Phytorestoration using Arbuscular Mycorrhizal Fungi in Association with Desert Plants in Boron-Contaminated Soils

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ABSTRACT

Borax mining at the Rio Tinto boron mine in Boron, California has caused pollution of soil, desertification of local habitats, and loss of biodiversity due to severe boron contamination. I conducted a phytoremediation study to test the effects arbuscular mycorrhizal fungi in association with native plant species. The goal of this study was to evaluate the effect of arbuscular mycorrhizal fungi on desert plants' ability to tolerate the boron pollution from Rio Tinto soils. I evaluated bush peppergrass (*Lepidium fremontii*), quail bush (*Atriplex lentiformis*), desert dandelion (*Malacothrix glabrata*), white cheesebush (*Hymenoclea salsola*), and cattle spinach (*Atriplex polycarpa*) in Rio Tinto soils containing three different levels of boron. Eight replicates of each species grew in each soil type, with half inoculated with arbuscular mycorrhizal fungi. After four weeks of growth in the Rio Tinto soils, I harvested, cleaned, and dried the plants. I measured fresh weights immediately after harvesting and dry weights after forty-eight hours of oven drying to determine biomass accumulation. The results of the study indicate that in general arbuscular mycorrhizal fungi acts as a parasite on plants in order to obtain nutrients rather than a symbiont under high boron conditions, leading to significantly lower survival and biomass accumulation in plants inoculated with arbuscular mycorrhizal fungi compared to those that were not inoculated. The results indicate that the addition of arbuscular mycorrhizal fungi to native plants is not a viable addition for increasing plant survival or biomass accumulation for phytoremediation purposes at the Rio Tinto boron mine.

KEYWORDS

Rio Tinto boron mine, Phytoremediation, boron pollution, desert soils, *Brassicaceae*

INTRODUCTION

Industrial mining of boron results in boron pollution that leads to desertification of habitats and loss of biodiversity. However, boron is necessary for many industrial and commercial uses, most notably the manufacturing of glass, laundry detergent, and insulation (Tanaka and Fujiwara 2008). Because boron is so widely used for industrial and commercial purposes, it is mined at high levels to supply the industry demand despite the negative impact that the practice has on native vegetation (Tanaka and Fujiwara 2008). When boron is mined, topsoil is stripped away, which directly removes vegetation and essential nutrients for plant growth from the soil. Furthermore, as the topsoil is removed toxic and nutrient-poor soil is exposed and left in place of the topsoil (Verce et al. 2012). It is difficult for native plants to survive in the soil that results from boron mining because the soils are stripped of the essential nutrients plants need to survive and are severely contaminated with boron (Stiles et al. 2011). Although in low levels boron is a necessary micronutrient for plants, once the level of boron surpasses an average of 5 mg/L in soil, its presence results in toxicity to plants (Nable et al. 1997). Boron pollution eventually leads to desertification as plants are unable to tolerate the heavy metal and quickly die off, leaving barren, polluted land and disrupting ecological function (Verce et al. 2012).

Boron pollution has become a significant problem at three actively mined boron deposits in California (Kistler and Helvaci 1994). The largest of the three deposits, the Kramer deposit, in Boron, California, has been mined intensely for the last 150 years, producing 10,000 tons of boron per day. The Rio Tinto Borax site, which mines boron from the Kramer deposit, has contributed to high levels of boron contamination of soils in areas surrounding the mine (Kistler and Helvaci 1994). Due to mining practices, the soils near the Rio Tinto Borax mine are severely contaminated with boron, which ranges in concentration from 7-700 mg/L boron (B) (Stiles et al. 2011). The soils near Rio Tinto are characterized by poor water retention and contain a high concentration of salts, which further inhibits plant growth (Verce et al. 2012). The poor characteristics of the Rio Tinto soil due to boron pollution have caused widespread desertification near the mining site. The absence of plants and roots caused by desertification has left the soil unanchored to the earth, which in combination with high winds results in plumes of boron-contaminated dust that can be detrimental to human health (Cetinkaya et al. 2010). The

soils must be restored to a more tolerable state to facilitate plant growth and prevent further damage due to desertification (Stiles et al. 2011). It is necessary to restore the Rio Tinto soils to their original state to create a wildlife habitat with high diversity that will also help with erosion control in the desert environment.

