved water availability in moisture-limited soils, the water-stress resistance of mycorrhizal plants is closely associated with the production or transport of substances active in plant response to water-stress. The concentration of compounds known to regulate osmotic adjustments, such as potassium and proline, are higher in mycorrhizal plants than in non-mycorrhizal plants (Ruiz-Lozano et al. 1995b). Suggesting a conditional genetic induction for improving water access for plants, a 2009 study found up-regulation of an AMF water-channel gene in response to water-stress (Aroca et al. 2009).

Plants are not passive in their relationship with AMF. Plant root exudates contain factors that stimulate spore germination in AMF and nodulation of N-fixing bacteria (Tsai and Phillips 1991). Akiyama et al. (2005) isolated strigolactones from root exudates of *Lotus japonicas* that induced hyphal branching in *Gigaspora margarita* and Horii et al. (2009) found other inductive compounds that were only released when plants were stressed. In addition to stimulating infection, plants may also inhibit colonization when P is not limited (Breuillin et al. 2010) and Menge et al. (1978) showed that this is due to adequate plant P rather than availability of P in the soil.

Soils are classified by texture and structure; soil texture is determined by the percentage of parent material, sand, silt, clay, or loam. Organic matter and microorganisms in soil adhere particles into aggregates that collectively makeup the soil structure (Encyclopedia of Geography, 2010). Soil structure influences water-holding capacity, gas movement, and nutrients (Rillig et al. 2002), traits critical to plant
performance. The literature suggests that soil quality must be linked to presence and abundance of bacteria and fungi. Indeed, fungicide and bactericide applications reduce formation of stable soil aggregates (Tang et al. 2011) and plant growth (Schreiner and Bethlenfalvay 1996). Conventional agriculture is particularly disruptive to soils. Plowed fields left fallow after cropping winter wheat had less microbial biomass than fields where no-till practices were used (Drijber et al. 2000). Another study found that, under conventional tillage, soil aggregates were scarcer than and not as stable as those in no-till conditions (Beare et al. 1994). The high correlation between percentage of soil aggregates and microbial population suggest that soil microbes are very important for aggregate formation (Diaz et al. 1994).

Plant water relations are both directly and indirectly affected by soil microorganisms. Meta-analysis shows that mycorrhizal plants outperform non-mycorrhizal plants in most measures of growth and yield under water-deficit (Jayne 2012). Wu et al. (2008) found that AMF increased the portion of water-stable macro-aggregates (>0.25mm), indirectly improving growth of plants under water stress. Auge (2004) demonstrated that mycorrhizal soil, i.e. soil that had previously hosted colonized plants, increased stomatal conductance in non-colonizing mutants grown subsequently. Mycorrhizae modify the soil environment by producing glomalin, a glycoprotein which is strongly correlated to soil aggregate stability. Rillig and Steinberg (2002) grew carrot (Daucus carota) in various sizes of glass beads to simulate different soil pore spaces. With small beads (<106µm) representing non-aggregated soil, hyphal lengths were
reduced more than 80% relative to the large bead (710–1180μm) test. Glomalin yields, however, were over seven times higher in the small bead environment.

The potential for horticultural applications of microorganisms is a promising option for organic gardens as a biological alternative for fertilization, disease prevention, and soil building (Azcon-Aguilar and Barea 1997). Sustainable systems minimize or omit the use of synthetic chemicals that broadly affect landscapes and ecosystems in lieu of integrated pest management. The broad spectrum of conventional treatments for destructive fungal parasites, for example, would also dispatch beneficial mycorrhizae. Microbial biotechnology would aspire to improve the environment by substituting reactive solutions to plant and soil health issues with proactive mechanisms to resist deficiencies and infections.

In 2009, 43 million US households planned to grow their own fruits, vegetables, or herbs, an increase of 19% from 2008 (Butterfield 2009). The National Gardening Association’s 2008 Environmental Lawn and Garden Survey also estimated that 12 million households used only all-natural fertilizer, pest, and weed controls in their home gardens that year. Organic gardening represents a trend in grass-roots environmental sustainability that could promote local and regional improvements in soil and water quality, health, and food security. The organic philosophy typically touts sustainability in support of its objective to reduce use of chemical fertilizers and pest controls. Until relatively recently, however, water conservation, which is vital to implementing a sustainable landscape, has mostly been a subtext. A key principle of water conservation
is improving soil quality to encourage root growth and improve water acquisition. Adding organic matter to garden soils is a common method to improve soil structure and water use efficiency in landscapes. Non-sterilized compost also adds beneficial soil microorganisms to deficient soils.

Americans extract about 10 billion gallons of water per day more than is returned to freshwater sources and, as a result, 36 states are predicting water shortages by 2013 (Hill 2003). Recommendations for low water gardens, or xeriscapes, are primarily ornamental plants and generally not useful as a human food source. Bridging the gap requires the application of xeriscape principles to plants not traditionally considered xeriscape species. In other words, growers and gardeners should look for ways to extend water resources through water use efficiency, selection of robust plant varieties, modification of soil structure with organic matter, and application of symbiotic microorganisms.

Enhancing microbial flora in both home and large scale food production should improve plants’ access to water, their ability to use applied water more effectively, prevent diseases without using chemicals, and reduce fertilizer applications. All of these effects can improve quality of life by producing nutritious food, protecting the environment, and conserving natural resources. Strawberry (*Fragaria x. ananassa*) is a common perennial fruit and important commercial crop, also widely cultivated in home gardens, and are known to develop mycorrhizal symbioses. Barbara Mosse described “fructifications associated with mycorrhizal strawberry roots” in 1953 (Mosse 1953).
The eastern plains of Colorado, including the populous Front Range Corridor cities of Ft. Collins, Boulder, Denver, and Colorado Springs metropolitan areas, receive less than 20” of average annual rainfall, placing them in Group BS of the Köppen Climate Classification. Group BS climates receive less precipitation than potential evapotranspiration but not extremely so. The Denver metro area is rated BSk, indicating it is in a semi-arid (B) steppe-climate (S) with at least one month of average temperatures below 0°C (k) (Encyclopedia of Geography, 2010).

The objective of this study is to investigate the individual and interactive effects of commercial microbial inoculants and soil structure amendment (peat) on growth of strawberry plants under water stress in the context of a typical home food garden. I expect the addition of peat and microbial inoculum to independently improve growth of strawberry plants. The improved soil structure produced by peat-amendment will also interact with symbiotic soil microorganisms to further augment growth improvements under water stress.

Methods

Strawberry plants, cv. Fort Laramie, in 2-1/4” pots were purchased from a Denver-area nursery and transported to the greenhouse at the University of Denver. Fort Laramie strawberry is purported to be both cold- and drought-tolerant. Plants were all approximately the same size. Temperature inside the greenhouse was 24C/18C day/night.
Microbial inoculum

Two brands of mixed inoculum containing endo- and ecto-mycorrhizae and rhizobacteria were purchased online. Bio-Organics Mycorrhizal Landscape Inoculant (Bio-Organics Mycorrhizae Inoculants, Palm Springs, CA), a mycorrhizae only mixture, is a multi-purpose product that includes *Glomus aggregatum*, *G. clarum*, *G. deserticola*, *G. intraradices*, *G. monosporus*, *G. mosseae*, *Gigaspora margarita*, *Paraglomus brasilianum*, *Lacaria laccata*, *Pisolithus tinctorius*, *Rhizopogon amylpogon*, *R. fulvigleba*, *R. rubescens*, *R. villosuli*, and *Scleroderma spp.* The second inoculum contains both mycorrhizae and bacteria, Bio-VAM (T&J Enterprises, Spokane, WA) include *G. mosseae*, *G. etunicatum*, *G. intraradices*, *G. clarum*, *Rhizopogon spp.*, plus the bacteria *Athrobacter globiformis*, *Azotobacter chrococcum*, *A. vinelandii*, *Bacillus subtilis*, *Pseudomonas alcaligenes*, *P. fluorescens*, *P. pseudoalcaligenes*, and *P. putida*.

