Edaphic sorting drives arbuscular mycorrhizal fungal community assembly in a serpentine/non-serpentine mosaic landscape

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Abstract. Serpentine soil generates distinct plant assemblages, but it is not known how this edaphically extreme environment affects arbuscular mycorrhizal fungal (AMF) assembly or how this may contribute to plant adaptation to serpentine. Our previous studies showed that serpentine and non-serpentine adapted ecotypes of Collinisa sparsiflora associates with distinct AMF assemblages, but a common garden experiment showed that this pattern was not due to host-fungal preference. We hypothesized that the observed differences in AMF associated with C. sparsiflora ecotypes was driven by edaphically defined AMF assemblages. To test this idea we employed a broader sampling of the plant community from five serpentine and five non-serpentine sites in close proximity (50–150 m between sites) and identified AMF and plant species associated with root samples by amplifying rDNA and cpDNA respectively, cloning, and sequencing. We compared AMF and plant assemblages, and measured the relative contribution of distance, plant and soil factors on AMF assembly. Analyses clearly showed that serpentine and non-serpentine AMF assemblages are distinct—with the complete absence of the non-serpentine dominant AMF taxon on serpentine. These results show strong edaphic sorting of serpentine tolerant/adapted AMF taxa in serpentine soil and indicate a strong ecological correlation between AMF and plant tolerance to serpentine soil.

Key words: arbuscular mycorrhizal fungi (AMF); community assembly; cpDNA; ecological sorting; edaphic factors; fungal community; McLaughlin Reserve, Lake County, California, USA; plant community; rDNA; serpentine; soil type.

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INTRODUCTION

Serpentine soils are well known for generating distinct plant assemblages. Derived from ultramafic rock, serpentine soils are characterized by a very low Ca:Mg ratio, low levels of essential nutrients (N, P, K), high to toxic levels of heavy metals (Fe, Cr, Co, Ni), and drought susceptibility (Brooks 1987, Brady et al. 2005). Serpentine soils often occur as discontinuous “island-like” areas within non-serpentine soil types generating an abruptly heterogeneous landscape sometimes at fine spatial scales (Davies et al. 2005). These abrupt changes in edaphic conditions pose severe challenges to plant growth leading to strong selection of serpentine tolerant and intolerant species, locally adapted ecotypes, as well as endemic (serpentine restricted) plant species (Kruckeberg 1984, Rajakaruna 2004, Brady et al. 2005). But very little is known about how...
serpentine edaphic factors shape assembly of important plant symbionts or what role a potentially serpentine tolerant/adapted symbiont assemblage may play in plant adaptation to serpentine.

AMF are common root symbionts that can increase their plant hosts’ establishment and growth in stressful environments by enhancing nutrient and water uptake and may provide protection against toxic conditions (Yost and Fox 1979, Habte and Manjunath 1987, Meharg 2003). Plant adaptation to complex serpentine edaphic factors is not fully explained by plant physiological and morphological traits alone (Brady et al. 2005, Wright and Stanton 2007). Therefore, plant traits (e.g., requirement for and response to AMF) and fungal traits (e.g., tolerance of or adaptation to edaphic stress) which affect the symbiotic functioning under nutrient and metal stress have the potential to contribute to plant growth and fitness in harsh serpentine edaphic conditions. Our 2008 study (Schechter and Bruns 2008) showed that serpentine and non-serpentine adapted ecotypes of Collinsia sparsiflora associated with distinct AMF assemblages. Since we did not find evidence of AMF dispersal limitation between C. sparsiflora ecotype locations we hypothesized that the distinction between plant ecotype AMF assemblages could be due to specificity between adapted plant genotypes and adapted fungal genotypes within a ubiquitous AMF assemblage. However, the distinction between the plant ecotype AMF assemblages was also correlated with the dissimilarity in rhizosphere soil chemistry associated with serpentine and non-serpentine plant ecotypes. Thus, the distinct AMF assemblages that we found associated with the two different ecotypes of C. sparsiflora may simply have been driven by edaphic factors rather than host genetic differences.

Both niche and neutral processes play roles in AMF community assembly (Dumbrell et al. 2010). Soil pH, soil texture and spatial distance (as a proxy for dispersal limitation) have all been shown to have a role in AMF assemblage structure and composition (Johnson et al. 1992, Lekberg et al. 2007, Dumbrell et al. 2010, Lekberg et al. 2011). Thus, heterogeneous extreme edaphic environments are also likely to generate dissimilarity in AMF assemblages. Ackerly (2003) described extreme edaphic habitats (e.g., serpentine, heavy metal mine tailings, acid bogs) as examples of environmental islands in which a species niche space is defined by adaptive evolution (i.e., the ability or inability to adapt to a specific edaphic stress). For instance, metal contaminated sites are associated with metal-tolerant AMF taxa (Gildon and Tinker 1981, Weissenhorn and Leyval 1995, Gonzalez-Chavez et al. 2002), thus variation between AMF species in tolerance of and/or adaptation to a specific metal contaminant can generate differences in assemblage composition (Meharg and Cairney 1999, Meharg 2003).

Serpentine soils provide an excellent system to study the effect of extreme edaphic factors on AMF assemblage structure and composition. Much like the anthropogenically contaminated sites discussed above, serpentine soils often have high concentrations of toxic metal ions, but unlike contaminated sites, serpentine has existed in particular areas for 10,000 to 10 million years (Kruckeberg 1984) and so provide a more sustained selective regime on AMF taxa. If plants are a good model, one would predict that the heterogeneous serpentine habitat would serve as an ecological filter for AMF taxa as well and generate serpentine tolerant and intolerant AMF taxa, locally adapted ecotypes, and/or unique and possibly endemic serpentine AMF. Distinct serpentine adapted AMF assemblages may provide specific services to plants in this harsh environment and facilitate plant adaptation to serpentine. However, it is difficult to isolate edaphic factors from the influence of plant community differences on AMF assemblage structure and composition (Johnson et al. 1992, Bever et al. 2002). We know that the extreme edaphic factors of serpentine soil generates distinct serpentine floras (Kruckeberg 1984, Brady et al. 2005). Therefore, any comparison of serpentine and non-serpentine AMF assemblages must also account for differences in associated plant assemblages.