Although there are several possible methods to remediate the Rio Tinto soils, phytorestoration is the best option given its relatively low cost and efficiency (Miransari 2011). Phytorestoration, the treatment of environmental problems using plants, is an ideal solution to remediate the Rio Tinto soils because the presence of plants that can tolerate the harsh conditions will improve soil over time (Verce et al. 2012). The plants used in phytorestoration will improve soil quality by enhancing aeration, water content, nutrient retention, and erosion prevention (Verce et al. 2012). Successive planting will increase organic matter in the soil as plants decompose, effectively diluting the boron levels in the soil and allowing the propagation of less boron-tolerant plants, which will improve biodiversity (Stiles et al. 2011). Furthermore, the presence of plant roots in the topsoil will support the growth of beneficial microbes in the Rio Tinto soils (Verce et al. 2012).

Several native, volunteer plants have been identified that can grow to some extent in the contaminated Rio Tinto Soils in their current condition. These native plants of the mustard (*Brassicaceae)* family include bush peppergrass (*Lepidium fremontii*), quail bush (*Atriplex lentiformis*), cattle spinach (*Atriplex polycarpa*), white cheesebush (*Hymenoclea salsola*), and desert dandelion (*Malacothrix glabrata*), which may be able to act as colonizer species whose presence over time will allow other species to grow in the contaminated soils as well, promoting biodiversity and helping to restore the habitat to its original state (Norman Terry Research Group, UC Berkeley, unpublished data).

To further improve the effectiveness of phytorestoration using native plants, the addition of Arbuscular Mycorrhizal (AM) fungi may increase plant tolerance to the harsh conditions of the Rio Tinto Soils. AM fungi are symbiotic root fungi that can help protect plants from heavy metal contamination by sequestering heavy metals into the fungi's hyphal cells walls (Giovannetti and Sbrana 1998). The addition of AM fungi may improve plant tolerance to the Rio Tinto soils by increasing the plants' root surface area available for the uptake of nutrients and water, effectively decreasing plant susceptibility to drought and nutrient stress caused by the the Rio Tinto soils. Phytorestoration utilizing AM fungi is an inexpensive remediation method

and has been proven an effective tool in the remediation of soils containing toxic levels heavy metals other than boron (Miransari 2011, Janouskova et al. 2006). It is possible that the native plants in conjunction with AM fungi could be very effective tools for remediation of the Rio Tinto soils if they form symbiotic associations. Although it has been suggested that the plants of the mustard family do not form associations with AM fungi (Giovannetti and Sbrana 1998), recent studies have shown that the associations may be possible (Norman Terry Research group, UC Berkeley, unpublished data). If AM fungi form an association with the boron-tolerant desert plants, the association should result in a significant increase in the ability of the plants to tolerate boron contamination, leading to a more efficient tool for the remediation boron-contaminated soils than the native desert plants alone.

The goal of this study is to evaluate the effect of AM fungi on desert plants' ability to tolerate the boron pollution from Rio Tinto soils containing three different levels of boron. I hypothesize that (1) the addition of AM fungi will increase the biomass of the desert plants, and (2) the addition of AM fungi will increase the survival rates of the desert plants compared to non-AM fungi plants in the Rio Tinto soils. This method should be proven a successful tool for remediating highly boron-contaminated soil in an efficient and cost-effective manner.

METHODS

Study Site

The boron-contaminated soil that I assessed for phytorestoration potential was collected from the Rio Tinto boron mine in Boron, California. Boron is located on the western edge of the Mojave Desert and receives an average precipitation of 95 mm/year in the winter months and 35 mm/year in the summer months (Hereford et al. 2006). The Rio Tinto boron mine is established on the Kramer Borate Deposit, which has been mined for borates since 1872. The process of boron mining removes nearly all nutrients from the soil, leaving it unsuitable for plant growth. Additionally, the land surrounding the mine is contaminated by boron through air and water-borne particle transport as well as transfer between soil horizons (Stiles et al. 2011). The combination of incredibly low precipitation, skeletal topsoil, and boron contamination from

mining practices that have continued for over one hundred years makes it nearly impossible for plants to grow successfully in areas near the mine (Verce et al. 2012).