Preparation of plant material and substrate

The substrate was either unmodified soil (‘collected’) or the same soil with the addition of 33% organic matter as peat (‘amended’). I collected soil from Panorama Park; a suburban park near northwest Denver established about 1950. The soil texture is silt-loam representative of the substrate in which an urban or suburban Denver food garden might be grown. I confirmed this soil texture by laser-diffraction particle size analysis (Beckman Coulter, Brea, CA). Peat was a compressed 1.0ft³ bale I purchased at a garden supply center. Both substrates were sterilized by autoclaving at 121°C for 20 minutes and stored in large, clean plastic bags before use. I made the amended substrate
by combining two parts (by volume) of the collected silt-loam soil with one part peat. Plants were grown in round-bottom cylinders (cones) approximately 5.0cm across and 25.4cm in length (~515cm³). Cones were filled with substrate to no more than 5.0cm from the top of the cone. 24 cones were filled with ‘collected’ soil substrate and 24 cones were filled with ‘amended’ soil substrate, eight each per mycorrhizal treatment. Once cones were prepared, roots were rinsed free of soil and debris with clean water by light agitation in a serial water bath (2X). Complete sterilization of living plant material other than seeds cannot be achieved even with fungicide or bactericide applications however I opted to further reduce surface organisms by soaking in a 1% sodium hypochlorite solution for five minutes. I then rinsed plants again with clean water by serial water bath (2X). Excess water was gently shaken from the plants and 2.0 grams of Bio-Organics Mycorrhizal Landscape Inoculant, Bio-VAM, or no inoculum was applied directly to the roots. I transplanted plants to cones filled with either ‘collected’ or ‘amended’ soil substrate, leaving a free space of 2.5cm from the top of the cone. I repeated these steps were until eight plants were treated and transplanted. Each cone was watered to field capacity at transplanting and every other day a 14 day establishment period.

**Stress induction**

I conducted preliminary observations in spring 2011 on non-mycorrhizal Fort Laramie strawberry plants to determine a drought threshold in unmodified soil. Sample plants were subjected to drought conditions for 3, 7, 10, 14, or 21 days. 3-day plants were unaffected; however moderate wilt was evident at the 7-day treatment. 10-day
plants showed severe wilt and some mortality while plants that had water withheld for 14 days or more all died. These observations led to the selection of the 7-day water restriction used in the final experiment; stress was appropriate but total plant loss was undesirable. After two weeks of establishment in the primary study, I watered plants according to the respective treatment watering regimen. Well-watered plants were saturated every third day for each 60 day experiment. Water-stressed plants were saturated every seventh day for the period.

**Data collection and Statistical Analysis**

After 60 days of growth, I harvested plants and counted flowers, fruits, and stolons. I measured leaf area (cm²) with a LI-3100 Leaf Area Meter (Li-Cor, Lincoln, NE) after clipping leaves where the petiole intersects the blade. I cut shoot from root; roots were rinsed free of soil with clean water again by light agitation in a serial water bath. I then placed shoots and cleaned roots separately in a drying oven at 60°C in clearly labeled brown paper sacks for one week, after which I weighed the dried plant tissues (Figure 12).

I grew a total of 96 plants (2 experiments x 2 soil amendments x 3 microbial treatments x 2 watering conditions x 4 replicates). In mid-August I started 48 plants and in mid-September started a duplicate set in a staggered start 30 days after the first set began. Each experiment ran for 60 days before harvest for final measurements (120 total experimental days, 90 calendar days). I log-transformed data to normalize distribution where appropriate and analyzed the results as a linear model with soil amendment,
mycorrhizal treatment, and water treatment as fixed effects. In cases where differences were observed, I followed with Tukey-Kramer Honestly Significant Different (HSD) tests to identify and sort them. I evaluated stolon counts by contingency analysis of a binomial score (absence or presence).

**Results**

**Overall Effects**

Independent of corresponding effects, peat amendment and microbial treatment significantly improved one or more measures of strawberry plant growth compared to controls (Table 3). Improved performance of colonized plants concurs with prior studies of microbial influence on plant growth (Janos 1980, Vidal et al. 1992, Glick 1995). Peat-amendment and inoculum did not interact to effect growth. When only water-stressed plants were analyzed, microorganisms did not increase growth significantly more than un-inoculated controls.

**Soil Effects**

Soil amendment with peat significantly increases all measures of plant growth except for the number of fruits and flowers (Table 4). Strawberry plants grown in peat-amended soil have much greater total dry weight biomass than plants grown in the unmodified, collected soil ($F_s = 34.92, df = 7, 91, P < 0.0001$). This was reflected in both increased shoot dry weight ($F_s = 23.77, df = 7, 91, P < 0.0001$) and increased root dry weight ($F_s = 26.29, df = 7, 91, P < 0.0001$) of amended-soil grown plants. Equivalent
increases in above- and below-ground growth are illustrated by root:shoot ratios that are unaffected by soil type ($F_s = 0.52, \text{df} = 7, 91, P = 0.47$). Leaf area was also increased in plants grown in the peat-amended soil ($F_s = 3.22, \text{df} = 7, 91, P = 0.0012$) and more than twice as many plants in the amended soil had stolons than the collected soil ($X^2_s = 5.86, \text{df} = 1, P = 0.016$). Fruit and flower counts were not increased by soil amendment.

**Inoculum Effects**

Addition of microbial inoculum has varying results (Table 4). Overall, total biomass of strawberry plants is increased by treatment ($F_s = 3.81, \text{df} = 7, 91, P = 0.026$). Total biomass was improved most by Bio-VAM however Bio-Organics was not significantly more than the non-microbial control. Leaf area was also increased by inoculation ($F_s = 5.90, \text{df} = 7, 91, P = 0.004$). In this case, both brands of inocula provided gains in leaf surface area. Independently, shoot and root dry weights are not increased meaningfully ($F_s = 2.84, \text{df} = 7, 91, P = 0.064$ and $F_s = 2.91, \text{df} = 7, 91, P = 0.059$, respectively) nor is the corresponding root:shoot ratio. Neither sexual nor vegetative reproductive structures were affected by microbial treatment.

**Water Effects**

The amount of water plants received has a strong effect on all measures of growth (Table 4). Well-watered plants are always significantly larger than water-stressed plants in terms of total dry weight biomass ($F_s = 139.02, \text{df} = 7, 91, P < 0.0001$), shoot dry weight ($F_s = 135.46, \text{df} = 7, 91, P < 0.0001$), root dry weight ($F_s = 56.90, \text{df} = 7, 91, P < 0.0001$), root:shoot ratio ($F_s = 37.38, \text{df} = 7, 91, P < 0.0001$), leaf area ($F_s = 139.58, \text{df} = 7, 91, P < 0.0001$), and leaf surface area ($F_s = 139.58, \text{df} = 7, 91, P < 0.0001$).
Discussion

In this study, microbial inoculation and peat-amendment of soil improved overall performance of strawberry plants, no deleterious effects were observed from either treatment. Not surprisingly, water-deficit had the most substantial impact on plant growth. Well-watered plants were significantly larger and had more reproductive structures than water-stressed plants. The materials and efforts used here are accessible even to inexperienced gardeners.

Soil modification through structural amendment is regularly employed by farmers and gardeners in marginal soils. Addition of organic matter improves water retention in loose sandy soils and drainage in impervious clay soils. Ros et al. (2003) observed spontaneous plant growth after addition of urban waste in abandoned, semi-arid farmland and reported increased organic carbon and enzyme activity compared to control sites. In another study, tomato plants treated with compost had more fruit and less disease than those in unamended plots (Abbassi, 2002). Soil quality is influenced by microbial activity (Forster 1990) and the chemical and physical properties of soil can even determine which microbial taxa colonize plants (Santos-Gonzalez et al. 2011). I saw increases in the size or number of all measured plant parts in peat-amended soil except
for the number of fruits and flowers. Measuring fruits and flowers, in hindsight, only represents a temporal snapshot of reproductive growth as flowers soon wilt if not pollinated and fruits rot or desiccate if not harvested. My count of these structures only totaled the fruits and flowers present at 60 days from transplanting when some may have developed before that time point. Stolons were slightly more persistent but I analyzed these as presence or absence due to a high number of zeroes in stolon count data.