The goal of this study is to examine if there are edaphically distinct AMF assemblages that associate with the broader plant communities present on serpentine and non-serpentine soils. This result will help explain the distinction between AMF assemblages observed associating with serpentine and non-serpentine C. sparsiflora eco-
types at the same location (Schechter and Bruns 2008). To carry out this study, we compared AMF assemblages associated with randomly sampled plant roots found in adjacent serpentine and non-serpentine soil types using molecular methods. We took advantage of the fine-scale mosaic of serpentine and non-serpentine soils associated with the McLaughlin Reserve Research Grid to sample serpentine and non-serpentine sites within a close geographical range (50–150 m between sites) in order to limit the influence of dispersal limitation on AMF assemblage structure (Lekberg et al. 2007, Lekberg et al. 2011). To directly link plant community to specific AMF assemblages, we used molecular methods to identify plant taxa from precisely the same root tissue from which the AMF taxa were sampled. We have two objectives in this study: (1) determine if serpentine and non-serpentine soil types harbor distinct AMF assemblages, and (2) to ascertain the relative influence of spatial distance, soil factors and plant communities on any AMF assemblage differences.

**MATERIALS AND METHODS**

**Study system**

This study was done at the Donald and Sylvia McLaughlin University of California Natural Reserve located in Napa, Lake, and Yolo counties in northern California (Fig. 1). The McLaughlin reserve is situated over a minor fault line that has produced a fine-scale mosaic of serpentine, volcanic, and valley sediment soil types occurring within meters of each other (Wright et al. 2006, Wright and Stanton 2007). In 2001, researchers from the University of California Davis used the unique geology of the research to establish a 27.5 ha research grid that encompassed this fine-scale mosaic of soil types. They established grid points 50 meters apart across the entire grid area in which they did soil physical and chemical analyses as well as vegetation surveys at each 50 meter grid point (http://nrs.ucdavis.edu/mcl/visitor/facilities.html). We used the McLaughlin research grid to establish five serpentine (Ca:Mg < 1) and five non-serpentine (Ca:Mg > 1) soil type sites for this study (Fig. 1) (Wright et al. 2006).
Sampling

In May 2007, we sampled roots from five serpentine (average Ca:Mg = 0.36) and five non-serpentine (average Ca:Mg = 6.35) grid points to compare soil type AMF assemblages. We collected four root samples (labeled A–D) at cardinal direction points along a 1.0 m diameter circle placed around each grid point (S1, S2, S3, S4, S5 and NS1, NS2, NS3, NS4, NS5) (Fig. 1). We sampled roots by taking a trowel slice 10 cm by 8 cm and 14 cm deep, at each grid point for a total of 40 root samples for the entire study (twenty serpentine and twenty non-serpentine). We also collected soil directly adjacent to the plant root collection in each sample point and combined equal amounts of soil from each sample point to equal one soil sample per grid point for soil analysis (10 soil samples for the entire study).

All root and soil samples were put directly into coolers and stored in a 4°C cold room within 8 hours of collection. Soil samples were sent to A&L Western Agricultural Laboratories (Modesto, CA) within 24 hours of collection for chemical analysis. All root samples were processed within two weeks of collection. We collected and washed all roots within each sample, took a small portion (0.5 g wet weight) to visually examine AMF colonization (Peters and Habte 2001), and then processed them for DNA extractions using the methods of Schechter and Bruns (2008).

Molecular analysis

DNA extraction.—We extracted DNA from washed root samples collected from each grid point (40 total). We crushed the dried and frozen roots by beadbeating (Mini-Beadbeater, Biospec Products) then extracted DNA from each root sample as described in Schechter and Bruns (2008).

Polymerase chain reaction (PCR): AMF.—We amplified a variable region of the 18S rDNA using *Pfu Turbo* DNA polymerase (Stratagene) and universal eukaryotic primer NS31 (Simon et al. 1992) paired with AM1 (Helgason et al. 1998) using methods described in Schechter and Bruns (2008). This primer pair is designed to avoid plant sequences and works well with direct (non-nested) amplification but it is thought to exclude taxa from the Paraglomeraceae and Archaeosporaceae (Redecker et al. 2000) We chose this primer set because of its past use at McLaughlin (Schechter and Bruns 2008) and our interest in comparing results across studies. Although the primer pair may miss some taxa it still provided a consistent comparison across samples and studies.

Polymerase chain reaction (PCR): Plants.—We amplified the cpDNA intergenic spacer region between the *trnL* (UAA) 3' exon and the *trnF* (GAA) gene using the primer pair *trnL*-e and *trnL*-f (Taberlet et al. 1991) and *Pfu Turbo* DNA polymerase (Stratagene) to identify plant communities associated with each root sample. Each 20 µl PCR reaction consisted of 14.4 µl of dH2O, 0.2 µl of 2.5 U *Pfu Turbo* DNA polymerase, 2 µl of manufacture’s buffer (Stratagene), 2 µl of 10x dNTPs, and 0.2 µl of each 50 µM primer and 1 µl of DNA. PCR conditions were the same as described by Taberlet et al. (1991).