Due to the harsh conditions of the land that must be remediated, it is necessary to use plants that are moderately tolerant of the soils in their present state. For my experiment I chose native species of plants that have been identified as volunteer species in the polluted soils at the Rio Tinto site (Stiles et al. 2011). The natural tolerance of the volunteer plants to the Rio Tinto soils and increased remediation ability from AM fungi holds potential for the most viable option to remediate the Rio Tinto soils.

Data collection

To test the effectiveness of AM fungi in association with desert plants in the Rio Tinto soils, I began by germinating five species of native desert plants. To germinate the desert plants, I planted seeds in a sterile sand and peat moss mixture until they grew to the four leaf stage. I prepared the soil by mixing three parts sand to one part peat moss. I then sterilized the soil by autoclaving it for twenty-five minutes three times. I planted three trays each of *L. fremontii*, *A. lentiformis*, *M. glabrata*, *H.salsola*, and *A. polycarpa* in the sterile soil mixture. The plants grew in the sterile soil for four weeks and were watered daily. I sprayed 50% dilute Hoagland's nutrient mixture on the plants twice a week for the four weeks that the plants grew in the sterile soil mixture to ensure that the plants were healthy before transplanting.

Transplanting

Once plants reached the four leaf stage, I transferred each plant to a pot prepared with either sterile control soil or a Rio Tinto soil. To prevent boron leakage, I lined each pot with two sterile Ziploc bags. I prepared 40 pots with 60 grams each of sterile peat moss potting soil (0 mg/L B), 40 pots of 200 grams each of Arkose Sand (AS) (120 mg/L B), 40 pots of 200 grams each of Highly Weathered Quartz Monzonite (HWQM) (7-14 mg/L B), and 40 pots of 200 grams each of Slightly Weathered Quartz Monzonite (SWQM) (500-700 mg/L B). I added 30 ml of sterile distilled, deionized (ddi) water to each pot. To ensure successful transplanting into the contaminated soil, I added a 20 ml plug of potting soil to all the pots containing a Rio Tinto soil.

I did not add a plug to the control soil, as it is primarily comprised of peat moss. I transplanted eight replicates of each species from the sand and peat moss mixture into each Rio Tinto soil and the control soil for a total of 160 (5 species x 4 soil types x 8 replicates of each type, half with AM fungi, half without AM fungi, replicates in the study. Immediately before transplanting, I weighed each plant and recorded its initial weight.

To compare the effects of AM fungi, I added AM fungi to half of the plants of each soil treatment. To add the AM fungi I mixed 2 grams of AM fungi spores with 10 ml of sterile ddi water, which I poured onto the root of each the plants when I transplanted them. I used "Myco Apply" AM fungi from Mycorrhizal Applications, Incorporated in Grants Pass, Oregon. The inoculum consisted of a combination of four naturally occurring desert species of AM fungi of the *Glomus* genera, including *Glomus intraradices, G. mosseae, G. aggregatum,* and *G. ettunicatum.* After transplanting and inoculation, I covered any exposed soils of all the treatments with perlite to help retain moisture in the soil. The plants grew for 4 weeks in these soil treatments. I watered the plants with sterile ddi water for the first week after transplanting and then with ddi water for the next three weeks. No nutrients were added to any of the treatments in order to promote associations, as AM fungi seek plant root associations in order to obtain nutrients (primarily phosphorous and nitrogen) (Giovannetti and Sbrana 1998).

Harvesting

After four weeks of growth in the soil treatments, I harvested and weighed the plants to determine changes in biomass that may have been dependent on the presence of AM fungi. To harvest the plants, I thoroughly cleaned soil off of the shoots and from the roots using ddi water. I weighed each plant to determine its fresh weight, which I used to determine each plants' change in biomass over the four week growing period.

Analysis

To determine if the AM fungi treatment affected the growth of the plants in contaminated soil, I ran a multi variable ANOVA for each species to test for significant differences between change in weight that is dependent on the presence or absence of AM fungi,

soil type, and the interaction between AM fungi and soil type. Each ANOVA evaluated differences in biomass as a function of soil type and presence of AM fungi for each species.

