Subsoil arbuscular mycorrhizal fungal communities in arable soil differ from those in topsoil

Moisés A. Sosa-Hernández[1,2], Julien Roy[1,2], Stefan Hempel[1,2], Timo Kautz[3], Ulrich Köpke[3], Marie Uksa[4,5], Michael Schloter[5,6], Tancredi Caruso[7], Matthias C. Rillig[1,2*].

[1] *Institute of Biology, Plant Ecology, Freie Universität Berlin, Berlin, Germany*

[2] *Berlin-Brandenburg Institute of Advanced Biodiversity Research, Berlin, Germany*

[3] *Institute of Organic Agriculture, University of Bonn, Bonn, Germany*

[4] *Institute of Soil Science and Land Evaluation, Soil Biology, University of Hohenheim, Stuttgart, Germany*

[5] *Research Unit for Comparative Microbiome Analysis, Helmholtz Zentrum München, Neuherberg, Germany*

[6] *Chair for Soil Science, Technische Universität München, Freising, Germany*

[7] *School of Biological Sciences and Institute for Global Food Security, Queen's University of Belfast, Belfast, Northern Ireland (UK)*

* Correspondence to Moisés A. Sosa Hernádez, Freie Universität Berlin, Plant Ecology, Altensteinstr. 6, D-14195 Berlin, Germany; Email: moisessh@zedat.fu-berlin.de Tel. +49 (0)30 838-53143

Abstract

Arbuscular mycorrhizal fungi are recognized as important drivers of plant health and productivity in agriculture but very often existing knowledge is limited to the topsoil. With growing interest in the role of subsoil in sustainable agriculture, we used high-throughput Illumina sequencing on a set of samples encompassing drilosphere, rhizosphere and bulk soil, in both top- and subsoil. Our results show subsoil AMF communities harbor unique Operational Taxonomic Units (OTUs) and that both soil depths differ in community structure both at the OTU and family level. Our results emphasize the distinctness of subsoil AMF communities and the potential role of subsoil as a biodiversity reservoir.

Keywords: subsoil; drilosphere; arbuscular mycorrhiza; agriculture; Illumina MiSeq; soil depth

Arbuscular mycorrhizal fungi (AMF) belong to the monophyletic subphylum Glomeromycotina (Spatafora et al., 2016) and form a symbiotic relationship with the roots of most land plants including the majority of agricultural crops (Smith and Read, 2008). This symbiosis is of great relevance for sustainable agriculture due to its ability to increase productivity (Lekberg and Koide, 2005), nutrient uptake (Smith and Smith, 2011), soil aggregation (Leifheit et al., 2014), and plant protection (Veresoglou and Rillig, 2012). In arable fields, the subsoil (i.e. the soil beneath the plough layer) contains more than two thirds of the soil nutrient pool (Kautz et al., 2013). Thus, understanding how AMF communities vary with depth and what factors drive their community assembly is a prerequisite for developing appropriate subsoil management strategies.

Previous studies have shown that AMF spore abundance and diversity in agricultural fields decrease with soil depth (Muleta et al., 2008; Oehl et al., 2005; Yang et al., 2010), however under some circumstances subsoil spore diversity may be greater than in the topsoil (Oehl et al., 2005). Moreover, some species' spores seem to be associated with particular soil layers, suggesting a vertical variation in community composition (Muleta et al., 2008; Oehl et al., 2005; Yang et al., 2010). These spore-based results have recently been supported by small ribosomal subunit (SSU) cloning-sequencing approaches, finding differences in community composition between AMF communities at different depths (Moll et al., 2016). Nonetheless, we are just starting to unearth subsoil AMF diversity, and the community assembly processes remain unknown.