Cloning and sequencing.—We gel purified and concentrated the AMF PCR products before cloning as previously described (Schechter and Bruns 2008) because using straight PCR products resulted in low cloning efficiency. It was not necessary to purify and concentrate plant PCR products. We then cloned AMF and plant PCR products (40 AMF and 40 plant PCR cloning reactions total) into pPCR-Script Amp SK(+) and transformed into Escherichia coli XL10-Gold Kan Ultracompetent cells (Stratagene). Transformants were screened for correctly sized inserts using plasmid primers T3/T7 under the same PCR conditions as described in Schechter and Bruns (2008). We cleaned these PCR products with ExoSAP-IT using the manufacturer’s instructions (USB), and sent the clean PCR products to the UC Berkeley Sequencing Facility (Berkeley, CA) to be sequenced from the AM1 primer. We edited the sequences using Sequencher 4.2.2 (Gene Codes) and eliminated vector sequences using VecScreen (http://www.ncbi.nlm.nih.gov/VecScreen/). Chimeras were detected using the methods of Schechter and Bruns (2008) and suspect sequences eliminated from the data set. AMF and plant sequences were deposited into GenBank (AMF: HQ342752–HQ342808; Plants: HQ342809–HQ342869).

Data analysis

Operational taxonomic unit (OTU) determination: AMF.—We determined AMF OTUs in this
experiment using the same combination of grouping by sequence similarity and phylogenetic analysis as described by Schechter and Bruns (2008). This included combining AMF sequences from each grid point root sample at 98% similarity and looking for >50% bootstrap or Bayesian posterior probability branch support for terminal clades that included the putative OTU sequences (98% sequence similarity groupings) (see Schechter and Bruns 2008 for details). We used these OTUs to determine the assemblages of AM fungi associated with each grid point root sample (40 total).

Operational taxonomic unit (OTU) determination: Plant.—We noted the aboveground plant taxa at each sampling site but felt that the only precise way to directly associate plant community with AMF assemblage was to use molecular markers to identify the plant taxa directly associated with the root samples. This way we are directly linking the specific AMF assemblages with the specific plant community associated with each root sample.

Our plant OTU determination is a variation on the methods described by Soininen et al. (2009) for the trnL intron. However, unlike Soininen et al. (2009), we did not have a sequence database of known local plants for our marker (intergenic spacer region between trnL and trnF). So, we determined plant OTUs in this experiment by using a combination of grouping by sequence similarity and phylogenetic analysis using sequences from GenBank. Sequences were combined at 97% rather than 98% similarity because a pilot study showed that the 97% similarity grouping was more conservative and resulted in better supported phylogenetic terminal clades. We aligned these sequences along with close BLAST matches and additional GenBank plant sequences of congeners known to be present at the research grid using ClustalX (Thompson et al. 1997) and then manually edited the sequences using MacClade v 4.08 (Maddison and Maddison 2005). Because of the difficulty aligning these plant sequences across all families, we used four separate alignments for phylogenetic analysis: Poaceae (outgroup: Streptochaeta sodiroa), Asterids (outgroup: Brassica nigra), Rosids/Asterids (outgroup: Nicotiana attenuata), and Onagraceae (outgroup: Rotala indica). For each alignment we performed two separate phylogenetic analyses: maximum likelihood (ML) analysis was conducted using Garli (Genetic Algorithm for Rapid Likelihood Inference) v 0.95 (Zwickl 2006), and Bayesian analysis was performed using MrBayes 3.1.1 (Ronquist and Huelsenbeck 2003). Phylogenetic analysis methods are detailed in Schechter and Bruns (2008).

We used the results of the phylogenetic analyses to confirm plant OTUs. We looked for consistency in topology between analyses and >50% bootstrap or Bayesian posterior probability branch support for terminal clades that included the putative OTU sequences (97% sequence similarity groupings). As above, we used these OTUs to determine the communities of plants associated with each grid point root sample (40 total). We assigned plant OTU names based on genus level in most cases, or to the family level in cases where it was not clear from phylogenetic analysis that genus could be assigned. Phylogenetic analyses were only used to help determine plant OTUs and help assign names, not as a means to determine the phylogenetic history or relationships between plant taxa.

Assemblage analyses.—We used the EstimateS 8.0 Mao Tau estimator (Colwell et al. 2004) to produce a rarefaction curve for both AMF and plant assemblages associated with each root sample to determine if clone sampling effort saturated the number of OTUs. We also computed Shannon-Wiener diversity (H’), richness, and evenness for each grid point AMF and plant assemblages using PRIMER 5, and used one-way ANOVA (JMP v. 5) to test for differences between soil types in the univariate indices, soil chemical data (log transformed) and colonization (arcsine transformed). Tukey HSD tests were used for all a posteriori comparison of means.

To determine if there are differences in AMF assemblage composition and structure between serpentine and non-serpentine root samples, we used the PRIMER 5 software (Plymouth Routines in Multivariate Ecological Research) (Clarke and Warwick 2001). We first prepared a relative abundance matrix of AMF OTUs present in each root sample based on the number of clones representing those OTUs present in each root sample based on the number of clones representing those OTUs within each sample. Dissimilarity between AMF assemblages was then measured using the Bray-Curtis measure after performing a square-root transformation on
the relative abundance matrix to down-weight the importance of highly abundant OTUs, and compared this with the presence/absence transformation to fully down-weight the influence of OTU abundance (Clarke and Warwick 2001). We used non-metric Multidimensional Scaling (MDS) ordinations to represent the dissimilarities in assemblage composition among grid point samples and the ANOSIM (analysis of similarities) routine to perform statistical analysis of assemblage data (Clarke and Warwick 2001). In addition, we used the SIMPER (Similarity Percentages) routine to determine the relative contribution of individual AMF OTUs toward dissimilarity between grid points.