RESULTS

Plant Survival

I found that the soil combination of Highly Weathered Quartz Monzonite with no added AM fungi resulted in the highest survivorship (Table 1). *Atriplex lentiformis* and *L. fremontii* had the highest survivorship overall (both 100%), while *M. glabrata* had the lowest survivorship (56.25%). The survivorships of *H.salsola* and *A. polycarpa* were 71.88% and 87.5%, respectively. More plant deaths resulted in replicates with AM fungi than replicates that lacked AM fungi. In *M. glabrata* 10 replicates died with AM fungi, while only 4 died that did not contain AM fungi. *H.salsola* showed similar trends to *M. glabrata*, and all replicate deaths (10) were in soils that included AM fungi. For *A. polycarpa*, 3 of the 4 deaths were in soil containing AM fungi. I found that the soil with the highest survivorship was Highly Weathered Quartz Monzonite, and the soil with the lowest survivorship was the Control Soil. Highly Weathered Quartz Monzonite only resulted in 3 deaths, while Slightly Weathered Quartz Monzonite and Arkose Sand resulted in 6 deaths each. The Control Soil resulted in 12 deaths (10 of which contained AM fungi) (Table 2).

Table 1. Comparison of plant survivorship rates with and without AM fungi inoculation per species (percentage)

Species	Overall Survivorship	Survivorship of replicates with no AM fungi	Survivorship of replicates with AM fungi
L. fremontii	100	100	100
A. lentiformis	100	100	100
A. polycarpa	87.5	100	75
H. salsola	71.88	100	43.75
M. glabrata	56.25	68.75	31.25

Table 2. Comparison of plant survivorship rates with and without AM fungi inoculation between soil types (number of replicates)

Soil Type	Number of Surviving Replicates No AM Fungi	Number of Surviving Replicates With AM Fungi
Control	18	10
HWQM	19	18
SWQM	20	16
Arkose Sand	19	16

Biomass Accumulation Trends

Contrary to my predictions, I found that overall the plants that were inoculated with AM fungi had significantly lower biomass accumulation than plants that were not inoculated. For *L. fremontii* I found that plants that lacked AM fungi had significantly higher biomasses than those that were inoculated with AM fungi ($p<0.0001$). Furthermore, I found that soil type had a significant effect on the biomass accumulation of *L. fremontii* ($p=0.0056$), and that there was a significant interaction affect between presence of AM fungi and soil type ($p=0.0049$). *A. lentiformis* had similar significant factors as *L. fremontii*. *Atriplex lentiformis* had significant differences between AM fungi presence or absence ($p<0.0001$), soil type ($p=0.0068$), and the combined effects of soil type and AM fungi presence or absence ($p=0.034$). Significant differences were apparent in *A. polycarpa* or AM fungi presence or absence as well ($p=0.00031$). I found significant differences between soil types for *H.salsola* ($p=0.76$) and between AM fungi presence and absence for *M. glabrata* $p=0.20$).

DISCUSSION

Overall, plants that were inoculated with AM fungi had significantly lower biomass accumulation and survival rates than plants that were not inoculated, which indicates that AM fungi are not a viable addition for increasing plant survival for remediation purposes in boron-contaminated soil. Although the application of AM fungi was expected to increase plant survival in the boron-contaminated soils, plants that received the inoculation had significantly lower survival and growth rates than those that did not revive the inoculation. Despite the fact that AM

fungi do not increase plant survival in the contaminated soils, it is evident that some of the species of plants alone can withstand moderate to high boron contamination. The species that indicate some boron tolerance may present a viable option for remediation without the use of AM fungi.

AM Fungi, Survival, and Biomass Accumulation

The addition of AM fungi was expected to improve plant survivorship and increase biomass accumulation, however the presence of AM fungi actually decreased survival for all species except *Lepidium fremontii* and *Atriplex lentiformis,* and resulted in significantly lower biomass accumulation for all species and soil combinations.