To shed light on subsoil AMF communities, we used Illumina MiSeq sequencing to analyze a set of soil samples encompassing drilosphere (soil directly influenced by earthworms), rhizosphere (soil directly influenced by roots) and bulk soil (without roots or earthworm burrows), in both top- (10-30 cm, i. e. above the ploughing layer) and subsoil (60-75 cm). Sampling the different compartments was intended to add greater resolution to our results. The first 10 cm were not sampled due to difficulties in differentiating the mentioned compartments at this depth. Samples were collected in May 2011 in a field planted with *Cichorium intybus* L. (see Uksa et al. (2014) for details).

We performed amplicon-based AMF specific metabarcoding using the AMF specific primer sets described in Krüger et al. (2009) and the primers LR3 and LR2rev (Hofstetter et al., 2002). The final product of amplification is a 350-440 bp region in the LSU including the variable D1-D2 region. Samples were paired-end sequenced on an Illumina MiSeq platform. Bioinformatics details are given in the supplementary material. Briefly, following quality filtering and chimera removal, sequences were *de novo*

clustered at a 97% similarity level into operational taxonomic units (OTUs). Representative sequences of these OTUs have been deposited at ENA under accession numbers LT855246-LT855309.

Taxonomic assignment of the OTUs was carried out using BLAST+ (Camacho et al., 2009) against Glomeromycotina (i.e. AMF) reference sequences published in Krüger et al. (2009) and against the EMBL nucleotide database (Kanz et al., 2005). Following a similar approach as in Martínez-García et al. (2015) for SSU sequences, we considered matches with ≥ 97% similarity a species level match, ≥90% a genus level match, ≥80% a family level match and ≥70% a subphylum level match. A species level match refers to how confidently we assign a name to our OTU based on known sequences, and does not imply that these OTUs are to be considered equivalent to those species. In total, 64 OTUs were confidently assigned to the subphylum Glomeromycotina. Of these, we were able to assign 11 OTUs at the species level, 34 at the genus level, 16 at the family level and 3 at the subphylum level.

The resulting OTU table was analyzed with R version 3.3.1 (R Core Team, 2016). Relative abundance data were obtained by rarefaction of all the samples to the lowest number of reads in a sample (34377 reads), by random subsampling without replacement. Details on the analyses are given in the supplementary material.

Our results present high-throughput molecular evidence that the subsoil AMF community is not simply a subset of the topsoil community, but harbors unique OTUs and that the two soil depths differ in structure both at the family level (**Fig. 1)** and at the OTU level (**Fig. 2**)**.** We detected 64 Glomeromycotina OTUs belonging to 7 families (Ambisporaceae, Archaeosporaceae, Claroideoglomeraceae, Diversisporaceae, Gigasporaceae, Glomeraceae and Paraglomeraceae, **Table S1**). OTU accumulation curves for both top- and subsoil reached a plateau, indicating that we captured the majority of the diversity (**Fig. S2**). We observed a highly significant community shift

97 when comparing the top- and the subsoil (PERMANOVA, $F_{1,16}$= 8.67, P<0.001, **Fig. 3**).
98 Most remarkably, Claroideoglomeraceae and Diversisporaceae exhibit inversely
99 proportional relative abundances across the studied soil profiles (**Fig. 1**). In topsoil
100 OTUs assigned to Diversisporaceae represented 41.8% of the reads whereas in
101 subsoil they represented 7.3% (GLM, $F_{1,15}$=50.83 P<0.001). Conversely, OTUs
102 assigned to Claroideoglomeraceae represented 15.0% of the reads in topsoil but
103 59.9% in subsoil (GLM, $F_{1,15}$=17.87 P<0.001). The greater relative abundance of the
104 family Claroideoglomeraceae in subsoil is not correlated with its nominal diversity but
105 with a modest increase in relative richness (**Fig. S3a,b**). This finding may point to some
106 species in the Claroideoglomeraceae family being subsoil specialists and particularly
107 dominant in this compartment. This hypothesis is supported by previous results where
108 *Claroideoglomus etunicatum* spores were more commonly found in deeper soil layers
109 (Oehl et al., 2005; Yang et al., 2010). Conversely, both nominal and relative diversity in
110 the Diversisporaceae decreased in subsoil showing no evidence for specialization in
111 subsoil within this family. The family Glomeraceae however, is detected at a mostly
112 constant relative abundance across topsoil (34.1%) and subsoil (28.4%), but decreases
113 in abundance from rhizosphere (49.0%) to bulk soil and drilosphere considered
114 together (23.6%; GLM, $F_{1,15}$=6.79 P=0.02) (**Fig. 1**). Members of the Glomeraceae are
115 known to preferentially allocate biomass inside the root while producing limited
116 biomass in the soil (Powell et al., 2009). Our observations broadly support the idea
117 that, due to producing a limited extraradical mycelium, Glomeraceae species are
118 expected to preferentially colonize the direct surroundings of the root and to rapidly
119 decrease in abundance outside the rhizosphere. We hypothesize that species with a
120 preferentially intraradical lifestyle are less responsive to abiotic factors outside the root
121 and therefore can readily colonize different soil horizons. In turn, those intraradical
122 lifestyles would be mostly affected by host characteristics.