The influence of arbuscular mycorrhizae on growth of plants under water-deficit is well established (Jayne 2012). These benefits may be caused by any of several measures reported in the literature including but not only the increased functional surface area of roots provided by extraradical hyphae (Safir and Boyer 1971), improved nutrition (Morte et al. 2000), enzyme activity (Marulanda et al. 2007), osmotic adjustments (Wu and Xia 2006), or better water availability through improved soil aggregate structure (Auge et al. 2001). Microbes may not always be able to prevent deleterious effects of water-deficit on plant growth at the time of the stress but may improve plant recovery when stress is relieved (Morte et al. 2001). Water is likely the most limiting factor in plant growth followed by primary nutrients nitrogen (N) and phosphorous (P). Soil microorganisms improve plant fitness by providing nutrients and increasing plant tolerance to abiotic stress. Rhizobium and other nodulating or N-fixing bacteria provide N to plants and soils and host plants are grown in both conventional and organic systems for this effect (Peoples et al. 2009). Mycorrhizae also contribute essential nutrition to
hosts in the form of P (Marschner and Dell 1994) which may itself contribute to improved drought tolerance of mycorrhizal plants (Nelsen and Safir 1982).

Strawberry plants were the only plant I examined and although it is an interesting model as a popular herbaceous perennial berry, it doesn’t represent the annual lifecycle of traditional home food garden plants outside the context of deliberate polyculture. The duration of experiment may also have been limiting. Ever-bearing cultivars of strawberry continue to grow and produce flowers and fruit for the summer after peak production in June. The 60-day duration I employed did not allow plants to mature to terminal size that might have resulted in more significant differences between treatments. Water-stressed plants generally had equivalent portions of above- and belowground biomass whereas well-watered plants had about twice as much aboveground biomass by dry weight. Fresh mass:dry mass ratio both alone and within aboveground and belowground parts might have been an interesting measure that could provide some insight into plant water by weight.

I did not control for some factors that I determined were not particularly relevant to the application, such as pH. The optimal pH range for strawberry is thought to be 5.5-6.5, on the low end of the 6.0-7.5 tolerance range for most plants other than acid-loving species. 1:1 mixtures of peat:soil can lower the mixed substrates pH up to one unit (Haynes and Swift 1986) but most Colorado soils have a pH of 7.0-8.3 (Whiting 2011) so even a decrease of one log(L/mol) would adjust pH of most soils toward the tolerable range. Home gardeners may test soil pH but any organic amendment to soil that is not
strongly alkaline will be beneficial to plant growth. Non-microbial control plants in this experiment were likely not completely devoid of microorganisms. Although I washed and surface-sanitized all roots in 1% bleach, even fungicide treatments cannot eliminate fungal organisms protected inside root cells. However, potted strawberry plants from commercial greenhouses are generally not highly colonized due to pest control and fertilizer regimes in these facilities (Robertson et al. 1988). Nevertheless, to avoid extraordinarily confounding variables, I did sterilize all of the substrates to control microbial inputs as much as possible. Again, this application was intended to duplicate activities that could reasonably be performed by a typical gardener.

Hyphae expand the volume of soil accessible by roots but both hyphal and root growth was artificially restricted in greenhouse pots. Water accessibility plus other factors, such as improved P access, likely have further increasing effects on plant growth when less water is available and soil-P can be reached by foraging hyphae. A corresponding field experiment would elucidate the role of mycorrhizae with unrestricted hyphae. In the field, test plots can be sterilized by fungicides to control microbial inputs but water restriction requires structures for rain protection. Given a large body of literature that describes the enhancement of plant resistance to water deficit by microbial symbioses, I suspect that experimental factors like test period length, pot limitations, and sample size coordinate to depress other improvements (e.g. shoot and root dry weights) of inoculation on water-stressed strawberry plants in this study. Another possibility is that strawberry does not express large improvements in growth or is not as receptive to
colonization as other species. It is clear, however, that, in general, amending soil and using applied soil microorganisms does improve strawberry growth overall.

Organic systems have challenges that are overcome in conventional models by increasing potentially environmentally harmful synthetic inputs. The organic philosophy advocates natural fertilizers and pest controls in support its objective of sustainability while water conservation, which is vital to implementing a sustainable landscape, is often a subtext. Use of soil microorganisms in backyard organic gardening could be a trend in grass-roots environmental sustainability that has the potential to promote local and regional improvements in soil and water quality, health, and food security. Inoculated strawberry plants in this experiment had greater total dry weight biomass and leaf surface area than un-inoculated plants. Plants grown in peat-amended soil had improved growth in all measures except number of fruits and flowers. My data show that modification of urban and suburban soil with peat and soil microorganisms can improve plant biomass.
Chapter Three: Effect of Mycorrhizae, Salinity, and Water on Performance of Native Plants for Tamarisk Site Re-vegetation

Introduction

AMF have been reported to improve salinity tolerance in many plant species, particularly food crops (Ruiz-Lozano et al. 1996, Feng et al. 2002, Hajiboland et al. 2010). Stress resistance of mycorrhized plants is closely associated with the production or transport of substances active in plant response to stress. The concentrations of compounds known to regulate osmotic adjustments are higher in mycorrhizal plants than in non-mycorrhizal plants (Ruiz-Lozano et al. 1995b). AMF symbiosis has been shown to improve tolerance of host plants to soil salinity by providing, inducing, or regulating production of antioxidant enzymes that can protect plants from the damage of activated oxygen species (Dionisio-Sese and Tobita 1998), plant hormones associated with stress response such as ethylene (Besmer and Koide 1999), or osmolytes, such as proline or glycinebetaine, made by plants in response to stress (Ashraf and Foolad 2007). In most cases, the accumulation of proline and glycinebetaine corresponds to better stress protection. Both compounds can reduce the osmotic potential of plants and glycinebetaine may also help to maintain photosynthetic efficiency in salt-stressed plants (Chen and Murata 2011).

Mycorrhizal growth and colonization of plant roots is also affected by salinity. Prior to colonization of a plant, roots must be discovered in the soil by fungal hyphae. Infection is initiated by spores or hyphae in the soil and AMF are stimulated by plant root
exudates that induce spore germination and hyphal branching (Akiyama et al. 2005). Upon contact with the root, hyphae adhere to the root surface and produce enzymes that digest the cell wall (Kubo 2005). Penetration is followed by the formation of a cytoplasmic column in the root cell, through which the hyphae grow (Siciliano et al. 2007). Hyphae can also grow out into the soil where they acquire nutrients, form spores, or colonize other roots (Sanders 2002). Inhibition of AMF in saline conditions may be a result of decreased spore germination or reduced hyphal growth and branching (McMillen et al. 1998). For some AMF, spore germination is delayed, but not prevented, in saline conditions and the rate at which hyphae grow through salty soils is reduced (Juniper and Abbott 2006). Pfeiffer and Bloss (1988) also found that salt decreases the number of arbuscules and vesicles in roots. AMF may, however, be able to acclimatize to saline conditions as shown by Sharifi et al. (2007). They found that salt-pretreated Glomus etunicatum colonized roots better than non-pretreated G. etunicatum in soybeans watered with 100mM NaCl. Shoot and root dry weights were higher in the plants infected with salt-pretreated inoculum.