Survivorship was low for inoculated plants in all soils, including the Control Soil (see Table 1), which indicates that plant survivorship is more dependent on AM fungi presence than soil type. However, the highest plant death rates were observed in the Control Soil with AM fungi, indicating that AM fungi caused more damage to host plants in soils with lower boron content. Because the Control Soil had no boron pollution, the soil may be more beneficial to the AM fungi than the host plants when AM fungi were present, as plant deaths in inoculated Control Soil may have been due to optimal conditions for high growth of AM fungi rather than host plants (Cartmill et al. 2013). It is possible that the highest number of plant deaths occurred in the Control Soil with AM fungi because the AM fungi were not inhibited by boron pollution as in the Rio Tinto soils, allowing the AM fungi to grow stronger and more efficiently at the expense of the host plant (Burrows and Pfleger 2002). Similarly to the control soil, inoculated plants in the Rio Tinto soils had lower survival rates than plants that were not inoculated. In the Rio Tinto soils AM fungi may have been stressed under the higher boron conditions, and in turn put more stress on the host plant for nutrients that were not available in the soil.

Similarly to survival, biomass accumulation was low for inoculated plants in all soils, including the Control Soil (Figure 1), which indicates that plant growth is more dependent on AM fungi presence than soil type. Presence or absence of AM fungi was the most significant factor in determining biomass accumulation for all species except *M. glabrata* (Table 3). For some species, soil type and the interaction between soil type and AM fungi were also significant factors that affected biomass accumulation (R Development Team 2009). However, AM fungi

presence or absence was the most significant factor in determining growth for species that had multiple significant factors (Table 3). Soil type and interaction factors were also significant determinants of biomass accumulation for both *L. fremontii* and *A. lentiformis* (R Development Team 2009). However, AM fungi presence is a more notable factor in the biomass trends of both species. Although biomass accumulation was progressively lower with increasing boron levels in plants without AM fungi for both species, biomass accumulation stayed constantly low for inoculated plants of both species, indicating that presence or absence of AM fungi was a more influential factor in determining biomass accumulation than soil type. Furthermore, because the combination of AM fungi and soil type *L. fremontii* and *A. lentiformis* was also a significant factor for biomass accumulation, it is clear that soil type alone did not determine biomass accumulation trends in *L. fremontii* and *A. lentiformis*.

Table 3. **ANOVA Generated P Values.** P values generated from five multivariable ANOVAs assess if there are significant differences in biomass accumulation between plants with AM fungi and plants that lack AM fungi, between soil type, and between combinations of soil type and presence or absence of AM fungi. Significance is indicated by asterisks, with more asterisks indicating more relative significance.

Species	AMF	Soil Type	Interaction
L. fremontii	5.70E-06 ***	0.005649**	0.00495**
A. lentiformis	4.27E-10 ***	0.006897**	0.034118*
A. polycarpa	0.0003102 ***	0.4486243	0.8705675
H. salsola	0.03972*	0.76473	0.93326
M. glabrata	0.08982	0.01589*	0.93396

The addition of AM fungi may have caused lower survival and biomass accumulation in all inoculated plants because the fungi may have acted as a parasite to host plants, rather than a symbiont as expected. AM fungi made it more difficult for host plants to survive and grow in all soils when inoculated with AM fungi, because the maintenance of the AM fungi by a host pant may result in a higher carbon cost for the plant, leading to lower plant growth and high plant death (Bryla and Eissenstat 2005). The ideal growth conditions of the Control Soil may have amplified the AM fungi's ability to parasitize the host plants, leading to the highest number of plant deaths in the Control Soil (Purin and Rillig 2008, Ryan et al. 2005). However, because biomass accumulation was low and relatively similar for all plants inoculated with AM fungi in all soil types, it appears that the AM fungi was less affected by boron levels that host plants without AM fungi, allowing the fungi to parasitize plant hosts in all boron levels, and effectively lowering biomass accumulation for all plants (Cartmill et al. 2013).

Furthermore, it is also possible that the AM fungi did not help plant survivorship and growth because they did not form associations with the plant roots. It has been suggest that plants of the family *Brassicaceae* may not form relationships with AM fungi because the plants lack factors in their rhizospheres that stimulate hyphal proliferation of AM fungi (Giovannetti et al. 1993). Furthermore, the Rio Tinto soils may have been responsible for the lack or associations because desert soil that has been disturbed by mining and other activities may be less conducive to AM fungi colonization than the soil of undisturbed desert land (Miller 1979). However, if it is the case that the AM fungi did not form associations with the plants, it seems that AM fungi presence would not have had any effect on biomass accumulation or survival rather than having detrimental effects (Cartmill et al. 2013). It is possible that a different strain of AM fungi rather than *Glomus* may form associations with the plants, which could lead to a symbiotic relationship resulting in enhanced growth. However, *Glomus* was specifically selected because it is a naturally occurring form of desert AM fungi, and it may not be appropriate to use strains of AM fungi that are not specifically adapted for a desert environment.