In our subsoil samples OTU richness (27.4 ± 5.9) was significantly lower than in topsoil (41.6 ± 6.0; GLM, $F_{1,15}$=23.83 P<0.001). Nonetheless we detected a total of 49 OTUs in subsoil, with two OTUs (OTU_40 subphylum Glomeromycotina and OTU_68 genus *Glomus*) exclusively found in the subsoil, also before normalization (data not shown). Agricultural soils are subjected to a set of disturbances including fertilization or plant removal during harvest; topsoils are additionally subjected to high disturbance in form of tillage, negatively influencing AMF diversity (Kabir, 2005). Applying the C-S-R (competitor, stress tolerator, ruderal) framework to AMF (Chagnon *et al.*, 2013), we would expect topsoil to be dominated by more ruderal species (i.e. elevated growth rates, rapid and abundant spore production, etc.) and subsoil by stress tolerators (i.e. low growth rates, long lived mycelium, etc.). We believe this may be one of the major factors explaining the observed differences in the communities across depth.

In our study no compartment effect was detected for the communities (PERMANOVA, $F_{2,16}$= 0.68, P=0.66), besides the mentioned change in relative abundance of the family Glomeraceae (see above). However, previous studies conducted with the same samples show that bacterial communities exhibit a clear compartmentation in the subsoil (Uksa et al., 2015). This difference might be related to the linear, hyphal growth habit of fungi and their unique ability to integrate over larger soil volumes.

We were able to show that subsoil communities are clearly different and not only a subset of topsoil communities, and found contrasting patterns of abundance for different families. Whether this shift in community composition also means a shift in function remains unknown; but the clear difference in dominant families with depth suggests turnover also in functional traits (Powell et al. 2009). Our results emphasize the need to account for subsoil when designing agricultural management strategies and highlight the potential role of deeper soil layers as a biodiversity reservoir.

Acknowledgements

MR acknowledges funding through the Federal Ministry of Education and Research (BMBF) initiative 'BonaRes - Soil as a sustainable resource for the bioeconomy' for the project Soil^3. TC was supported by the project SENSE (Structure and Ecological Niche in the Soil Environment; EC FP7 - 631399 - SENSE). Illumina sequencing was carried out at the Berlin Center for Genomics in Biodiversity Research (BeGenDiv, Berlin, Germany). All authors thank Doreen Fischer and Marta Fogt for their involvement in the sampling and sample preparation processes, respectively. MS-H thanks Margarita Moreno Bayón for constant support.

References

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., Madden, T.L., 2009. BLAST+: architecture and applications. BMC Bioinformatics 10, 421. doi:10.1186/1471-2105-10-421

Chagnon, P.L., Bradley, R.L., Maherali, H., Klironomos, J.N. 2013. A trait-based framework to understand life history of mycorrhizal fungi. Trends in Plant Science 18: 484–491.