We also used the PRIMER 5 software to look for differences in plant community and soil characteristics between serpentine and non-serpentine root samples. Differences in plant communities between root samples were determined as described above for AMF assemblage differences. Briefly, root sample plant community differences were measured by Bray-Curtis dissimilarity after square-root and presence/absence transformations, MDS ordination to represent these dissimilarities, ANOSIM statistical analysis, and SIMPER to determine the contribution of individual plant OTUs toward dissimilarity. To compare soil characteristics between serpentine and non-serpentine grid points, we included soil texture data collected in 2001 along with the soil nutrient analysis from the same grid points. A similarity matrix of soil data (log transformed) associated with serpentine and non-serpentine grid points was produced using Euclidean distance (Clarke and Warwick 2001). We then used non-metric MDS and ANOSIM to explore differences in soil characteristics between grid points. Finally, we used the BIO-ENV routine to explore which subset of abiotic variables best matched the observed biotic patterns.

We tested for correlations between AMF assemblage similarity and distance between sites (as a proxy for dispersal limitation), soil variable and plant community similarity using several different tests. We performed a Mantel test (Monte Carlo 1000 runs; PC-ORD v. 5; McCune and Mefford 2006) to test for a correlation between AMF assemblage similarity and distances between grid points. We used the RELATE procedure and Mantel tests to test for a correlation between AMF assemblage similarity matrix and the soil data similarity matrix, between the AMF assemblage similarity matrix and the plant assemblage similarity matrix, and between the plant assemblage similarity matrix and the soil data similarity matrix. Finally, we used multivariate variance partitioning as described by Borcard et al. (1992) and implemented in CANOCO for Windows 4.5 (Ter Braak 1988) to determine how much of the AMF assemblage variation could be accounted for by soil variables, plant assemblage, and a combination of the two.

**RESULTS**

**Are serpentine and non-serpentine AMF assemblages distinct?**

AMF assemblage identification.—All 40 grid point root samples were highly colonized (48–57% root length) by AMF with no significant differences in colonization between samples ($F_{1,9} = 1.02, P < 0.41$). We sequenced 1,071 clones to determine grid point AMF assemblages (91% AMF sequences, 2% ascomycota origin, 0.3% bacterial origin, and 0.2% chimeric sequences). Each grid point sampled was represented by similar numbers of AMF sequences ($NS1 = 137$, $NS2 = 92$, $NS3 = 94$, $NS4 = 87$, $NS5 = 88$, $S1 = 140$, $S2 = 81$, $S3 = 89$, $S4 = 90$, $S5 = 80$).

Phylogenetic analysis: AMF.—We detected only two AMF genera in this study (Table 1). *Glomus* species were the overwhelming dominants, representing 99.8% of the sequences. The only other AMF genus detected was *Archeospora* (0.2% of the sequences). We established 12 AMF OTU (Table 1), 7 of which matched OTUs found in Schechter and Bruns (2008) and the common garden study (Schechter and Bruns, unpublished manuscript). In this present study, two OTUs were most dominant in the root samples, *Glomus* 1 (34% of sequences) and *Glomus* 5 (29%).

AMF assemblage analysis

Comparing AMF assemblages.—The rarefaction analysis shows that sequence sampling effort saturated the AMF diversity in grid points NS3, S1, S2, and S4, but curves associated with the other grid points were still increasing (Appendix: Fig. A3). However, the Chao1 richness estimator suggested that further sequence sampling in
these grid points would have only added one to two AMF OTUs (representing a 8–16% increase in OTUs). This indicates that the sequence sampling effort captured a large portion (approximately 84–92%) of the AMF diversity associated with grid point root samples.

The MDS ordination shows that the AMF assemblages found in the serpentine soil type root samples were much more similar to each other than the non-serpentine soil type AMF assemblages and vice versa (Fig. 2). This distinction was supported by ANOSIM analysis ($R = 0.513, P < 0.001$). The distinction between soil type AMF assemblages was still highly significant after presence/absence transformation of the relative abundance matrix data ($R = 0.339, P < 0.001$) indicating that the presence and/or absence of specific AMF OTUs in the root samples contributed to the distinction between serpentine and non-serpentine assemblages. These results clearly show that AMF assemblages associated with serpentine and non-serpentine soil types are distinct from each other.

**OTU contribution to AMF assemblage differences.**—SIMPER analysis shows that two AMF OTUs contributed the most to the distinction between soil type AMF assemblages: *Glomus* 1 (36%) and *Glomus* 5 (23%). *Glomus* 1 showed the most conspicuous pattern, being completely absent in the root samples from the serpentine soil type but highly abundant in root samples from the non-serpentine soil type with the exception of the NS3 grid point (Table 1). In

![Fig. 2. Non-metric multi-dimensional scaling ordination of AMF assemblages associated with plant roots found in serpentine and non-serpentine grid points. The non-metric multi-dimensional scaling ordination is a configuration of the samples in which relative positions are assigned based on the Bray-Curtis similarity matrix of the data so that samples closer together have a higher similarity of component taxa than samples farther apart and overlapping samples are highly similar.](image-url)
contrast, *Glomus* 5 was present in root samples from every grid point but was much more abundant in the serpentine soil type root samples (Table 1). Interestingly, *Glomus* 6, denoted as a serpentine-only OTU (Schechter and Bruns 2008), was also found in the non-serpentine soil type root samples but abundance was higher in serpentine grid point samples. Species diversity ($F_{1,9} = 0.04, P < 0.84$), evenness ($F_{1,9} = 1.10, P < 0.30$), and richness ($F_{1,9} = 0.13, P < 0.73$) were not significantly different between serpentine and non-serpentine soil types.