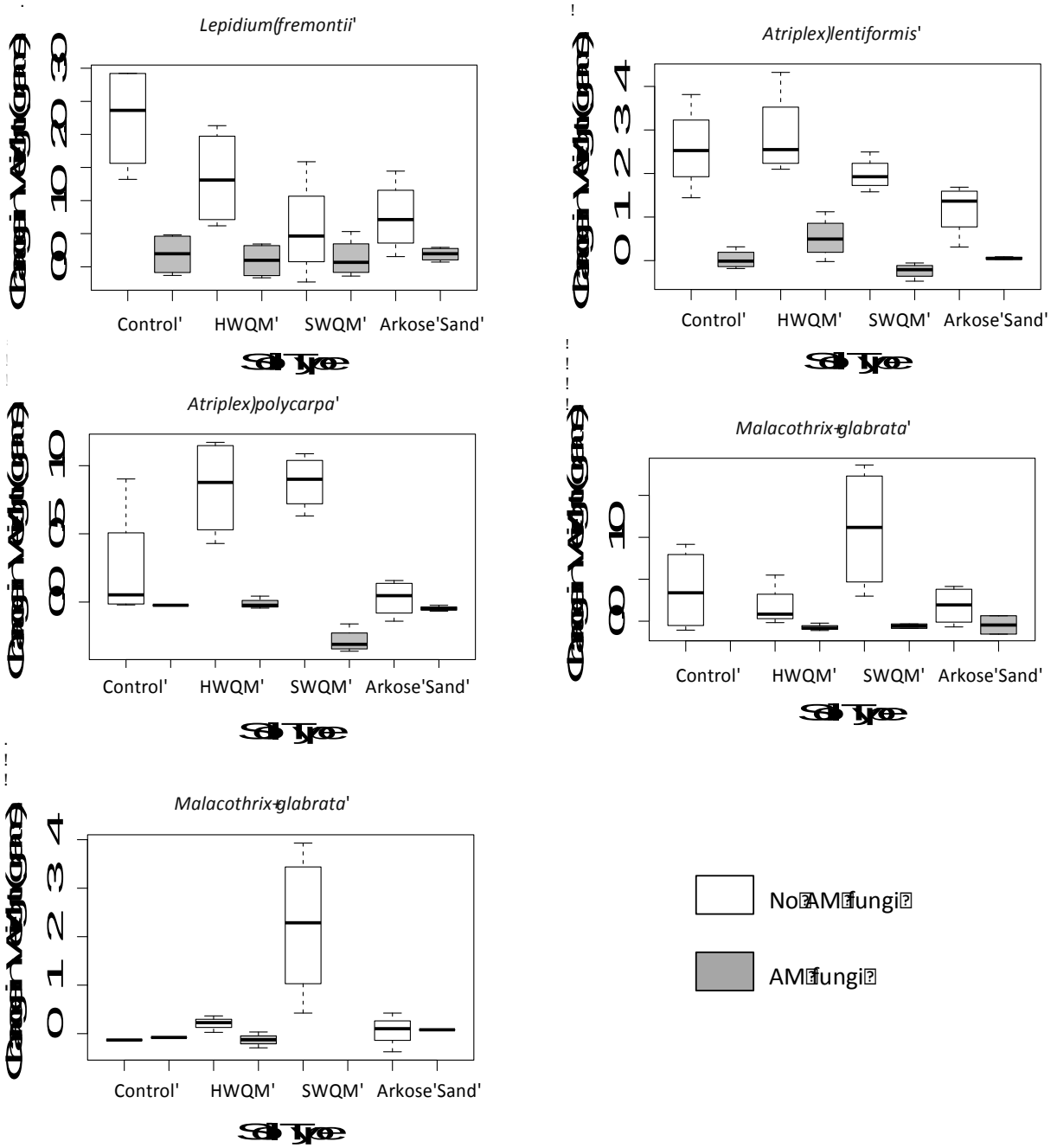

Figure 1. Difference in biomass accumulation between non-inoculated plants and inoculated plants. Box and whisker plot comparisons of three species with and without the addition of AM fungi: *L. fremontii*, *A. lentiformis*, *A. polycarpa*, *H. salsola*, and *A. polycarpa*.