Hofstetter, V., Clémençon, H., Vilgalys, R., Moncalvo, J.-M., 2002. Phylogenetic analyses of the Lyophylleae (Agaricales, Basidiomycota) based on nuclear and mitochondrial rDNA sequences. Mycological Research 106, 1043–1059. doi:10.1017/S095375620200641X

172 Kabir, Z. 2005. Tillage or no-tillage: impacts on mycorrhizae. Canadian Journal of Plant
173 Science 85: 23–29.

174 Kanz, C., Aldebert, P., Althorpe, N., Baker, W., Baldwin, A., Bates, K., Browne, P., van
175 den Broek, A., Castro, M., Cochrane, G., Duggan, K., Eberhardt, R., Faruque, N.,
176 Gamble, J., Diez, F.G., Harte, N., Kulikova, T., Lin, Q., Lombard, V., Lopez, R.,
177 Mancuso, R., McHale, M., Nardone, F., Silventoinen, V., Sobhany, S., Stoehr, P.,
178 Tuli, M.A., Tzouvara, K., Vaughan, R., Wu, D., Zhu, W., Apweiler, R., 2005. The
179 EMBL Nucleotide Sequence Database. Nucleic Acids Research 33, D29-33.
180 doi:10.1093/nar/gki098

181 Kautz, T., Amelung, W., Ewert, F., Gaiser, T., Horn, R., Jahn, R., Javaux, M., Kemna,
182 A., Kuzyakov, Y., Munch, J.C., P??tzold, S., Peth, S., Scherer, H.W., Schloter, M.,
183 Schneider, H., Vanderborght, J., Vetterlein, D., Walter, A., Wiesenberg, G.L.B.,
184 Köpke, U., 2013. Nutrient acquisition from arable subsoils in temperate climates: A
185 review. Soil Biology and Biochemistry 57, 1003–1022.
186 doi:10.1016/j.soilbio.2012.09.014

187 Krüger, M., Stockinger, H., Krüger, C., Schüssler, A., 2009. DNA-based species level
188 detection of *Glomeromycota*: one PCR primer set for all arbuscular mycorrhizal
189 fungi. New Phytologist 183, 212–23. doi:10.1111/j.1469-8137.2009.02835.x

190 Leifheit, E.F., Veresoglou, S.D., Lehmann, A., Morris, E.K., Rillig, M.C., 2014. Multiple
191 factors influence the role of arbuscular mycorrhizal fungi in soil aggregation-a
192 meta-analysis. Plant and Soil 374, 523–537. doi:10.1007/s11104-013-1899-2

193 Lekberg, Y., Koide, R.T., 2005. Is plant performance limited by abundance of
194 arbuscular mycorrhizal fungi? A meta-analysis of studies published between 1988
195 and 2003. New Phytologist 168, 189–204. doi:10.1111/j.1469-8137.2005.01490.x

196 Martínez-García, L.B., Richardson, S.J., Tylianakis, J.M., Peltzer, D.A., Dickie, I.A.,

2015. Host identity is a dominant driver of mycorrhizal fungal community composition during ecosystem development. New Phytologist 205, 1565–1576. doi:10.1111/nph.13226

Moll, J., Hoppe, B., König, S., Wubet, T., Buscot, F., Krüger, D., 2016. Spatial Distribution of Fungal Communities in an Arable Soil. PLoS ONE 11, 1–17. doi:10.1371/journal.pone.0148130

Muleta, D., Assefa, F., Nemomissa, S., Granhall, U., 2008. Distribution of arbuscular mycorrhizal fungi spores in soils of smallholder agroforestry and monocultural coffee systems in southwestern Ethiopia. Biology and Fertility of Soils 44, 653–659. doi:10.1007/s00374-007-0261-3

Oehl, F., Sieverding, E., Ineichen, K., Ris, E.A., Boller, T., Wiemken, A., 2005. Community structure of arbuscular mycorrhizal fungi at different soil depths in extensively and intensively managed agroecosystems. New Phytologist 165, 273–283. doi:10.1111/j.1469-8137.2004.01235.x

Powell, J.R., Parrent, J.L., Hart, M.M., Klironomos, J.N., Rillig, M.C., Maherali, H., 2009. Phylogenetic trait conservatism and the evolution of functional trade-offs in arbuscular mycorrhizal fungi. Proceedings of the Royal Society B: Biological Sciences 276, 4237–45. doi:10.1098/rspb.2009.1015

R Core Team, 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Smith S. E., Read D., 2008. Mycorrhizal Symbiosis, 3rd Edn. San Diego, CA: Academic Press.