Tamarisk (Tamarix spp.) invasion is considered one of the most critical biological threats to southwestern river ecosystems. Introduced from Europe, Asia, and Africa in the 1800s as an ornamental, windbreak and stream-bank stabilizer, it is now present in every major watershed in the Southwest US in a variety of native communities (Zouhar et al. 2008). Tamarisk has been linked to changes in surface and groundwater quality and quantity, plant and animal biodiversity, wildlife habitat, soil conditions, and fire regimes
However, it is unclear whether tamarisk is the cause or the effect of these ecosystem changes (Sher and Quigley, in press).

It is generally assumed that tamarisk is the cause of many of the environmental problems with which it is associated, particularly increased soil salinity (e.g. Shafroth et al. 1995). Tamarisk collects salts from the soil and groundwater and excretes it on the leaf surface. Salt is then deposited on the soil surface when the leaf falls. Salty soils inhibit the establishment of native seedlings and facilitate tamarisk establishment (Busch and Smith 1995) but some argue that soil salinization and corresponding allelopathy of tamarisk may be overestimated (Lesica and DeLuca 2004). If natural flooding patterns were allowed, salts would be leached away; however, dams and water diversions have greatly altered the natural hydrology of watersheds in the Southwest (Ohrtman et al., in press). Alterations in the timing, extent, or existence of flooding events has caused higher salinity in soil, lower water tables, and a buildup of debris in riparian systems (Zouhar et al. 2008). All of these changes create an environment where native species are not well adapted (Briggs and Cornelius 1998) and must compete with invasive non-native plants for resources.

A variety of salts seep from the leaves of tamarisk, including sodium, potassium, and chloride (Di Tomaso 1998), but the actual composition of salt exudate is dependent upon the composition of salts in the surrounding soil and water table (Thomson et al. 1969, Hagemeyer and Waisel 1988). High concentrations of salts in soil have an adverse effect on plant seed germination and growth (Pandya et al. 2004). Water moves from high to
low water potential and high salinity creates a low water potential in soil. The diminished ability of plants to acquire water causes water stress in the rhizosphere by means of this lower water potential (Mahajan and Tuteja 2005). Two of the negative effects of salinity on plants are the water-stress condition created by the decrease in soil water potential and buildup of ions to toxic levels in plant tissues, particularly leaves (Gomez-Cadenas et al. 1998).

Plant restoration can be difficult in salt-affected areas because low soil water potential and high ionic content is harmful to the fitness of re-introduced native plants. Efforts may be hampered by loss of soil microbial richness especially in tamarisk monocultures where biotrophic AMF species lack a serviceable host. AMF may be able to increase survival of restoration species in degraded areas. Genera used for re-vegetation in southwestern riparian areas such as *Leymus* (Zhang et al. 2011), *Prosopis* (Scambato et al. 2010), and *Bouteloua* and *Pascopyrum* (Allen et al. 1984), have responded positively to inoculation. Middleton and Bever (2012) showed that AMF-inoculated nurse plants had a positive growth effect on uninoculated plants within one meter of the nurse plants and suppressed non-mycorrhizal colonizing species. Beauchamp et al. (2005) reported AMF influence on competition between exotic tamarisk and cottonwood, a riparian native; in cultures composed of both tamarisk and cottonwood, they found that tamarisk growth was much lower in AMF-treatments than controls. This antagonistic effect on growth of non-host species corroborates the findings of other authors (e.g. Francis and Read 1995, Rinaudo et al. 2010).
Reestablishment of stable, diverse native plant communities on tamarisk-infested sites is a process that is dependent upon having functional, salt-tolerant microbial communities present as part of the plant-soil system. AMF increase plant uptake of nutrients and water and, as a result, are often able to improve plant tolerance to drought and saline soils (e.g. Mohammad et al. 2003).

To provide information on the mycorrhizal inoculation potential of soil, it is necessary to determine potential for mycorrhizal infection of native species under varying salinity and moisture gradients. The specific objective of this study was to determine the potential usefulness of mycorrhizal inoculations for establishment of desirable plant species during restoration of riparian areas after tamarisk removal. This was accomplished through evaluating mycorrhizal effect on growth (height and biomass) of plants across varying salinity and water treatments and by rate of infection of plant roots by AMF.

**Methods**

**Experimental Design**

In 2004, seven native species were selected for evaluation, comprising five grasses, one forb, and one shrub: *Bouteloua curtipendula* (Sideoats Grama, grass), *Elymus trachycaulus* (Slender Wheatgrass, grass), *Heliotropium curassavicum* (Salt heliotrope, forb), *Leymus triticoides* (Creeping Wild Rye, grass), *Lycium andersonii* (Anderson Wolfberry, shrub), *Pascopyrum smithii* (Western Wheatgrass, grass), and *Sporobolus airoides* (Alkali Sacaton, grass). All of these species are native to the Colorado Plateau.
in riparian sites infested with tamarisk and have shown potential for growth in past re-vegetation projects.

One of two commercially available mycorrhizal inoculants (Glomus intraradices or a mixture of G. intraradices, G. mosseae, and G. aggregatum) (Reforestation Technologies International, Salinas, CA) were uniformly mixed with sterile sandy-loam soil at a rate of ~1.2 g/0.65 L. Ten seeds of each species were planted in D40 Deepots (Steuwe & Sons, Tangent, OR) lined to prevent soil loss. In the greenhouse, pots were randomly placed in species blocks and rotated weekly to minimize edge and position effects. During propagation, the greenhouse was kept between 16°-26°C daily, with humidity ranging between 30%-45%. Before salinity and watering treatments were applied, seedlings were thinned to two per pot and then down to one seedling per pot 3-4 weeks later.

Once established, seedlings were randomly split into two watering regimes: 50 ml every 3 days (well-watered) or 100 ml every 6 days (pulsed or water-stressed) to simulate drought conditions. Three levels of salinity were applied within each watering treatment. Salinity solutions of 38.7% CaSO4, 36.9% NaHCO3, 15% MgCl2, 8.5% NaCl, and 1.8% K2SO4 (Shafroth et al. 1995) were mixed at electrical conductivity levels commonly observed in the field and known tolerance limits of halophytic species (1.3 dS/m [control], 8 dS/m [low], and 15 dS/m [high]). For each species, all mycorrhizal, watering, and salinity treatments were replicated four times for a total of 72 pots per species.
Data Collection

After 10 weeks of treatment all plants were harvested then above and below ground biomass was dried and weighed to the nearest 1/100th of a gram. Every 2 weeks throughout the study, height of all plants was measured to the nearest 1/10th of a centimeter. Above ground biomass was dried at 70°C for 48 hours. For evaluation of mycorrhizal colonization, ~0.2 grams of fresh root tissue was fixed in 2.5% KOH at 90°C for 60 minutes, placed in 3% HCl at 90°C for 45 minutes, then soaked in Trypan Blue at 90°C for 30 minutes, and finally rinsed with water. Stained roots were then and affixed to glass microscope slides with mounting solution and a cover slip. This portion used for microscopic analysis was accounted for in dry weight measurements, calculated based on the percentage of wet material removed for mycorrhizal evaluation. Remaining below ground tissue was dried and weighed like above ground biomass. This portion of the experiment was conducted between 2004 and 2005 at the Denver Botanic Gardens in collaboration with the Bureau of Reclamation. The majority of the data was collected following the conclusion of the greenhouse experiment as well as some analysis. It was considered afterward that mycorrhizal analysis procedures used presence of hyphae as a determinant of mycorrhizal infection. Hyphae, however, are the vegetative portion (collectively known as mycelium) of all fungi, not exclusively AMF, and septate and non-septate hyphae were not distinguished. Any potential analysis considering infection, therefore, would be dubious. All data was shelved until the opportunity to re-evaluate AMF colonization could be completed. In 2012, I established a new scoring protocol that
distinguished mycorrhizal from non-mycorrhizal structures. I determined AMF infection by presence of arbuscules or vesicles during microscopic analysis of root specimens taken from each species. I examined each slide at 10-40X magnification using a Zeiss Axioplan (Carl Zeiss & Co., Germany). Conditions to qualify as ‘infected’ required convincing observations of arbuscules or vesicles within root tissue. Due to their age, some slides had deteriorated beyond potential for analysis. To address this, I applied a quality score of 1-3 for each slide that explained the omission of an infection score for some specimens. I gave a quality score of “1” to slides in good condition; most of the cover solution was retained and the tissue was definitely score-able. A quality score of “2” meant that the slide had deteriorated but could still be scored; some of the cover solution had been lost but score-able tissue was present. A score of “3” indicated that the slide was not usable; most of the cover solution had been lost and specimens were desiccated with no score-able tissue present. A quality score of 3 always corresponded to no infection score.