**Did soil factors and plant communities influence AMF assemblage differences?**

**Comparing root sample plant communities.**—We sequenced 664 clones to determine plant assemblages associated with grid point root samples (93% plant sequences and 0.4% chimeric sequences). Each grid point sampled was represented by similar numbers of plant sequences (NS1 = 60, NS2 = 61, NS3 = 80, NS4 = 57, NS5 = 64, S1 = 61, S2 = 59, S3 = 61, S4 = 59, S5 = 56).

**Phylogenetic analysis: Plants.**—We detected 13 plant families within the grid point root samples. The most abundant family was Poaceae (66% of sequences), followed by Asteraceae (17%), Scrophulariaceae (4%), Onagraceae (3%), Geraniaceae (2%), Juglandaceae (2%), Solanaceae (2%), Polemoniaceae (1%), Rosaceae (1%), Apiaceae (0.8%), Caryophyllaceae (0.5%), Linaceae (0.4%), and Lamiaeae (0.3%). We established 31 plant OTUs, the four most dominant were: *Avena* 1 (33% of sequences), *Aster* 2 (11%), *Bromus* 1 (8%), and *Vulpia* 1 (6%).

All plant families identified by root sequence are known to be at the McLaughlin Reserve (http://herbarium.ucdavis.edu/flora/mclaughlin.htm) and sequences matched aboveground plant species. For example, two rare plant OTUs found only on serpentine were good matches to serpentine plants: *Navarretia* 1 is a 99% match to *Navarretia jepsonii*, a native and rare serpentine endemic, and *Hesperolinon* 1 is a 99% match to *Hesperolinon californicum*, a native strong serpentine indicator species (http://www.calflora.org).

**Comparing plant communities.**—The rarefaction analysis shows that sequence sampling effort saturated plant taxa diversity associated with root samples from grid points S1, S4, and S4, but curves associated with the rest of the grid points were still increasing, especially for the NS2 and NS4 grid points (Appendix: Fig. A2). However, in all but NS2 and NS4, the Chao1 richness estimator suggested that further sequence sampling would have only added one to two plant OTUs. This indicates that, in all but NS2 and NS4, the plant sequence sampling effort captured a large portion of the plant taxa diversity associated with grid point root samples. The MDS ordination of all the grid point root samples showed a slight separation between plant communities sampled from serpentine and non-serpentine soil types (Fig. 3). The ANOSIM analysis was significant ($R = 0.145, P < 0.001$), even after the presence/absence transformation ($R = 0.138, P < 0.003$). These data show that the root sample plant communities were different between soil types.

**OTU contribution to plant community differences.**—Three plant OTUs contributed the most to the soil type distinction between root sample plant communities (SIMPER analysis): *Avena* 1 (19%), *Aster* 2 (12%), and *Bromus* 1 (7%). *Avena* 1 was found predominantly in non-serpentine samples, *Aster* 2 was absent from non-serpentine samples, and *Bromus* 1 also showed preference for the non-serpentine soil type (Table 2). Species diversity ($F_{1,9} = 1.52, P < 0.23$), evenness ($F_{1,9} = 1.27, P < 0.27$), and richness ($F_{1,9} = 4.11, P < 0.06$) were not significantly different between root sample plant assemblages from serpentine and non-serpentine soil types.

**Comparing soil type soil factors.**—ANOSIM analysis of the soil variables showed that serpentine and non-serpentine soil types are clearly distinct from each other ($R = 0.972, P < 0.008$). When comparing individual variables across soil type, only Ca concentration ($F_{1,9} = 30.95, P < 0.0008$) Mg concentration ($F_{1,9} = 56.49, P < 0.0001$), and Ca:Mg ratio ($F_{1,9} = 15.91, P < 0.005$) levels were significantly different between soil types, although average P concentration was nearly two times higher in non-serpentine than serpentine soil (Appendix: Table A1). Using the BIO-ENV routine to explore which subset of abiotic variables best “matched” the observed biotic patterns, soil concentrations of K, Ca, Mg, Ca:Mg, B had the highest Spearman’s rank correlation score.

**Contribution of distance, soil factors and plant communities to AMF assemblage differences.**—Spa-
tial distance was not correlated to AMF assemblage similarity \((r = -0.039, P < 0.235)\) showing that distance (as a proxy for dispersal limitation) was not a significant factor shaping AMF assemblage differences. The correlation between soil factors and AMF assemblages was significant (RELATE: Rho = 0.370, \(P < 0.02\); Mantel \(r = 0.457, P < 0.02\); Rho and Mantel \(r\) range from a complete agreement of +1 to complete opposition −1 between the two data sets). However, the RELATE and Mantel tests between soil factors and plant communities was not significant (Rho = 0.021, \(P < 0.39\); \(r = 0.019, P < 0.332\)).

The RELATE and Mantel tests were also used to test for a relationship between root sample plant communities and AMF assemblages. The RELATE test was significant (Rho = 0.186, \(P < 0.007\), but marginally insignificant in the Mantel test \((r = 0.278, P < 0.055\)). In addition, the low Rho and \(r\)-value indicate a weak relationship between plant and AMF assemblages. Finally, variance partitioning analysis showed that 33.5% of the variance between AMF assemblages could be uniquely described by soil nutrients alone, 25.6% could be described by plant assemblage alone, and 28.9% shared between soil nutrients and plant communities.