Smith, S.E., Smith, F.A., 2011. Roles of Arbuscular Mycorrhizas in Plant Nutrition and Growth: New Paradigms from Cellular to Ecosystem Scales. Annual Review of

221 Plant Biology 62, 227–250. doi:10.1146/annurev-arplant-042110-103846

222 Spatafora, J.W., Chang, Y., Benny, G.L., Lazarus, K., Smith, M.E., Berbee, M.L.,
223 Bonito, G., Corradi, N., Grigoriev, I., Gryganskyi, A., James, T.Y., O'Donnell, K.,
224 Roberson, R.W., Taylor, T.N., Uehling, J., Vilgalys, R., White, M.M., Stajich, J.E.,
225 2016. A phylum-level phylogenetic classification of zygomycete fungi based on
226 genome-scale data. Mycologia 108, 1028–1046. doi:10.3852/16-042

227 Uksa, M., Fischer, D., Welzl, G., Kautz, T., Köpke, U., Schloter, M., 2014. Community
228 structure of prokaryotes and their functional potential in subsoils is more affected
229 by spatial heterogeneity than by temporal variations. Soil Biology and
230 Biochemistry 75, 197–201. doi:10.1016/j.soilbio.2014.04.018

231 Uksa, M., Schloter, M., Endesfelder, D., Kublik, S., Engel, M., Kautz, T., Köpke, U.,
232 Fischer, D., 2015. Prokaryotes in subsoil-evidence for a strong spatial separation
233 of different phyla by analysing co-occurrence networks. Frontiers in Microbiology
234 6, 1–13. doi:10.3389/fmicb.2015.01269

235 Veresoglou, S.D., Rillig, M.C., 2012. Suppression of fungal and nematode plant
236 pathogens through arbuscular mycorrhizal fungi. Biology Letters 8, 214–217.
237 doi:10.1098/rsbl.2011.0874

238 Yang, F.Y., Li, G.Z., Zhang, D.E., Christie, P., Li, X.L., Gai, J.P., 2010. Geographical
239 and plant genotype effects on the formation of arbuscular mycorrhiza in Avena
240 sativa and Avena nuda at different soil depths. Biology and Fertility of Soils 46,
241 435–443. doi:10.1007/s00374-010-0450-3

242

243

Figures

Fig. 1 Relative abundance of reads per family in different soil compartments. Proportion of reads assigned to each family for depth (panel A), compartment (B) and depth and compartment (C). Families are coded by color. The category "Others" comprises the families Ambisporaceae, Archaeosporaceae, Gigasporaceae and Paraglomeraceae, as well as OTUs assigned only at the subphylum level. Topsoil = 10-30 cm, subsoil = 60-75 cm. Extended results are presented in the supplementary materia