**Statistical Analysis**

I evaluated shoot dry weight (SDW), root dry weight (RDW), total biomass (TB), terminal height (Ht), and root-to-shoot ratio (R:S) by ANOVA in JMP version 9 (SAS Institute Inc., Cary, NC). I analyzed the interactions between salinity×water, salinity×infection, water×infection, and salinity×water×infection in addition to the main effects of salinity, water, and infection. I analyzed the success of inoculation by examining infection of inoculated treatments and calculated the percentage of inoculated
plants that were observed to be colonized. Other than this calculation, I did not use the treatment factor of mycorrhizal inoculation in subsequent analyses because it only indicated the application of AMF to pots not the colonization of plants. Using infection as a main effect provided the empirical observation of mycorrhizal status of the plants studied. I evaluated the rate of infection by contingency analysis reported as $\chi^2$ value (Pearson’s).

**Results**

When analyzed separately for each species, interaction effects of salinity×water were present in the root dry weight of *B. curtipendula* and the shoot dry weight and total biomass of *E. trachycaulus* (P < 0.05, Table 1). With frequent watering the effect of salinity on both species was similar to the main effects of salinity in other species, with less biomass as salinity increased. With pulsed watering, however, there was little to no effect of salinity on biomass. *B. curtipendula* exhibited a loss of root dry weight biomass only at the highest salinity which was similar to root dry weight at the highest salinity treatment with frequent watering (Figure 14A). *E. trachycaulus* showed no difference in biomass between the lowest and highest salinity levels on shoot dry weight or total biomass (Figures 14B-C). The interaction of salinity×infection was observed in the root-to-shoot ratio of *L. andersonii* and the height of *P. smithii* (Table 5). Non-colonized *L. andersonii* expressed a trend of less root biomass relative to shoot biomass with increasing salinity. When *L. andersonii* is colonized by AMF, however, root-to-shoot
ratio is highest at the highest salinity level (Figure 15A). For *P. smithii*, there was little
difference in height between salinity levels but plants under low and high salt treatments
were significantly taller than controls when colonized (Figure 15B). Water×infection
and salinity×water×infection interactions did not affect growth of the species in this
study.

No main effects of infection were observed in any of the species. Four of seven
species (*B. curtipendula, E. trachycaulus, H. curassavicum, L. andersonii*) show a strong
main effect of salinity on two or more growth parameters (P < 0.05, Table 1) that
illustrates the decrease in plant growth with increasing salinity (Figure 13). Salinity had
an influence on height, shoot dry weight, root dry weight, and/or total biomass in these
four species. Water had a strong main effect on total biomass of *B. curtipendula*, root dry
weight and root-to-shoot ratio of *E. trachycaulus*, and the shoot dry weight of *H.
curassavicum* and *L. andersonii* (P < 0.05, Table 1).

When all species are analyzed together, height of plants is significantly reduced by
high salinity (*F*$_{7, 396}$ = 3.43, P = 0.03) but is not affected by any other interactions or main
effects. Shoot dry weight is also affected by salinity (*F*$_{7, 396}$ = 10.3, P < 0.0001) only.
The highest level of salt significantly reduces shoot biomass. Root dry weight is
significantly affected by the interaction of salinity×water (*F*$_{7, 396}$ = 5.49, P = 0.005).
When well-watered, root dry weight decreases with increasing salinity similar to the
expected trend of main effects of salinity. However, under pulsed watering, plant root
biomass is reduced at the lowest level of salinity while root weights at the two higher
salinity levels are unchanged between water regimes (Figure 16). Total biomass of all plants was also affected by the interaction of salinity×water \((F_{7, 396} = 4.33, P = 0.014)\) following a very similar trend as root biomass with water having no effect on total plant biomass at the higher salinities (Figure 17).

Infection rates ranged from 0-61% of plants inoculated with one or the other mycorrhizal treatment (Table 6). No mycorrhizal structures were observed in any \(B.\ curtipendula\) roots regardless of inoculum type. AMF colonization of plants was affected by soil salinity treatments as indicated by significantly lower infection rates in high salinity treatments \((\chi^2 = 16.4, df = 2, P = 0.0003)\). In most cases, colonization rates were higher in plants inoculated with \(G.\ intraradices\) only \((\chi^2 = 15.4, df = 2, P = 0.0005)\) than with the mixed species treatment.

**Discussion**

The commercially available inocula used here do not appear to be beneficial to the species investigated. If mycorrhizae were increasing plant salinity tolerance, we would expect significantly more biomass in infected plants at higher salinities. Height and root-to-shoot ratio were the only aspects of growth affected by infection and only as an interaction with salinity. The increased growth of only two infected plant species at the higher salinity levels also suggests that AMF improvements in growth of salt-exposed plants may only occur in some growth parameters in certain species. The results do not necessarily suggest that AMF are not important in this system. The species used in this study were for general purpose use and were applied “off the shelf”. Some mycorrhizae
confer better plant growth improvements with specific hosts (van der Heijden et al. 1998) and these plant species may not have been the ideal hosts for the AMF species applied. Klironomos (2003) found that AMF influence on plant growth is dependent on the plant with which they are associated and that the relationships span a continuum from mutualism to parasitism; a single species of AMF (*Glomus etunicatum*) had an effect on the growth of 64 different plant species that ranged from a 49\% reduction in plant biomass to a 46\% increase in plant biomass depending on the plant species. The three species of AMF used in our experiment may not benefit any of the seven plant species tested; rather our observations suggest that they are likely to have little to no effect on growth and may even harm growth of these plant species if no other factors are contributing to the lack of improvement.

Reduced leaf growth or necrosis is often the first and strongest observable plant response to salinity (Abbruzzese et al. 2009) and if AMF conferred tolerance to salinity in plants we should see corresponding improvements in above ground growth. We saw the root-to-shoot ratio of *L. andersonii* increased, i.e. more root biomass relative to shoot biomass (Figure 3A) which would suggest that AMF either did not protect leaf growth from salinity or that AMF improved root growth more in the high saline treatments. In *P. smithii*, plant height at the higher two salt treatments was about the same in plants with colonized roots as plants that were not infected. The height of *P. smithii* plants at the lowest salt treatments was significantly less than the other measures. This also seems to
indicate little improvement of above ground plant growth by AMF and possibly a parasitic effect at the least stressful salt condition.

AMF have been shown to be susceptible to salinity as indicated here by reduced infection rates at the highest salinity level. Some species, however, have putative salt-tolerant isolates (Jahromi et al. 2008) while others may be conditioned to be more tolerant of salt, increasing plant benefits (e.g. Sharifi et al. 2007). It is unclear why B. curtipendula was completely free of mycorrhizal infection. Prior studies show infection in other Bouteloua species (Monz et al. 1994) as well as B. curtipendula although the infection rate may be less than other grass species (Hetrick et al. 1991). There is also no known explanation for the increased colonization success of G. intraradices. Although, some species may infect plant roots at a greater rate, G. intraradices was included in the mixed species inoculum.

The literature overwhelmingly shows that AMF improve plant tolerance to soil salinity so we must consider that elements of the experiment didn’t allow for that to be properly illustrated. The period of time passing since the execution of the experiment played some role in the quantity of infection data collected and corresponding loss of statistical power as a result of the reduced sample size. Overall, 142 out of 932 total slides (15.2%) were deemed unscoreable as a result of deterioration in storage. Had these specimens provided valid scores, inclusion may have resulted in clearer result. Moreover, the effects of mycorrhizal fungi also change throughout the life the plant (e.g.
Bellei et al. 1992) and this study may not have been extensive enough to see the long-term effects of AMF.