**DISCUSSION**

**Distinction between soil type AMF assemblages**

We found that AMF assemblages associated with serpentine and non-serpentine soil types are distinct from each other. Soil type had a large effect on presence and abundance of certain AMF OTUs. *Glomus* 1 was the overwhelming dominant AMF OTU in non-serpentine soil just as it was in our previous study (Schechter and Bruns 2008) (Table 1). The fact that *Glomus* 1 is still the non-serpentine dominant at different sites, on different plant types, and two years after the *C. sparsiflora* sampling, implies that *Glomus* 1 is the best AMF associate on non-serpentine soils at McLaughlin Reserve. In contrast, the complete absence of *Glomus* 1 on serpentine soils in this study and its low abundance in serpentine *C. sparsiflora* roots (only 3% of *Glomus* 1 sequences were found in serpentine; Schechter and Bruns 2008), indicate that serpentine has a strong negative effect on *Glomus* 1. This is also consistent with the results of a greenhouse study in which *Glomus* 1 was dominant in every non-serpentine soil type, but found at very low presence and abundance in serpentine soil. (Schechter and Bruns, *unpublished manuscript*).
The negative effect of serpentine soil on *Glomus* 1 becomes more intriguing if one considers the presence of a new OTU in this study: *Glomus* 1A. This very closely related OTU was only found in serpentine soil at high abundance at two serpentine grid points (Appendix: Fig. A1). If serpentine soil does indeed have a strong negative effect on *Glomus* 1, the appearance of *Glomus* 1A may represent a serpentine-evolved relative of *Glomus* 1.

The effect of soil variables on AMF assemblage composition found in this study was not restricted to serpentine versus non-serpentine soil types. The AMF assemblage associated with the non-serpentine grid point NS3 was an outlier even to the rest of the non-serpentine AMF assemblages (Table 1). While NS3 was chosen as a “non-serpentine” soil type based on Ca:Mg, it varies considerably from the other non-serpentine as well as serpentine soils in pH (Appendix: Table A1). Soil pH is known to alter AMF composition, and therefore may be the soil factor driving this unique AMF assemblage (Dumbrell et al. 2010).

It is interesting to note that while the AMF assemblages differed at this grid point, the plant assemblages were similar to those sampled in other non-serpentine grid points (Table 2).

### Relative influence of plant communities and soil on AMF assemblages

We found that plant root assemblages differed between soil types. We had expected the plant root assemblages to be quite distinct between soil types, but the low R-value of the ANOSIM analysis indicates very little distinction between assemblages. This may be caused by the high number of rare OTUs found in the root samples (26 of 31 plant OTUs were only found once; Table 2), the fact that our plant OTUs likely encompass more than one species, or the presence of invasive species that are common in both serpentine and non-serpentine grid points at McLaughlin (Davies et al. 2005). For example, sequences of *Avena* 1 found in both soil types matched *Avena barbata* GenBank sequences at 99% similarity, and this species is an invasive species known to occupy both serpentine and non-serpentine soils (Harrison et al. 2001).

Basing our plant sample on roots rather than on above ground presence, provided a useful perspective to the study. We also identified aboveground plant assemblages found in the immediate sample area, and this resulted in 12 potential plant hosts for this study (data not shown). In contrast, by sequencing the cpDNA of the actual roots contained in the sample we conservatively identified 31 plant taxa from our sample. This shows that identification of above-ground plants at or around the sampling site may not be reflective of the identity of the root. However, in our case, in all but one sample the identity of dominant aboveground plants matched dominant sequences of plant roots.

Using only the aboveground plant taxa in the RELATE and Mantel analysis improves the Rho and r values of the correlations between plant and AMF assemblages but these statistics still show a weaker relationship than soil variables and AMF assemblages (Appendix: Table A2).

---

Table 2. Relative abundance matrix of plant taxa associated with roots collected from five serpentine (S1–S5) and five non-serpentine (NS1–NS5) grid points.

<table>
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<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
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<th>NS2</th>
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Notes: Boldface operational taxonomic units (OTUs) show soil type affects based on SIMPER analysis. Relative abundance equals the proportion of sequenced clones assigned to an OTU within the sample.
Soil-plant interactions shaped AMF assemblages in this study. However, RELATE, mantel tests and variance partitioning analysis suggest that soil factors better explained differences in AMF composition and structure than plant assemblage. This is best illustrated by comparing AMF and plant assemblages associated with serpentine grid point root samples S3 and S4. These root samples from these grid points were both dominated by the *Avena* 1 plant OTU, but they were not associated with *Glomus* 1 as was found in the other root samples dominated by *Avena* 1 (Table 1 and 2). This is of particular interest since several studies have shown that invasive plants (like *Avena barbata* and *A. fatua*) typically have an effect on microbial community composition including AMF (Batten et al. 2006, Hawkes et al. 2006). But, even though soil had a stronger effect, there is clearly a combined effect of both soil factors and plant assemblage on AMF assembly as these two variables (alone and together) accounted for 88% of the AMF assemblage variance.

Our finding is similar to that of Dumbrell et al. (2010) and Lekberg et al. (2011), who both showed a stronger effect of soil pH than plant community composition on AMF assemblage composition. However, each study tested extremes in soil chemistry. In our case, the average Ca:Mg ratios between test soils differed by 17 fold, the Dumbrell et al. (2010) study pH ranged from 3.72 to 8.04, and Lekberg et al. (2011) pH range was 4.0–7.9. Thus, the safest conclusion is that extreme soil differences drive differences in AMF assemblages. However only our study showed the conspicuous ecological sorting pattern of dominance and absence within a small-scale heterogeneous environment. Lekberg et al. (2011) found several AMF types were restricted to low pH sites but they suggested that these patterns were due to dispersal limitation.