Plant Species, Survival, and Biomass Accumulation

Although the addition of AM fungi may not increase plant survival, the higher survival rates of desert plants without AM fungi indicate that *Atriplex lentiformis* and *L. fremontii* may be

effective remediators on their own. *Atriplex lentiformis* and *Lepidium fremontii* exhibited the highest tolerance to the contaminated soils with and without AM fungi inoculation (Figure 1). Because the AM fungi had less of a negative effect on *Atriplex lentiformis* and *Lepidium fremontii*, it is possible that these species are more tolerant of extreme stressors, including both boron and AM fungi. Because of their ability to survive in all tested soil and AM fungi combinations, *Atriplex lentiformis* and *Lepidium fremontii* are likely the best options for remediating the sub-optimal soils of the Rio Tinto Borax Mine by acting as habitat colonizers and adding more organic matter to soils.

Study Limitations

The results of this study indicate that AM fungi may reduce plant survivorship and growth in the Rio Tinto soils, but this may be due to the limitations of the study set up. It is unclear whether the AM fungi directly or indirectly lead to decreased plant growth and survival, because it is unknown if the AM fungi actually formed associations with the plants. A solid conclusion about the effect of the AM fungi cannot be drawn if the status of the colonization is not known. Future studies may assess the associations between the AM fungi and the plant roots through root samples and microscopy. If the associations did form, it is likely that the AM fungi directly lead to decreased plant survival because of a higher carbon cost (Cartmill et al. 2013). If the association did not form then it is unclear what factors lead to significantly lower growth and survival rates.

It is also possible that the AM fungi chosen for the study were inappropriate. A lower growth strain of native desert AM fungi may have formed more positive associations with the plants (Cartmill et al. 2013). Furthermore, the AM fungi used in the study may have been inappropriate as they are a sporulating strain, despite the fact that a high percentage of AM fungi in that occur in desert environments are nonsporulating (Stutz and Morton 1996). The boron in the soils may have caused excess strain on the nonsporulating strain utilized in this study, resulting in a parasitic relationship with the desert plants (Bryla and Eissenstat 2005). The fungi may have encountered further stress due to the fact that they generally thrive under soil conditions characterized by higher levels of organic content, unlike the soils used in this study (Khaleil 1985).

The plants chosen for the study may have been inappropriate as well. Although the plants were chosen for their preexisting tolerance to the Rio Tinto soils, they may not have been able to form associations with the fungi (Verce et al. 2012). Perhaps boron-tolerant plants of a different family would perform better under the same conditions (Giovannetti and Sbrana1998).

The results of this study may be skewed due to the high instance of plant deaths, which lead to unequal variances and data that was not transformed for normality. The data were not strictly normal for all species, and I chose not to transform the data for each species to maintain continuity given that the box and whisker plots (Fig. 1) showed relative normality for *L. fremontii*, *A. lentiformis*, and less so for *A. polycarpa*. The lack of normality and appropriate transformation was partially due to the number of plant deaths that occurred in *A. polycarpa*, *H.salsola*, and *M. glabrata*. Plant deaths resulted in low replicate numbers that made data sets patchy and unable to be transformed for normality. Data were not transformed to ensure continuity between species' individual biomass accumulation data sets and box and whisker plots. Furthermore, a logistic regression could be performed for the survival data, to help understand the effect of each soil type and AM fungi on survival, or potential predict the outcome of various soil and AM fungi combination

Further analysis to determine the boron content of the harvested plants may have given insight into the effect of the AM fungi. It would be beneficial to compare boron content between plants that were inoculated with AM fungi to those that were not inoculated. The level of boron in the plants could give key insights into the effect of the AM fungi in the Rio Tinto soils. I was unable to analyze the boron content due to inadequate funding for inductively coupled plasma mass spectrometry.