Other treatments may augment the conditions for improving fitness of restoration species. While salt-tolerant or pre-conditioned inocula may be better adapted for growth and plant infection in saline soil, broadening the microbial inputs further may create synergies between microorganisms that yield more success. Soil bacteria including the Mycorrhiza Helper Bacteria (MHB) interact with AMF to help establish and improve mycorrhizal infection possibly by helping the symbionts communicate with each other chemically or facilitating interactions between mycorrhizae and plants (Garbaye 1994). Rhizobia could also be considered, as Fabaceous plants, such as Acacia and Prosopis, other native riparian species, may nodulate if rhizobia are present. Ectomycorrhizae, another fungal symbiont, provide nutritional (Bending and Read 1995) and stress-resistance benefits (Parke et al. 1983) to some, mostly woody, species. Calcium is also thought to play some role in increasing salinity tolerance of plants both alone (Mahajan and Tuteja 2005) and in relation to AMF. Under salt-stress, Ca\(^{2+}\) ion concentrations are increased in plants to initiate stress-signal transduction (Evelin et al. 2009). Cantrell and Linderman (2001) found higher calcium uptake in AMF-colonized lettuce and Yano-Melo et al. (2003) reported the same result in banana. If AMF increases plant calcium uptake more than sodium under salt stress, reducing ion toxicity, and exogenous calcium alone can reduce harmful effects of NaCl (Rabie and Almadini 2005), it may be worth
investigating the influence of AMF/calcium co-treatment of seeds or plants in re-vegetation efforts of salt-affected areas.

Salinity clearly affects seedling growth for all species such that high salinity is detrimental to their growth. The effects of these commercially available mycorrhizal fungi on salinity and drought tolerance could be somewhat species-specific and may not be significant at all for the species evaluated here. Further study is needed on more microbially-responsive restoration species and a broader inoculum input. This information will be beneficial for land managers as they develop revegetation protocols for formerly tamarisk-infested areas. Additionally, it will enable more effective assessment of possible effects and interactions occurring between the seeded plant species and the mycorrhizal community in large-scale, land treatment applications.
Works Cited


Acacia-Albida) and Acacia-Nilotica in Sterile and Nonsterile Soils. Biology and Fertility of Soils 14:159-165.


Tables and Figures

Figure 1

Hyphae (hy) and arbuscules (arb) in Chilopsis linearis (desert willow). Photo by B. Jayne.
Figure 2

Vesicles (ves) and subtending hyphae (hy) in *Heliotropium curassavicum* (salt heliotrope). Photo by B. Jayne.
Figure 3

Detail of log response ratio (LRR ±SE) of all plant growth responses to mycorrhizal symbiosis under water-deficit. Positive values (black-filled circles) indicate an increase in the plant growth measure; negative values (open circles) indicate treatment is deleterious to plant growth. Gray-filled circles are not significant. Measures with n=1 were excluded. Overall LRR = 0.324 ± 0.020 indicating a positive effect of mycorrhizal symbiosis.
Figure 4

Log response ratio (±SE) of plant growth response to mycorrhizal symbiosis controlled for growth category. Aboveground (AG) LRR = 0.341±0.029, belowground (BG) LRR = 0.204±0.036, Reproductive (RP) LRR = 0.313±0.051, and whole plant (WP) LRR = 0.430±0.054. Plant growth response varied significantly between categories stressed ($F_s = 3.50$, df = 3, 601, $P = 0.015$). Values sharing the same letter do not differ significantly after Tukey’s HSD.
Log response ratio (±SE) of plant growth response to mycorrhizal symbiosis controlled for lifecycle. Annuals overall LRR = 0.272±0.024, perennials overall LRR = 0.426±0.036. Overall, drought-stressed perennials responded significantly better to colonization. WP growth is significantly greater in perennials (Fₙ = 8.74, df = 1, 92, P = 0.004). Low sample size (n=4) for perennial reproductive measures contributed to high SE. AG = aboveground, BG = belowground, RP = reproductive, WP = whole plant.
Log response ratio (±SE) of plant growth response to mycorrhizal symbiosis controlled for tissue type. Herbaceous overall LRR = 0.288±0.023, woody overall LRR = 0.324±0.020. Plant growth response varied significantly between herbaceous and woody plants ($F_{s} = 6.47$, df = 1, 117, $P = 0.012$). AG = aboveground, BG = belowground, RP = reproductive, WP = whole plant.
Figure 7

Log response ratio ±SE of plant growth response to mycorrhizal symbiosis controlled for study site. Field overall LRR = 0.287±0.033, greenhouse overall LRR = 0.335±0.025. Overall, there is no difference between study sites. Low sample size (n=10) for field belowground measures contributed to the high SE. AG = aboveground, BG = belowground, RP = reproductive, WP = whole plant.
Log response ratio ±SE of plant growth response to mycorrhizal symbiosis controlled for functional group. Forb overall LRR = 0.385±0.039, grass overall LRR = 0.221±0.033, legume overall LRR = 0.213±0.032, and tree/shrub overall LRR = 0.578±0.057. Plant growth response varied significantly between functional groups (F₃ = 14.08, df = 3, 601, P < 0.0001). AG = aboveground, BG = belowground, RP = reproductive, WP = whole plant. Values sharing the same letter do not differ significantly after Tukey’s HSD.
Log response ratio (±SE) of plant growth response to mycorrhizal symbiosis controlled for the seven most often applied species of Glomus inoculum. There is no overall difference between species. AG = aboveground, BG = belowground, RP = reproductive, WP = whole plant. Values sharing the same letter do not differ significantly after Tukey’s HSD.
Figure 10

Log response ratio (±SE) of plant growth response to mycorrhizal symbiosis controlled for water regime (well-watered or water-stressed, not accounting for the degree of water stress. Well-watered overall LRR = 0.250±0.034, water-stressed overall LRR = 0.324±0.020. There is no overall difference in plant growth between water regimes. AG = aboveground, BG = belowground, RP = reproductive, WP = whole plant.
Figure 11

Log response ratio (±SE) of plant growth response to mycorrhizal symbiosis controlled for water stress level. Low stress overall LRR = 0.336±0.058, moderate stress overall LRR = 0.324±0.023, and high stress overall LRR = 0.300±0.040. There is no difference between water stress levels on overall plant growth. AG = aboveground, BG = belowground, RP = reproductive, WP = whole plant. Values sharing the same letter do not differ significantly after Tukey’s HSD.
Figure 12

Strawberry plants after leaf area measurement and fruit, flower, and stolon counts prepared for drying. All aboveground portions were included in Shoot Dry Weight (SDW) measurements. Belowground portions were Root Dry Weight (RDW).
Figure 13

Total biomass in dry weight (g) of *B. curtipendula* by salinity level (Mean+SE, P < 0.0001, n = 64). *B. curtipendula* shows the strongest response to high salinity than all other species but the graph is representative of general trends over species with significant (P<0.05) salinity main effects, including *B. curtipendula* height (Ht), shoot dry weight (SDW), root dry weight (RDW), and TB; *E. trachycaulus* Ht, SDW, RDW, and TB; *H. curassavicum* SDW, RDW, and TB; and *L. andersonii* Ht, SDW, RDW, and TB. Salinity is measured in the electrical conductivity through soil in deci-Siemens per meter (dS/m).
Interaction between water and salinity treatments for *B. curtipendula* root dry weight (Mean+SE, P = 0.0005, n = 64). Salinity is measured in the electrical conductivity through soil in deci-Siemens per meter (dS/m). Pulsed water treatments were every six days, well-watered treatments were every three days.
Interaction between water and salinity treatments for *E. trachycaulus* total biomass (Mean+SE, $P = 0.0057$, n= 72). Salinity is measured in the electrical conductivity through soil in deci-Siemens per meter (dS/m). Pulsed water treatments were every six days, well-watered treatments were every three days.
Figure 14C