The host effect on AMF assemblages has certainly been seen in other studies (Bever et al. 1996, Vandenkromhuyse et al. 2003, Hausmann and Hawkes 2009) and should not be underrated from the current results. We do see a host effect, and although it is less pronounced than the soil effect, it must be viewed with the knowledge that the soil differences at our site are extreme. With less severe heterogeneity in soil chemistry, it would not be surprising if the host effects dominate.

**Ecological sorting**

The AMF assembly patterns found in this study suggest that the main mechanism generating the distinction between soil type AMF assemblages is ecological sorting (Weiher and Keddy 1995) of serpentine intolerant and tolerant/adapted AMF taxa. The sorting effect of serpentine soil on AMF taxa at this site is supported by the complete absence of the non-serpentine dominant AMF taxon on serpentine within a small-scale mosaic (50–150 m between sites) of soil types as well as similar patterns found in previous field (Schechter and Bruns 2008) and greenhouse experiments (Schechter and Bruns, unpublished manuscript) from the same site. In fact, the primary edaphic factors (low calcium and high levels of magnesium) that are involved in plant adaptation and ecological sorting of serpentine tolerant and intolerant plant species in heterogeneous environments (Kruckenberg 1984, Brady et al. 2005) are the same ones correlated with AMF assemblages in this study.

Calcium is known to be important in maintaining a functioning mycorrhiza (Hepper and Oshea 1984, Habte and Soedarjo 1995). In fact, A hydroponic study by Jarstfer et al. (1998) showed that independent of phosphorus nutrition, low Ca:Mg (low Ca and high Mg) dramatically reduced AMF colonization and sporulation in onion. Hepper and Oshea (1984) suggested that low calcium could limit the ability the AMF inoculum to develop in the rhizosphere and colonize roots or stimulate the host to limit colonization. Thus the absence of non-serpentine dominant *Glomus* 1 in serpentine may be due direct or indirect effects of the low Ca:Mg limiting its successful colonization and therefore build-up and maintenance of inoculum in serpentine soil. Highlighting that along with soil pH and soil texture (Johnson et al. 1992, Lekberg et al. 2007, Dumbrell et al. 2010, Lekberg et al. 2011), extremes in soil Ca:Mg can also affect AMF assembly.

The presence of ecological sorting based on edaphic factors is reinforced by the lack of evidence for dispersal limitation in this study. This is an unusual result as four prior studies of the processes that effect mycorrhizal fungal assembly have all shown some level of spatial...
autocorrelation (related to dispersal limitation) (Lekberg et al. 2007, Dumbrell et al. 2010, Peay et al. 2010, Lekberg et al. 2011) even within a 20 m by 7 m plot (Dumbrell et al. 2010). Thus, if dispersal was not limited in our study then the AMF communities were assembled from a common pool. Yet, Glomus 1 (the non-serpentine dominant AMF taxon) was excluded from serpentine soils. This fulfills Weiher and Keddy’s (1995) criteria for a “filtered” species and supports ecological or more specifically edaphic sorting as the primary factor driving AMF community assembly in this study.

**Conclusion**

The primary goal of this study was to examine if there are edaphically distinct AMF assemblages that associate with the broader plant communities present on serpentine and non-serpentine soils. This study clearly shows that serpentine and non-serpentine AMF assemblages are distinct from each other even on a fairly fine spatial scale. Both soil nutrients and plant communities shaped this distinction between serpentine and non-serpentine AMF assemblages, but soil factors had a more pronounced effect in this system. This study indicates that edaphic sorting of serpentine intolerant and tolerant/adapted AMF taxa played a large role in structuring fungal assemblages.

**ACKNOWLEDGMENTS**

We thank John Taylor, Bruce Baldwin and anonymous reviewers for helpful comments. We thank Nhu Nguyen, Cathy Koehler and Paul Aigner for their generous help during sampling and those involved with creating the Donald and Silvia McLaughlin Natural Reserve Research Grid, without which this work could not be done. This research has been supported by a grant from the U.S. Environmental Protection Agency’s Science to Achieve Results (STAR) program, by a grant from the University of California Natural Reserve System, a grant from the Mycological Society of San Francisco, and NSF Grant #2036096 and DBI1046115 (T. D. Bruns).

**LITERATURE CITED**


and Soil 175:233–238.
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**SUPPLEMENTAL MATERIAL**

**APPENDIX**

Table A1. Soil chemical variables (S = serpentine, NS = non-serpentine) associated with McLaughlin Reserve grid points. Nitrogen (as NO₃) phosphorus (P, Weak Bray), potassium (K), magnesium (Mg), calcium (Ca), zinc (Zn), iron (Fe), copper (Cu), and boron (B) are reported in parts per million (ppm). Cation exchange capacity (CEC) is reported as milliequivalents per 100 grams of soil. Highlighted numbers indicate Ca:Mg ratio; serpentine soils have a ratio much less than one and non-serpentine soils have ratios greater than one. An asterisk indicates significant differences between soil types at *P* < 0.05.

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Table A2. Comparison of ANOSIM, RELATE, and Mantel test results between plant root assemblages (determined by cloning and sequencing cpDNA) and aboveground plant assemblages (determined by visual identification).