Broader Implications and Remediation Recommendations

The best option for the restoration of the boron-contaminated Rio Tinto soils is to augment the soil either directly, through weathering, or through plants. To augment the soil additions should be mixed into the contaminated soil to dilute the boron content. Mixtures of peat moss, gypsum, and organic material should be tested to determine the optimum combination of soil additives to promote plant growth.

Additionally, converting severely contaminated soils to less contaminated soils, such as Highly Weathered Quartz Monzonite, could augment the soil enough to promote plant growth. Despite the overall trend of the ineffectiveness of AM fungi for increasing plant survival or biomass, plants displayed high survival rates and growth in Highly Weathered Quartz Monzonite without AM fungi. Of the Rio Tinto soils, Highly Weathered Quartz Monzonite had the lowest boron content (7-14 mg/L B) and resulted in the highest survivorship for inoculated plants and high survivorship and growth for non-inoculated plants (Table 2). Highly Weathered Quartz Monzonite is much more tolerable than Slightly Weathered Quartz Monzonite (500-700 mg/L B), and is the product of leaching boron out of Slightly Weathered Quartz Monzonite through wind, rain, and other weather forces over time. By digging out large masses of Slightly Weathered Quartz Monzonite and leaving them to be weathered into Highly Weathered Quartz Monzonite, the soil could be augmented to the point that it is high enough quality to promote plant colonization and habitat restoration.

The Rio Tinto soils could also be augmented by adding organic material indirectly through successive planting of the most tolerant species without the use of AM Fungi. *Lepidium fremontii* and *A. lentiformis* demonstrated the highest growth in the contaminated soils, and would be good options for initial colonizer plants. These species should be planted for several seasons and as they grow, die, and decompose they will contribute to more biomass in the soil, making it more habitable for less boron-tolerant plants (Khaleil 1985, Ramadan and Omar 2000). *L. fremontii*, however, may not be a good colonizer plant as it can produce an allelopathic growth inhibitor, which acts as a toxin and prevents other plants from growing to minimize competition (Kato-Noguchi et al. 2002). Because of this growth inhibitor *A. lentiformis* and *A. polycarpa*, which had the next highest survival and growth rates, should be used as the colonizer species. *H. salsola* should be planted in succession to *A. lentiformis* and *A. polycarpa*. *M. glabrata* however, had significantly lower growth and survival rates than any of the other species that were more dependent on soil type than AM fungi presence, and may not be an appropriate option for remediation. None of the plants used for restoration should be inoculated with AM fungi, as it will likely result in reduced growth and survival rates at Rio Tinto as in this study.

ACKNOWLEDGEMENTS

I would like to thank the ES 196 team comprised of Patina Mendez, Kurt Spreyer, Carrie Cizauskas, Rachael Marzion, Anne Murray, and Vicki Wojcik for their continued support over the course of the last year. I would specifically like to thank Patina for grading and editing all of my drafts and guiding me throughout the entire thesis process when I often felt completely lost, and Carrie for her help with R studio and understanding the tedious statistical problems associated with my project. Maria Suarez of the Terry Lab served as my mentor, and guided and supported me throughout my entire project. The Terry Lab at UC Berkeley gave me the opportunity to work on my project, which would not have without Professor Norman Terry and the many undergraduate lab assistants at the Terry Lab. I received critical feedback and support for the last year from my peer editing group, the Plant Mob: Paz Lozano, Fatemeh Adlparvar, Kanesha Pompey, and Matthew Perryman.

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APPENDIX A: REPLICATE SURVIVORSHIP

Species	Soil Type	Number of Surviving Replicates No AMF Fungi	Number of Surviving Replicates With AMF Fungi
L. fremontii	Control	4	4
	HWQM	4	4
	SWQM	4	4
	Arkose Sand	4	4
A. lentiformis	Control	4	4
	HWQM	4	4
	SWQM	4	4
	Arkose Sand	4	4
A. polycarpa	Control	4	1
	HWQM	4	4
	SWQM	4	4
	Arkose Sand	4	4
H. salsola	Control	4	0
	HWQM	4	3
	SWQM	4	2
	Arkose Sand	4	2
M. glabrata	Control	2	1
	HWQM	3	3
	SWQM	4	0
	Arkose Sand	3	2

Table A1. Plant Survivorship of each species in all soil types (per replicate)