Interaction between water and salinity treatments for *E. trachycaulus* shoot dry weight (Mean+SE, $P = 0.0098$, $n=72$). Salinity is measured in the electrical conductivity through soil in deci-Siemens per meter (dS/m). Pulsed water treatments were every six days, well-watered treatments were every three days.
Interaction between salinity and mycorrhizal status for *L. andersonii* root-to-shoot ratio (Mean+SE, P = 0.0498, n = 71). Salinity is measured in the electrical conductivity through soil in deci-Siemens per meter (dS/m). Colonized plants were only those where arbuscules and/or vesicles were observed.
Figure 15B

Interaction between salinity and mycorrhizal status for *P. smithii* height (Mean+SE, P = 0.0166, n = 83). Salinity is measured in the electrical conductivity through soil in deci-Siemens per meter (dS/m). Colonized plants were only those where arbuscules and/or vesicles were observed.
Interaction between water and salinity treatments for all plants root dry weight (Mean+SE, P = 0.0098, n= 398). Salinity is measured in the electrical conductivity through soil in deci-Siemens per meter (dS/m). Pulsed water treatments were every six days, well-watered treatments were every three days.
Figure 17

Interaction between water and salinity treatments for all plants total biomass (Mean+SE, P = 0.013, n= 398). Salinity is measured in the electrical conductivity through soil in deci-Siemens per meter (dS/m). Pulsed water treatments were every six days, well-watered treatments were every three days.
Table 1

Mean log response ratio (LRR) (±SE) of plant growth category by variable. LRR values are sorted high to low. AG = aboveground, BG = belowground, RP = reproductive, WP = whole plant. WS = water-stressed, WW = well-watered. * indicates significance (P < 0.05). Values sharing the same letter do not differ significantly after Tukey’s HSD.

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Table 2

Mean log response ratio (LRR) (±SE) of variable by plant growth category. LRR values are sorted high to low. AG = aboveground, BG = belowground, RP = reproductive, WP = whole plant. WS = water-stressed, WW = well-watered. * indicates significance (P < 0.05). Values sharing the same letter do not differ significantly after Tukey’s HSD.

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<td>19</td>
<td>0.175 ± 0.10</td>
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</tr>
<tr>
<td>WP</td>
<td>WW</td>
<td>93</td>
<td>0.414 ± 0.06</td>
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</tr>
<tr>
<td></td>
<td>WS</td>
<td>47</td>
<td>0.284 ± 0.11</td>
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<table>
<thead>
<tr>
<th>Growth Category</th>
<th>Glomus spp.</th>
<th>n</th>
<th>LRR (±SE)</th>
<th>Tukey's HSD</th>
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<tbody>
<tr>
<td>WP</td>
<td>versiforme</td>
<td>5</td>
<td>1.217 ± 0.28</td>
<td>a</td>
</tr>
<tr>
<td>a</td>
<td>etunicatum</td>
<td>14</td>
<td>0.610 ± 0.16</td>
<td>ab</td>
</tr>
<tr>
<td></td>
<td>intraradices</td>
<td>13</td>
<td>0.607 ± 0.15</td>
<td>ab</td>
</tr>
<tr>
<td></td>
<td>Mixed spp</td>
<td>15</td>
<td>0.518 ± 0.14</td>
<td>ab</td>
</tr>
<tr>
<td></td>
<td>deserticola</td>
<td>8</td>
<td>0.371 ± 0.14</td>
<td>ab</td>
</tr>
<tr>
<td></td>
<td>fasciculatum</td>
<td>13</td>
<td>0.336 ± 0.11</td>
<td>a</td>
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<tr>
<td></td>
<td>mossea</td>
<td>0</td>
<td>0.000 ± 0.00</td>
<td></td>
</tr>
<tr>
<td>AG</td>
<td>Mixed spp</td>
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<td>0.576 ± 0.16</td>
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</tr>
<tr>
<td>b</td>
<td>versiforme</td>
<td>33</td>
<td>0.466 ± 0.08</td>
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<tr>
<td></td>
<td>fasciculatum</td>
<td>24</td>
<td>0.395 ± 0.11</td>
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<td></td>
<td>mossea</td>
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<td>0.317 ± 0.06</td>
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<td>0.312 ± 0.21</td>
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<td>0.293 ± 0.08</td>
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<td>intraradices</td>
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<td>0.189 ± 0.05</td>
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<td>Mixed spp</td>
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</tr>
<tr>
<td>b</td>
<td>fasciculatum</td>
<td>6</td>
<td>0.344 ± 0.20</td>
<td>b</td>
</tr>
<tr>
<td></td>
<td>intraradices</td>
<td>24</td>
<td>0.327 ± 0.09</td>
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</tr>
<tr>
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<td>mossea</td>
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<td>etunicatum</td>
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<td>versiforme</td>
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<td>0.000 ± 0.00</td>
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</tr>
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<td>BG</td>
<td>etunicatum</td>
<td>1</td>
<td>0.693 ± 0.00</td>
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Table 3


<table>
<thead>
<tr>
<th>Water regime</th>
<th>Soil type</th>
<th>Inoculum</th>
<th>Replicates</th>
<th>TB (g)</th>
<th>SDW (g)</th>
<th>RDW (g)</th>
<th>R:S ratio (log)</th>
<th>LA (cm²)</th>
<th>FF</th>
</tr>
</thead>
<tbody>
<tr>
<td>WS A BO</td>
<td></td>
<td></td>
<td>8</td>
<td>3.1 ± 0.44</td>
<td>1.6 ± 0.32</td>
<td>1.5 ± 0.19</td>
<td>0.8 ± 0.11</td>
<td>94.5 ± 24.5</td>
<td>0.8 ± 0.49</td>
</tr>
<tr>
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<td></td>
<td></td>
<td>8</td>
<td>3.1 ± 0.37</td>
<td>1.5 ± 0.26</td>
<td>1.6 ± 0.16</td>
<td>0.8 ± 0.08</td>
<td>80.6 ± 13.2</td>
<td>1.4 ± 0.71</td>
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<td>8</td>
<td>2.6 ± 0.47</td>
<td>1.3 ± 0.42</td>
<td>1.3 ± 0.13</td>
<td>1.0 ± 0.19</td>
<td>37.6 ± 9.10</td>
<td>0.3 ± 0.16</td>
</tr>
<tr>
<td>N BO</td>
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<td></td>
<td>8</td>
<td>1.8 ± 0.48</td>
<td>0.9 ± 0.27</td>
<td>1.0 ± 0.23</td>
<td>0.9 ± 0.11</td>
<td>33.1 ± 15.8</td>
<td>0.8 ± 0.37</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8</td>
<td>2.3 ± 0.36</td>
<td>1.0 ± 0.25</td>
<td>1.3 ± 0.23</td>
<td>1.0 ± 0.15</td>
<td>57.2 ± 17.5</td>
<td>0.0 ± 0.00</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>8</td>
<td>1.2 ± 0.30</td>
<td>0.5 ± 0.15</td>
<td>0.7 ± 0.16</td>
<td>0.9 ± 0.14</td>
<td>33.5 ± 14.8</td>
<td>0.1 ± 0.13</td>
</tr>
<tr>
<td>WW A BO</td>
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<td></td>
<td>8</td>
<td>6.7 ± 0.32</td>
<td>4.1 ± 0.26</td>
<td>2.6 ± 0.19</td>
<td>0.5 ± 0.05</td>
<td>253.3 ± 19.5</td>
<td>3.8 ± 0.56</td>
</tr>
<tr>
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<td></td>
<td></td>
<td>8</td>
<td>6.3 ± 0.65</td>
<td>3.6 ± 0.35</td>
<td>2.7 ± 0.42</td>
<td>0.6 ± 0.06</td>
<td>209.0 ± 28.5</td>
<td>3.4 ± 0.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8</td>
<td>6.0 ± 0.22</td>
<td>3.6 ± 0.20</td>
<td>2.4 ± 0.12</td>
<td>0.5 ± 0.03</td>
<td>195.7 ± 23.6</td>
<td>3.9 ± 0.88</td>
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<tr>
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<td></td>
<td>8</td>
<td>4.5 ± 0.47</td>
<td>2.7 ± 0.37</td>
<td>1.8 ± 0.19</td>
<td>0.5 ± 0.06</td>
<td>176.7 ± 11.8</td>
<td>3.5 ± 0.33</td>
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<td>8</td>
<td>5.2 ± 0.43</td>
<td>3.2 ± 0.32</td>
<td>2.0 ± 0.20</td>
<td>0.5 ± 0.05</td>
<td>213.6 ± 22.5</td>
<td>3.4 ± 1.18</td>
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<td>8</td>
<td>3.7 ± 0.68</td>
<td>2.0 ± 0.42</td>
<td>1.6 ± 0.32</td>
<td>0.6 ± 0.07</td>
<td>121.0 ± 28.6</td>
<td>2.0 ± 0.63</td>
</tr>
</tbody>
</table>