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</tr>
<tr>
<td>Mantel Test§</td>
<td><em>r</em> = 0.278, <em>P</em> &lt; 0.055</td>
<td><em>r</em> = 0.325, <em>P</em> &lt; 0.027</td>
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† Comparison between soil types.
‡ Relationship between AMF and plant assemblages.
§ Relationship between AMF and plant assemblages.
Fig. A1. Consensus tree (50% majority rule) from Mr. Bayes analysis showing the phylogenetic relationship of the AMF sequences obtained from roots sampled from McLaughlin research grid from five serpentine (MLG_S1, MLG_S2, MLG_S3, MLG_S4, MLG_S5) and five non-serpentine (MLG_NS1, MLG_NS2, MLG_NS3, MLG_NS4, MLG_NS5) grid points, in bold. Additional sequences from roots sampled from three serpentine (S1, S2, S3) and three nonserpentine (NS1, NS2, NS3) ecotype populations of *Collinsia sparsiflora* field experiment were included (Schechter and Bruns 2008). Letters directly behind site designation refers to an individual sample; no letter means that the sequence is a representative from a 98% consensus of sequences found in multiple samples within that site. Grey blocks encompass groups of sequences that are 98% similar and designate experiment OTUs (in white by Genus affiliation). Other sequences are GenBank accessions of closely related BLAST matches as well as Glomeromycota voucher sequences (Schussler 2001). Letters behind GenBank accessions refer to origin of the sequence (S = spore, E = environmental). The values above the branches are Bayesian posterior probabilities (bold) followed by bootstrap values (100 replicates in Garli maximum likelihood analysis), only support greater than 50 is shown. *Olpidium brassica* was used as an out-group. Topology was similar between Bayesian and Garli analyses and did not affect OTU delimitation.
Fig. A2. Rarefaction curve of the total number of AMF sequences sampled from plant roots from serpentine (S) and non-serpentine (NS) grid points. Rarefaction curves were produced by the EstimateS version 8.0 Mao Tau estimator (Colwell et al. 2004).

Fig. A3. Rarefaction curve of the total number of plant sequences from plant roots sampled from serpentine (S) and non-serpentine (NS) grid points. Rarefaction curves were produced by the EstimateS version 8.0 Mao Tau estimator (Colwell et al. 2004).
Fig. A4. Consensus tree (50% majority rule) from Mr. Bayes analysis showing the phylogenetic relationship of the Poaceae plant sequences obtained from roots sampled from McLaughlin research grid from five serpentine (GP_S1, GP_S2, GP_S3, GP_S4, GP_S5) and five non-serpentine (GP_NS1, GP_NS2, GP_NS3, GP_NS4, GP_NS5) grid points, in bold. Letters directly behind site designation refers to an individual sample; no letter means that the sequence is a representative from a 98% consensus of sequences found in multiple samples within that site. Grey blocks encompass groups of sequences that are 98% similar and designate experiment OTUs (in white by Genus affiliation). Other sequences are GenBank accessions of closely related BLAST matches. The values above the branches are Bayesian posterior probabilities (bold) followed by bootstrap values (100 replicates in Garli maximum likelihood analysis), only support greater than 50 is shown. *Streptochaeta sodiroa* was used as an outgroup. Topology was similar between Bayesian and Garli analyses.
Fig. A5. Consensus tree (50% majority rule) from Mr. Bayes analysis showing the phylogenetic relationship of the Asterids plant sequences obtained from roots sampled from McLaughlin research grid from five serpentine (GP_S1, GP_S2, GP_S3, GP_S4, GP_S5) and five non-serpentine (GP_NS1, GP_NS2, GP_NS3, GP_NS4, GP_NS5) grid points, in bold. Letters directly behind site designation refers to an individual sample; no letter means that the sequence is a representative from a 98% consensus of sequences found in multiple samples within that site. Grey blocks encompass groups of sequences that are 98% similar and designate experiment OTUs (in white by Genus affiliation). Other sequences are GenBank accessions of closely related BLAST matches. The values above the branches are Bayesian posterior probabilities (bold) followed by bootstrap values (100 replicates in Garli maximum likelihood analysis), only support greater than 50 is shown. *Brassica nigra* was used as an out-group. Topology was similar between Bayesian and Garli analyses.
Fig. A6. Consensus tree (50% majority rule) from Mr. Bayes analysis showing the phylogenetic relationship of the Rosids/Asterids plant sequences obtained from roots sampled from McLaughlin research grid from five serpentine (GP_S1, GP_S2, GP_S3, GP_S4, GP_S5) and five non-serpentine (GP_NS1, GP_NS2, GP_NS3, GP_NS4, GP_NS5) grid points, in bold. Letters directly behind site designation refers to an individual sample; no letter means that the sequence is a representative from a 98% consensus of sequences found in multiple samples within that site. Grey blocks encompass groups of sequences that are 98% similar and designate experiment OTUs (in white by Genus affiliation). Other sequences are GenBank accessions of closely related BLAST matches. The values above the branches are Bayesian posterior probabilities (bold) followed by bootstrap values (100 replicates in Garli maximum likelihood analysis), only support greater than 50 is shown. *Nicotiana attenuata* was used as an out-group. Topology was similar between Bayesian and Garli analyses.
Fig. A7. Consensus tree (50% majority rule) from Mr. Bayes analysis showing the phylogenetic relationship of the Onagraceae plant sequences obtained from roots sampled from McLaughlin research grid from five serpentine (GP_S1, GP_S2, GP_S3, GP_S4, GP_S5) and five non-serpentine (GP_NS1, GP_NS2, GP_NS3, GP_NS4, GP_NS5) grid points, in bold. Letters directly behind site designation refers to an individual sample; no letter means that the sequence is a representative from a 98% consensus of sequences found in multiple samples within that site. Grey blocks encompass groups of sequences that are 98% similar and designate experiment OTUs (in white by Genus affiliation). Other sequences are GenBank accessions of closely related BLAST matches. The values above the branches are Bayesian posterior probabilities (bold) followed by bootstrap values (100 replicates in Garli maximum likelihood analysis), only support greater than 50 is shown. *Rotala indica* was used as an out-group. Topology was similar between Bayesian and Garli analyses.