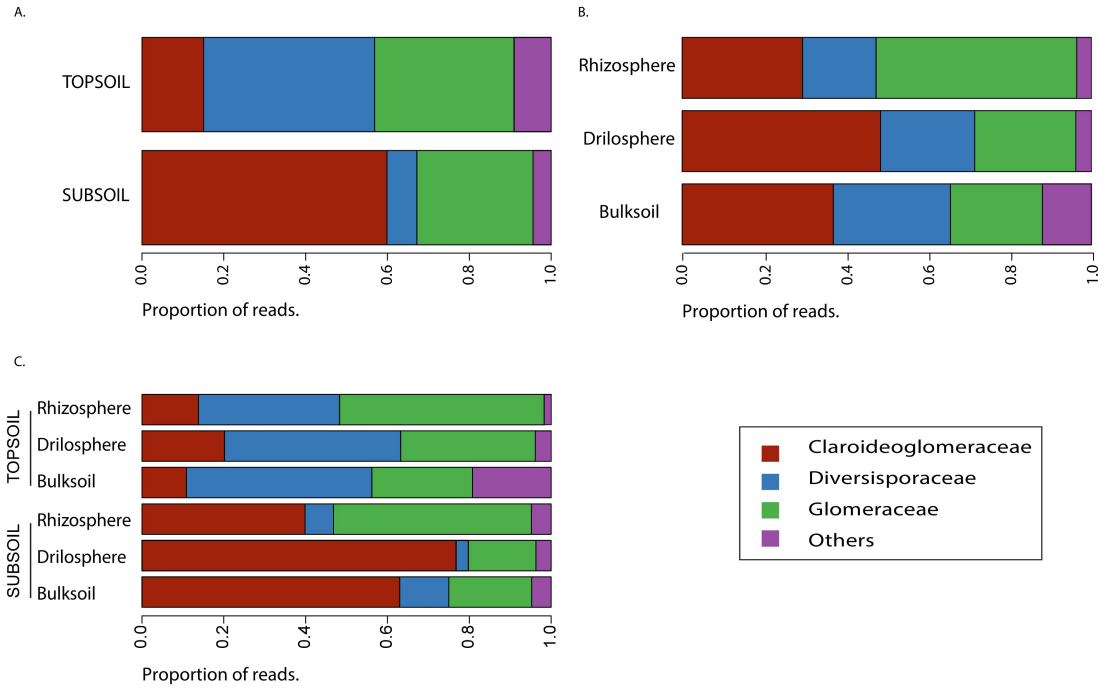
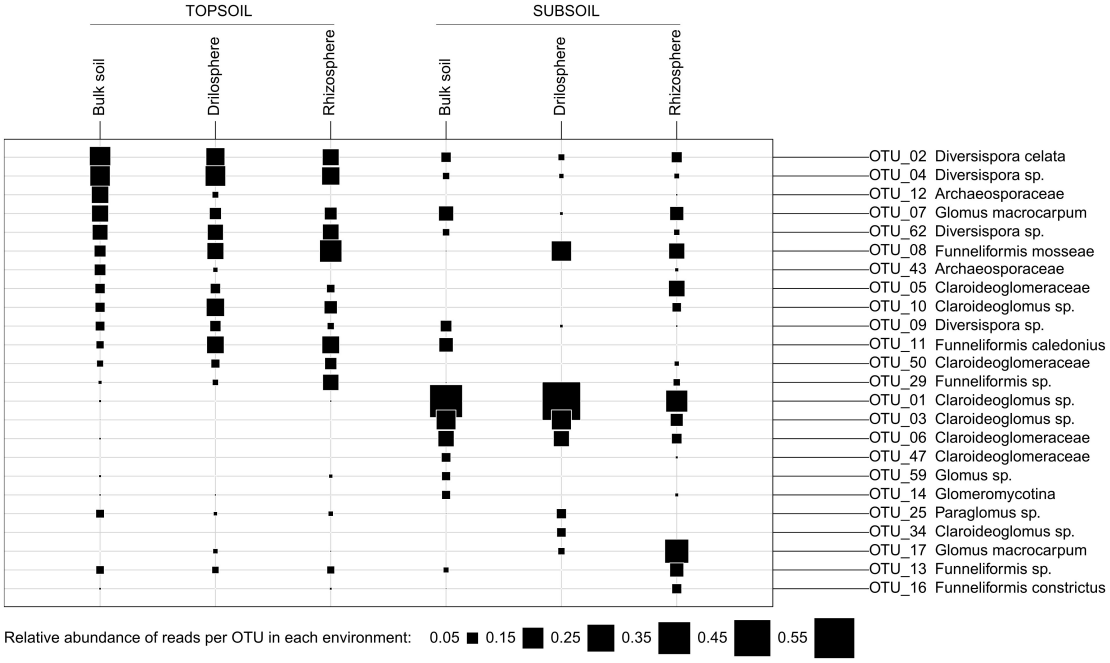