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Table 4

Mean ± SE of plant growth parameters of strawberry in peat-amended or unamended soil, microbial inoculum or none, and water-stressed or well-watered treatments. WS = water-stressed, WW = well-watered, A = peat-amended soil, N = unmodified soil, BO = Bio-Organics inoculum, BV = Bio-VAM inoculum, CN = no inoculum. Root:shoot ratio is natural log transformed. * indicates significantly improved values (P < 0.05), ** indicates significantly improved values (P < 0.01). Values sharing the same letter do not differ significantly after Tukey’s HSD.

<table>
<thead>
<tr>
<th>Soil type</th>
<th>Replicate</th>
<th>TB (g)</th>
<th>SDW (g)</th>
<th>RDW (g)</th>
<th>R:S ratio (log)</th>
<th>LA (cm2)</th>
<th>FF</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>48</td>
<td>4.6 ± 0.30**</td>
<td>2.6 ± 0.21**</td>
<td>2.0 ± 0.12**</td>
<td>0.7 ± 0.05</td>
<td>145.1 ± 14.0**</td>
<td>2.2 ± 0.33</td>
</tr>
<tr>
<td>N</td>
<td>48</td>
<td>3.1 ± 0.28</td>
<td>1.7 ± 0.19</td>
<td>1.4 ± 0.11</td>
<td>0.7 ± 0.05</td>
<td>105.8 ± 12.7</td>
<td>1.6 ± 0.31</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Replicate</th>
<th>TB (g)*</th>
<th>SDW (g)</th>
<th>RDW (g)</th>
<th>R:S ratio (log)</th>
<th>LA (cm2)**</th>
<th>FF</th>
</tr>
</thead>
<tbody>
<tr>
<td>BO</td>
<td>32</td>
<td>4.0 ± 0.38 a</td>
<td>2.3 ± 0.26</td>
<td>1.7 ± 0.14</td>
<td>0.7 ± 0.05</td>
<td>139.4 ± 17.3 a</td>
<td>2.2 ± 0.33</td>
</tr>
<tr>
<td>BV</td>
<td>32</td>
<td>4.2 ± 0.36 ab</td>
<td>2.3 ± 0.24</td>
<td>1.9 ± 0.16</td>
<td>0.7 ± 0.06</td>
<td>140.1 ± 16.4 a</td>
<td>2.0 ± 0.46</td>
</tr>
<tr>
<td>CN</td>
<td>32</td>
<td>3.4 ± 0.38 b</td>
<td>1.9 ± 0.25</td>
<td>1.5 ± 0.15</td>
<td>0.8 ± 0.07</td>
<td>96.9 ± 15.5 b</td>
<td>1.6 ± 0.38</td>
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</table>

<table>
<thead>
<tr>
<th>Water regime</th>
<th>Replicate</th>
<th>TB (g)</th>
<th>SDW (g)</th>
<th>RDW (g)</th>
<th>R:S ratio (log)</th>
<th>LA (cm2)</th>
<th>FF</th>
</tr>
</thead>
<tbody>
<tr>
<td>WS</td>
<td>48</td>
<td>2.4 ± 0.19</td>
<td>1.1 ± 0.12</td>
<td>1.2 ± 0.09</td>
<td>0.9 ± 0.05**</td>
<td>56.1 ± 7.3</td>
<td>0.5 ± 0.17</td>
</tr>
<tr>
<td>WW</td>
<td>48</td>
<td>5.4 ± 0.24**</td>
<td>3.2 ± 0.16**</td>
<td>2.2 ± 0.12**</td>
<td>0.5 ± 0.02</td>
<td>194.9 ± 10.7**</td>
<td>3.3 ± 0.31**</td>
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</tbody>
</table>
Table 5

F- and P-values of significant main effects and interactions for salinity (S), water (W), and infection status (I). Ht = height, SDW = shoot dry weight, RDW = root dry weight, TB = total biomass, R:S = root-to-shoot ratio.

<table>
<thead>
<tr>
<th>Species</th>
<th>Ht</th>
<th>SDW</th>
<th>RDW</th>
<th>TB</th>
<th>R:S</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. curtipendula</td>
<td>S (F₂,41 = 29.0, P &lt; 0.0001)</td>
<td>S (F₂,41 = 78.1, P &lt; 0.0001)</td>
<td>SₓW (F₂,41 = 11.9, P &lt; 0.001)</td>
<td>SₓW (F₂,41 = 4.0, P &lt; 0.05)</td>
<td></td>
</tr>
<tr>
<td>E. trachycaulus</td>
<td>S (F₂,60 = 3.3, P &lt; 0.05)</td>
<td>SₓW (F₂,60 = 5.6, P &lt; 0.01)</td>
<td>S (F₂,60 = 4.8, P &lt; 0.05)</td>
<td>SₓW (F₂,60 = 5.8, P &lt; 0.01)</td>
<td>W (F₁,60 = 13.2, P &lt; 0.001)</td>
</tr>
<tr>
<td>H. curassavicum</td>
<td>S (F₂,64 = 10.4, P &lt; 0.001)</td>
<td>S (F₂,64 = 7.7, P &lt; 0.01)</td>
<td>S (F₂,64 = 14.9, P &lt; 0.0001)</td>
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<tr>
<td>Ly. andersonii</td>
<td>S (F₂,54 = 4.3, P &lt; 0.05)</td>
<td>S (F₂,54 = 22.2, P &lt; 0.0001)</td>
<td>S (F₂,54 = 16.8, P &lt; 0.0001)</td>
<td>S (F₂,54 = 28.5, P &lt; 0.0001)</td>
<td>SₓI (F₁,54 = 4.2, P &lt; 0.05)</td>
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</table>
Table 6

Percentage of inoculated plants colonized by AMF (observed arbuscules or vesicles). Single species consisting of *Glomus intraradices* or mixed species consisting of *G. intraradices*, *G. aggregatum*, and *G. mosseae*.

<table>
<thead>
<tr>
<th>Species</th>
<th><em>G. intraradices</em></th>
<th>Mixed spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bouteloua curtipendula</em></td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td><em>Elymus trachycaulus</em></td>
<td>50%</td>
<td>30%</td>
</tr>
<tr>
<td><em>Heliotropium curassavicum</em></td>
<td>40%</td>
<td>23%</td>
</tr>
<tr>
<td><em>Lycium andersonii</em></td>
<td>35%</td>
<td>22%</td>
</tr>
<tr>
<td><em>Leymus triticoides</em></td>
<td>47%</td>
<td>13%</td>
</tr>
<tr>
<td><em>Pascopyrum smithii</em></td>
<td>28%</td>
<td>7%</td>
</tr>
<tr>
<td><em>Sporobolus airoides</em></td>
<td>45%</td>
<td>61%</td>
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