Fig. 2 Relative abundance of the 10 most abundant OTUs for each soil compartment. Relative abundance of the OTU is represented by the area of the square (see scale below figure panel). The OTU list corresponds to the 10 most abundant OTUs for each environment. Topsoil = 10-30 cm, subsoil = 60-75 cm. Taxonomic assignment of each OTU is given (also see Table

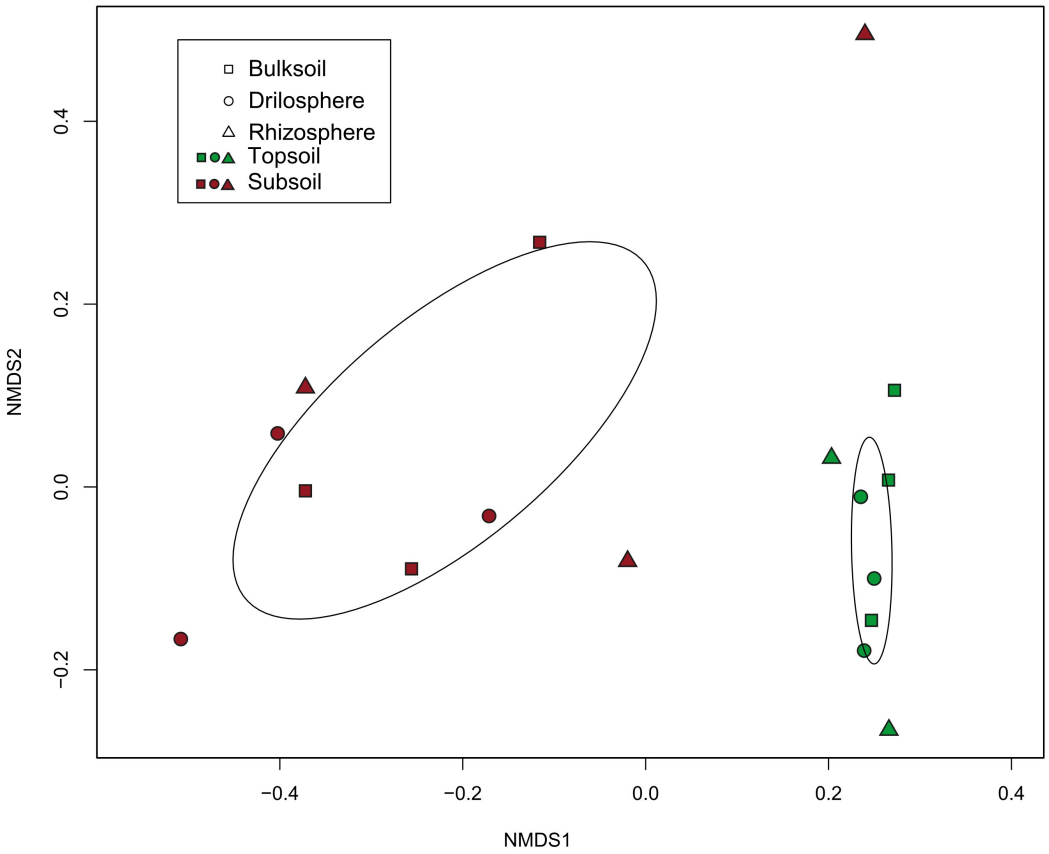
258 S1).



259

260

261 **Fig. 3 Community ordination of AMF in different soil compartments.** Non-metric
262 multidimensional scaling (NMDS) of Bray-Curtis pairwise community dissimilarities.
263 The OTU table was normalized to the minimum amount of reads per sample. Ellipses
264 represent one standard deviation around the centroid of each soil depth. Lines link
265 each sample to the centroid of the group. Topsoil = 10-30 cm, subsoil = 60-75 cm.
266 Depth is coded by color and compartment by symbol shape.



267

268